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3.3.2 Number of research papers published per teacher in the Journals notified on UGC website during the last five years

2023

S. No.	Title of paper	Name of the author/s	Department of the teacher	Name of journal	Year of publication	ISSN number	Link to the recognition in UGC enlistment of the Journal /Digital Object Identifier (doi) number		
							Link to website of the Journal	Link to article/paper/abstract of the article	Is it listed in UGC Care list/Scopus/Web of Science/other. mention
1	Green Synthetic of Silver Nanoparticles using plants extract for Diabetic wound healing	Nayany Sakalle, Mahendra Singh Rathod, Dinesh Kumar Mishra, Shivani Burman	Pharmaceutes	Journal of the Maharaja Sayajirao University of Baroda	Nov-23	ISSN : 0025-0422			
2	Antidiarrhoeal activity ziziphus mauritiana leaf extraction in rodents	Rohit Sahu, Pritesh Paliwal & Praveen Sharma	Pharmacy	Journal of the Maharaja Sayajirao University of Baroda	Nov-23	ISSN: 0025-0422			
3	An in vitro pharmacognostical study on gluconeogenesis and glucose	Praveen Sharma,	Pharmacy	Journal of Advanced Zoology	Nov-23				
4	(Knowledge, Attitudes and Practices) Study on Medicine and Health Infrastructure use in Pregnant Women of Rural Areas of Ujjain Madhya Pradesh, India: A Cross-Sectional Survey	Sandeep Singh Bhadoriya1*, Prashant Wadagbalkar2 , Praveen Sharma3 , RekhaBisht3 ,Nayany Sharma Sakalle3 , Amol R Chandekar4		Journal of Chemical Health Risks	Nov-23	JCHR (2023) 13(4), 2293-2298 ISSN:2251-6727		file:///C:/Users/NILESH/Downloads/Sandeep_Article_1_.pdf	
5	IN SILICO PHARMACOKINETIC, BIOACTIVITY AND TOXICITY STUDIES OF SEVERAL SELECTED ANTI-VIRAL DRUGS	Rohit Kumar Trivedi, Datta Madhavrao Avhad, Rajesh E Jesudasan, Yogesh Tiwari, D.T Sakhare, Rekha Bisht, Pratibha Lakhera, Jhansi Jhansi	Pharmacy Practice	European chemical bulletin	Sep-23	ISSN 2063-5346	https://www.eurchembull.com/issue-content/in-silico-pharmacokinetic-bioactivity-and-toxicity-studies-of-several-selected-anti-viral-drugs-5288		Scopus
6	Phytoconstituent and Pharmacological screening for anti-diabetic activity of Tephrosia villosa	Sheik Nasar I, Azra Aisha. M, Naveen Kumar, Hemant P. Suryawanshi, Rekha Bisht, Sushila Gupta, Yashvardini S., Mohammad Khalid	Pharmacology	European chemical bulletin	Sep-23	ISSN 2063-5346	https://www.eurchembull.com/issue-content phytoconstituent-and pharmacological-screening-for-anti-diabetic-activity-of-tephrosia-villosa-6881		scopus
7	Formulation Development and Evaluation of Herbal Tablet of Diplocyclos palmatus (L.) Jeffry	Sumeet Dwivedi, Devendra S. Lodhi, Deepak Kumawat, Pradeep Golani, Anup K. Chakraborty, Rekha Bisht		INTERNATIONAL JOURNAL OF DRUGT DELIVERY AND TECHNOLOGY	Sep-23	ISSN: 0975 4415	https://ijddt.com/volume13issue3/		UGC approved
8	Reishi(Ganoderma lucidum) A potential Sources of Nutraceuticals and prebiotics	Pritesh Paliwal & Praveen Sharma	Pharmacy	Journal of the Maharaja Sayajirao University of Baroda	Sep-23	ISSN: 0025-0422			

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9	Research progress of small-molecule drugs in targeting telomerase in human cancer and aging	Dinesh K. Mishra5, Rupesh K. Gautam	Pharmacology	Chemico biological Interaction	Sep-23			https://doi.org/10.1016/j.cbi.2023.110631	Elsevier
10	Nanomedicine: Innovative Strategies and Recent Advances in Targeted Cancer Therapy	Dinesh K. Mishra5, Rupesh K. Gautam	Pharmacology	Current Medicinal Chemistry	Aug-23	0929-8673	Current Medicinal Chemistry, XXXX, XX, 1-00		Scopus
11	Phytochemical, Ethnobotanical, and Global Perspectives of Genus Echinacea: A Panoramic Review	Dinesh K. Mishra5, Rupesh K. Gautam	Pharmacology	Current Traditional Medicines	Aug-23	2215-0838	Current Medicinal Chemistry, XXXX, XX, 1-01		Scopus
12	Review on Nanosuspension as a Novel Drug Delivery System for the Treatment of Fungal Infection	Ritu Kumari1*, Nadeem Farooqui1 , Dinesh Kumar Mishra1 , Arti Majumdar2	Pharmaceutcs	Journal For Basic Sciences	Jul-23	ISSN NO : 1006-8341	https://drive.google.com/file/d/1BXEP0Pgbpo_KXuP2BkLWOd71FWTmOvla/view?pli=1		scopus
13	Celiac disease: Pathogenesis, disease management and new insights into the herbal-based treatments	Dinesh K. Mishra5, Rupesh K. Gautam	Pharmacology	Naaraj	Jul-23		10.52225/narra v3i2.147		
14	Computerized system validation- A Regulatory requirementin pharmaceutical Industry	Niel Ravi Daniel, Dr. Gurmeet Singh Chhabra, Gaurav Sarsodiya	Pharmaceutical Chemistry	International Journal of Research and Analytical Reviews	Jun-23	2349-5138	www.ijrar.org	Print/online	
15	Evaluation of In vitro Antioxidant, Antimicrobial and Cytotoxicity Activities of Orthosiphon Pallidus Royle	Mukesh Kr. Singh, Gurdeep Singh, Ritesh Patel, Amrita Mishra , Arun Kr. Mishra, Sushil Kumar	Pharmaceutical Chemistry	International Journal of Pharmaceutical Sciences and Nanotechnology(IJPSN)	Jun-23	0974-3278	https://www.ijpsnonline.com	https://www.ijpsnonline.com/index.php/ijpsn/article/view/2619	scopus
16	Mechanism-Based Suppression of Cancer by Targeting DNA-Replicating Enzymes	Rupesh K. Gautam*, Dinesh Kumar Mishra	Pharmacology	Current Protein & Peptide Science	June , 23	875-5550	https://benthamscience.com/public/journals/current-protein-and-peptide-science	http://dx.doi.org/10.2174/1389203724666230512144011	Scopus, Pubmed/IF-3.118
17	Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021	Rupesh K. Gautam*	Pharmacology	The Lancet	June , 23	0140-6736 (print); 1474-547X (web)	https://www.thelancet.com/journals/lancet/home	https://doi.org/10.1016/S0140-6736(23)01301-6	Scopus/ IF-202
18	The unfinished agenda of communicable diseases among children and adolescents before the COVID-19 pandemic, 1990–2019: a systematic analysis of the Global Burden of Disease Study 2019	Rupesh K. Gautam*	Pharmacology	The Lancet	June , 23	0140-6736 (print); 1474-547X (web)	https://www.thelancet.com/journals/lancet/home	https://doi.org/10.1016/s0140-6736(23)00869-7	Scopus/ IF-202
19	Fused Deposition Modelling (FDM) in Personalized Medicine-An Overview on the Rise of Fused Deposition Mode	Ms. Parul Vaishnav, Mr.Kuldeep Vinchurkar, Dr. Dinesh Kumar Mishra	Pharmaceutics	International journal of pharmaceutical science and nanotechnology (IJPSN)	May, 23		International Journal of Pharmaceutical Sciences and Nanotechnology(IJPSN)	https://doi.org/10.37285/ijpsn.2023.16.2.8	Scopus
20	Current Insight on antifungal activity of Nanocarriers	Neha Kamalpuria, Nayany Sharma, Dinesh Kumar Mishra, Mahendra singh Rathore	Pharmaceutics	Dogo Rangsang Research Journal	Apr-23	2347-7180	https://www.journal-dogorangsang.in/	print journal	UGC Care Group I
21	Promising Repurposed Antiviral Molecules to Combat SARS-CoV-2: A Review	Rupesh K. Gautam*, Dinesh Kumar Mishra	Pharmacology	Current Pharmaceutical Biotechnology	April , 23	ISSN (Print): 1389-2010 ISSN (Online): 1873-4316	https://benthamscience.com/public/journals/current-pharmaceutical-biotechnology	https://doi.org/10.2174/1389201024666230302113110	Scopus/ IF-2.829

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22	Formulation and Characterization of of herbal hair oil for hair growth protection	Bharthi khichi Gurmet Chabra, Darshan Jamindar, Sumeet dwivedi,Mahavir Chajed	Pharmaceutics	Latin American Journal of Pharmacy	Apr-23	326-2383	www.latamjpharm.org	Print/online	
23	Pharmacological Evaluation of Different Extracts of Asparagus officinalis (Asparagaceae) as an Analgesic, Anti-Inflammatory and Anti-arthritic Agent in Rats	Rupesh K. Gautam	Pharmacy	Pharmacognosy Research	March-2023	0974-8490	www.phcogres.com	https://www.phcogres.com/article/2023/15/1/105530097484900333	Web of science
24	Edible Mushroom assisted synthesis and applications of metal nanoparticles: A comprehensive review	Hitesh Chopra, Rajat Goyal, Navidha Aggarwal, Dinesh Kumar Mishra, Rupesh K. Gautam	Pharmacy	Journal of Integrated Science & Technology	Mar-23	2321-4635	https://pubs.iscience.in/journal/index.php/jist	https://pubs.thescienceim.org/journal/index.php/jist/article/view/427	Scopus
25	Recent advancement of polymeric transdermal drug delivery system for emetic drug therapy	Praveen Sharma,	Pharmacy	European Chemical Bulletin	Mar-23	ISSN2063-5346			Scopus
26	Antiulcer effect of Euphorbia nerifolia Linn leaf Extract	Rohit Sahu, Pritesh Paliwal & Praveen Sharma	Pharmacy	European Chemical Bulletin	Mar-23	IISN00 1891-1899			Scopus
27	Isolation, Characterization and Docking Studies of Isolated Compounds as Antidiabetic Molecules from Scindapsus officinalis (Roxb)	Pawan Tiwari*, Gurdeep Singh, Ritesh Patel, Mukesh Kr. Singh	Pharmaceutical Chemistry	Research Journal of Pharmacy and Technology (RJPT)	Feb-23	0974-360X and 0974-3618	https://www.rjptonline.org/	https://www.rjptonline.org/AbstractView.aspx?PID=2023-16-2-37	scopus
28	Natural Products-based Drugs: Potential Drug Targets Against Neurological Degeneration	Rupesh K. Gautam*	Pharmacology	Current Neuropharmacology	February , 23	1875-6190	https://benthamscience.com/journal/26	http://dx.doi.org/10.2174/1570159X21666230220102605	Scopus, Pubmed/IF-7.7
29	Dietary Supplementation with Resveratrol Attenuates Serum Melatonin Level, Pro-Inflammatory Response and Metabolic Disorder in Rats Fed High-Fructose High-Lipid Diet under Round-the-Clock Lighting	Rupesh K. Gautam*	Pharmacology	Pathophysiology	February , 23	1873-149X	https://www.mdpi.com/journal/pathophysiology	https://doi.org/10.3390/pathophysiology30010005	Scopus, Pubmed
30	3D Printing : A Promising Revalutionary Technology in pharmaceutical drug development and health care	Deepika Bhawsar	Pharmaceutics	IJPSN	February , 23	16(1):6337-6349	https://www.ijpsnonline.com/index.php/ijpsn/article/view/3383		SCOPUS
31	Development and optimization of floating alginate microspheres of repaglinide using Box-Behnken experimental design	Kuldeep Vinchurkar | Jitendra Sainy | Masheer Ahmed Khan	Pharmacy	International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN)	Feb-23	16(1):6337-6349	https://www.ijpsnonline.com/index.php/ijpsn	http://www.ijpsnonline.com/index.php/ijpsn/article/view/2526	SCOPUS
32	Nanocarriers and Diabetes: New Vistas and the way ahead	Dinesh Kumar Mishra*, Rupesh K. Gautam*	Pharmacology	Current Pharmaceutical Biotechnology	January , 23	ISSN (Print): 1389-2010 ISSN (Online): 1873-4316	https://benthamscience.com/public/journals/current-pharmaceutical-biotechnology	https://doi.org/10.2174/1389201024666221227140728	Scopus/ IF-2.829
33	Spinacia Oleracea- An Overview of Its Chemical, Nutritional, Phytochemical and Pharmacological Profile	Rohit Sahu	Pharmacy	High Technology Letters	Jan-2023	1006-6748	https://gjstx-e.cn/	https://gjstx-e.cn/?page_id=789	Others
34	Synthesis and pharmacological evaluation of new quinazolinone derivatives as anti diabetic agents	Ritesh Patel, Pritesh Paliwal, Praveen Sharma, Rohit Sahu, Gourav Agrawal, Dheeraj Jain,	Pharmacy	European Chemical Bulletin	Jan-23	ISSN2063-5346			Scopus

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35	Medicines for Management of Psoriasis: Current Status	Dinesh Kumar Mishra	Pharmacy	Pharmacognosy Research	Nov-23	0974-8490	www.phcogres.com	https://www.phcogres.com/article/2023/15/1/105530_09748490026	Web of science
				2022					
36	Medicalization of sexuality and sexual health: A perspective review	Rupesh K. Gautam	Pharmacy	Journal of Experimental Biology and Agricultural Sciences	2022	2320 – 8694	https://jebas.org/ojs/index.php/jebas	https://jebas.org/ojs/index.php/jebas/article/view/1146	UGC Care, Scopus
37	The role of nanomaterials in enhancing natural product translational potential and modulating endoplasmic reticulum stress in the treatment of ovarian cancer	Rupesh K. Gautam	Pharmacy	Frontiers in Pharmacology	2022		https://www.frontiersin.org/journals/pharmacology	https://www.frontiersin.org/articles/10.3389/fphar.2022.987088/full	Scopus and Web of science
38	Nanocarriers and Diabetes: New Vistas and the Way Ahead	Pankaj V Dixit, Dinesh K Mishra, Sanjay Sharma, Rupesh K Gautam	Pharmacy	Current Pharmaceutical Biotechnology	2022	1389-2010	https://www.eurekaselect.com/journal/30/about-journal#:~:text=Current%20Pharmaceutical%20Biotechnology%20is%20an,latest%20and%20most%20important%20developments.	https://pubmed.ncbi.nlm.nih.gov/36578251/	Scopus
39	ROCK2 inhibition: A futuristic approach for the management of Alzheimer's disease	Rupesh K. Gautam	Pharmacy	Neuroscience and Biobehavioral Reviews	2022	0149-7634	https://www.sciencedirect.com/journal/neuroscience-and-biobehavioral-reviews	https://www.sciencedirect.com/science/article/abs/pii/S0149763422003608?via%3Dihub	Scopus and Web of science
40	A Comprehensive Review on Rising Concern of Transmission Potential of Monkeypox Virus on Healthcare System	Rajat Goyal, Mohini Devi, Rupesh K. Gautam, Sumeet Gupta	Pharmacy	Indo Global Journal of Pharmaceutical Sciences	2022	2249-1023	https://www.iglobaljournal.com/	https://igjps.com/index.php/IGJPS/article/view/22935	Web of science
41	An Insight View on the Role of Herbal Medicines in Infectious Diseases	Smriti Parashar, Rupesh K Gautam*, Rajat Goyal, Sanjay Sharma, Sumeet Gupta and Pooja Mittal	Pharmacy	Current Traditional Medicine	2022	2215-0846	https://www.eurekaselect.com/journal/156	http://www.eurekaselect.com/article/126815	Bentham Sciences
42	Comparative Highlights on Mers-CoV, Sars-CoV-1, Sars-Cov-2, and Neo-CoV	Rajat Goyal , Rupesh K. Gautam , Hitesh Chopra, Ankit Kumar Dubey, Rajeev K. Singla, Rehab A. Rayan, Mohammad Amjad Kamal	Pharmacy	EXCLI Journal	2022	1611-2156	https://www.excli.de/index.php/excli	https://www.excli.de/index.php/excli/article/view/5355	Scopus and Web of science
43	Green Chemistry: Current Applications In Pharmaceutical Industry	Priyalata, Kuldeep Vinchurkar, Saloni Yadav, Gurmeet Chhabra, Dinesh K. Mishra	Pharmacy	Dogo Rangsang Research Journal	2022	2347-7180	https://www.journal-dogorangsang.in/	Not found online	UGC Care
44	An Overview of Quality Audit: A Boon for Pharmaceutical Sector	Anita Patidar, Gurmeet S. Chhabra, Dinesh K. Mishra	Pharmacy	International Journal of Pharma and Bio Sciences	2022	0975-6299	https://www.ijpbs.com/	Not found	UGC Care
45	Formulation and Evaluation of Trolamine Salicylate Microemulsion	Prachi Sharma, Nadeem Farooqui, Darshan Jamindar, Pritesh Paliwal, Dinesh Kumar Mishra	Pharmacy	Asian Journal of Pharmaceutics	2022	1998-409X	https://www.asiapharmaceutics.info/index.php/ajp	https://www.asiapharmaceutics.info/index.php/ajp/article/view/4487	UGC Care

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46	3D Printing for Drug Delivery and its Pharmaceutical Applications – Recent Findings and Challenges	Neelima Mandloi, Kuldeep Vinchurkar, Dinesh K Mishra, Pankaj Dixit	Pharmacy	International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN)	2022	0974-3278	http://www.ijpsnonline.com	https://ijpsnonline.com/index.php/ijpsn/article/view/2668	Scopus
47	Six Sigma – A Managerial Tool To Improve Pharmaceutical Quality	Ankita Bhadoriya, Dr. Gurmeet Singh Chhabra	Pharmacy	International Journal for Research Trends and Innovation	2022	2456-3315	https://www.ijrti.org/f	https://www.ijrti.org/viewpaperforall.php?paper=IJRTI2208070	UGC Care

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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PLANTS EXTRACT FOR DIABETIC WOUND HEALING: A REVIEW

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Mahendra Singh Rathod, Geetanjali Institute of Pharmacy, Geetanjali University, Udaipur
Dinesh Kumar Mishra, Central University Bilaspur Chhattisgarh
Ms. Shivani Burman Indore Institute of Pharmacy, Indore

ABSTRACT
The utilization of plant-mediated green synthesis for nanomaterial production is experiencing a surge in popularity due to its dual advantages of environmental sustainability and cost-effectiveness. Towards different kinds of metallic nanoparticles, Silver Nanoparticles (AgNPs) have garnered primary goal and are widely recognized as one of the most extensively researched nanomaterials. This era highlights the pivotal aspect of plants in the green synthesis of nanoparticles, drawing considerable attention for its ability to create stable and environmentally friendly nanoparticles. The synthesis of silver nanoparticles holds particular significance owing to their wide array of potential applications, including catalysis, plasmonics, antimicrobial properties, biosensing, and biomedical usage. The interaction between Ag+ ions and phytochemicals present in plant extracts catalyzes the production of bioactive AgNPs molecules, fostering their creation and stability. In fact, these synthesized AgNPs have demonstrated superior efficacy in inhibiting bacterial growth when compared to the plant extract itself. Notably, the application of silver nanoparticles has found its place in wound healing, addressing various conditions such as cuts, chronic ulcers, and wounds. In light of these developments, a comprehensive review is presented to elucidate the bio-inspired mechanisms behind the synthesis of silver nanoparticles. This approach not only provides an avenue beyond conventional physical and chemical techniques but also champion's ecological friendliness, cost-efficiency, and enhanced effectiveness in various applications, prominently including antimicrobial wound healing activities. The integration of plant-mediated synthesis of AgNPs offers a promising solution that aligns with contemporary environmental and economic considerations, heralding a paradigm shift in nanomaterial production and its diverse utilities.

Keywords-
Silver nanoparticles, Green synthesis, Wound healing, Plant extract, Antimicrobial.

1. INTRODUCTION
Diabetes is one of the primary reasons of decreased healing of the affected region. Diabetes Mellitus (DM) is a multi farious metabolic disease that affects millions, and around 20% of people suffer from diabetic wounds worldwide (Patel *et al.,* 2019). In general, DM is characterized by decreased insulin secretion and causes due to hyperglycemia. Among the significant serious consequences of diabetes is a decrease in self-repairing defects (Simona *et al.,* 2020) (Armstrong *et al.*,2017). Diabetic wounds can develop due to several variables, incorporating poor glycemic control and peripheral nerve damage vascular disease, and suppression of the immune system (Aumiller *et al.*,2015).
Invasion of ulcers from diabetes involving Multi-Drug Resistance (MDR) bacteria can delay wound healing, enhance hospitalization, treatment expenses, and raise patient death (Gómez *et al.*,2009). There are many other approaches to chronic diabetic wounds had also managed to fail should provide particular effects in terms of recovery from wounds, acute discomfort legislation, and beverage loss, along with indispensable capabilities, such as malleability, dependability, and utility within which the entirety of aforementioned variables have affected compliance the possibility of nanotechnology-based technological advances, leading to may additionally make a significant impact on diabetic wound people with illnesses (Hamdan *et al.*,2017).

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www.phcogres.com

Review Article

Phytotherapeutic Potential of Natural Herbal Medicines for Management of Psoriasis: Current Status

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ABSTRACT

Psoriasis is a complex multifunctional inflammatory autoimmune skin disease, which is mainly characterized by activation of T-cell (T-lymphocyte), abnormal proliferation keratinocyte, local vascular changes and activation of the neutrophil. A number of therapies are being used to treat psoriasis including topical, systemic and phototherapy recpectively but none of them is able to cure the disease completely, precluding the long-term serious side effects for the human body. In contrast to these, herbal therapies can play an important role for treatment of psoriasis. With this endeavor, this review reports the recent developments and patent showing potential of herbal therapy for treatment of psoriasis along with future prospect in the field of traditional and novel drug delivery system (NDDS) for treatment of psoriasis.

Keywords: Psoriasis, Keratinocyte, Hyperproliferation, Herbal drug, Novel drug delivery systems.

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INTRODUCTION

The immune system is a complex of various tissues, organs, proteins and special cells, which helps to protect the human body against disease. The largest part of our body (skin) perform various function including immunological and physical protection through some cellular and humor constituents including keratinocyte, dendritic cells (DCs), β-defensins, mannose-binding lectins and immunoglobulin.[1] These components are responsible to generate adaptive immune responses against the exogenous injuries.

Psoriasis is a most common and chronic autoimmune disease among various skin diseases including lupus, scleroderma and atopic dermatitis (AD) which has significantly negative effect on quality of life style of patient along with their families resulting in emotional, physical and social burden.[2]

The term psoriasis is originated from the Greek word *psora* which reflect with "itching".[3] Psoriasis is a complex multifunctional inflammatory autoimmune skin disease, which is characterized by activation of T-cell (T-lymphocyte), abnormal proliferation in keratinocyte, local vascular changes and activation of the neutrophil.[4,5]

According to Epicast report, there were approximately 36.5 million prevalent cases of psoriasis in 2012 across the world and it was estimated that this number will reach approximately 40.93 million prevalent cases by 2030.

With a prevalence of India, it is observed that most of the psoriatic patients are individuals between 30-40 years of age.[6] Understanding the morphological difference between normal and diseased skin can make the discussion more clear. The lack of safe and effective treatment for psoriasis has directed many researchers towards the development of novel therapies. Parallel to various topically or systemically used synthetic medicines; herbal therapy can play potential role for treatment of psoriasis.[7,8] Against this background, the present review emphasizes the future prospect of herbal therapy and recent developments along with patents reported in the field of traditional and novel drug delivery system (NDDS) for treatment of psoriasis.

CAUSES OF PSORIASIS

Various environmental factors including stress, hypocalcemia infections (e.g., streptococcal pharyngitis) physical trauma (Koebner or isomorphic phenomenon) and some medications including beta blockers, IFNs, antimalarials and systemic corticosteroids are responsible for psoriasis. Apart from these some other factors such as higher body mass index (BMI) rapid weight changes, alcohol consumption, vitamin D deficiency and habit of tobacco are also associated to increase psoriatic inflammation.[9]

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GENERAL SYMPTOMS

The general symptom of this disease includes silvery scales on bright red, pitting on nails, genetic predisposition, well demarcated plaques (usually on the *sclap*, elbows and knees) and chronic inflammation. People with psoriasis are also have risk of coronary artery diseases.[3,10]

TYPES OF PSORIASIS

Historically, the disease has been classified on the basis of clinical appearance (Table 1). According to morphology and localization, plaque psoriasis is a most common type of psoriasis, covers around 85% of psoriatic population. In this type of psoriasis, scalp, trunk and buttocks are mainly infected but inflammation may occur at anywhere on the body. The degree of severity of disease is classified as mild, moderate, and severe, which is further measured by means of patient's body surface area (BSA) affected by psoriatic lesions. Normally 3-10% BSA is considered to be moderate while more than 105 is considered to be severe.[11,12]

PROBABLE MECHANISMS OF PSORIASIS

The mechanism of the occurrence of psoriasis is not clear yet. Progression of psoriasis is assumed to be based on number of cumulative events which are involved in the activation of immune cell along with secretion of various signaling molecules such as cytokines, chemokine and growth factors that lead to congealing of epidermis, hyperkeratosis and neovascularization.[23,24] External factors, such as medication, infection or trauma can stimulate the formation of complex antimicrobial peptides (AMPs) which are released from keratinocytes in genetically

Table 1: Clinical classification of psoriasis.

Clinical Classification	Characterization	Associations
Chronic Plaque Psoriasis[13,14]	Well circumscribed, erythematous, silvery scale, either as single lesions or as a generalized disease. Usually involving the scalp, knees, elbows, low back, umbilicus, and gluteal cleft.	Most common; accounting for approximately 90% of all cases of psoriasis
Flexural[15]	Also known as *inverse psoriasis*, minimally scaly, affecting predominantly the axillae, groin, submammary area, genital, natal cleft region.	Prone to secondary bacterial or yeast infections
Nail[16]	Pitting, distal onycholysis, oil drop sign, splinter haemorrhages, leukonychia, crumbling, red lunula.	More common in people with psoriatic arthritis.
Scalp[17]	One of the most common sites of psoriasis that is difficult to treat.	-
Palmoplantar[18]	Localized to the hands and soles of feet with yellow-brown macules. Confluent redness and scaling without obvious plaques to poorly defied scaly or fissured areas to large plaques covering the palm or sole.	Commonly associated with sterile inflammatory bone lesions
Guttate[19]	The acute eruption of "dew-drop," salmon-pink, fine-scaled, small papules on the trunk or limbs.	Second most common type (2%) in children and young adults. Associated with group A *Streptococcus* infections.
Pustular[20]	Sheets of monomorphic pustules on painful, inflamed skin Most commonly localized to the palms or soles. Confluent pustules on an erythematous base von Zumbusch type: generalized with acute fever, chills, nausea, headache, and joint problems possible. Acrodermatitiscontinua of Hallopeau: distal fingers; fingernails possibly floating away on lakes of pus and permanent nail destruction common.	Life-threatening Potential complications include high-output cardiac failure, sepsis, and hypercalcemia
Erythroderma[21]	Erythema with scaling over more than 80-90% of the body surface area. Life-threatening emergency.	Potential complications include high-output cardiac failure, renal failure, hypothermia, sepsis hypoalbuminemia
Annular[22]	Subacute or chronic; systemic symptomsdemarcated erythematous scaly plaques with central clearing.	Systemic symptoms are less common than with the Hallopeau type.

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predisposed individuals. For example, antigen presenting cells including Toll-like receptor 7 (TLR7) and (TLR9) respectively can bind to cathelicidin antimicrobial peptide (CAMP) on the surface of plasmacytoid dendritic cells (pDCs), which helps local expansion and the activation of antigen specific CD8+ T cells in the dermis and local lymph nodes.[25] Consequently, these activated cells migrate into the epidermis and attack on the major histocompatiblity complex (MHC) receptors on to the surface of keratinocytes, which triggers the release of soluble factors locally such as innate immune mediators, chemokine's and cytokines that could be able to increase proliferation of keratinocyte and local inflammation.[26] On the other hand some inflammatory mediators are released such as interferon-α (IFNα) and IFNβ which indirectly increase the secretion of additional pro inflammatory mediators such as IL-12, IL-23 and tumor necrosis factor (TNF) that helps to release additional chemokines and cytokines and promotes the defense mechanism of the host which leads to recruitment of extrainflammatory cells towards new lesion.[27] Some interleukins are responsible to contribute the characteristic psoriatic histological phenotype, including acanthosis, parakeratosis (incomplete keratinization with retention of nuclei) and epidermal hyperplasia. Activation of key transcription factors in psoriasis such as Janus kinase (JAK)–signal transducer, nuclear factor-κB (NF- κB) and cyclic AMP leads to further production of IL-17 and TNF respectively.[28] The Overexpression of growth factor of vascular endothelial receptor promotes the vascular proliferation into the skin which leads to the establishment of chronic psoriatic inflammation.[29] The pictorial representation for mechanism is given in Figure 1.

CO-MORBIDITIES ASSOCIATED WITH PSORIASIS

Moderate to severe psoriasis is linked with some other diseases, which may have a significant impact on patients.[30] Co-morbidities may increase with age and it is believed that nearly 3/4 population of psoriatic patients may have 1-3 co-morbidities. In one study it was found that patients with high severity of disease have 3-4 year shorter life than healthy people.[30] Patients older than 65 years had a statistically significant higher incidence of cardiac disease with increased risk for myocardial infarction and increased risk of higher blood glucose level which leads to diabetes mellitus.[31] Other important co-morbidities associated with this disease are psoriatic arthritis, metabolic syndrome, Crohn's disease, dyslipidemia, obstructive sleep apnea, ulcerative colitis, depression, liver disease, chronic obstructive pulmonary disease and cancer, which lead to impairment in quality of life.[32] Thus, psoriasis leads to significant psychological and psychosocial co-morbidities, which further lead to poor treatment outcomes and worsening of disease.

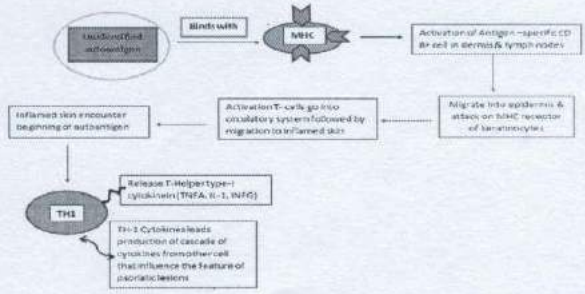

Figure 1: Mechanism for Psoriasis.

TREATMENT STRATEGIES

Psoriasis is a long-term diseased condition, which requires a long-term treatment therapy. The aim for the treatment of psoriasis is to reduce the severity and extent of the disease as well as to improve patient care with a major emphasis on their health-related quality of life (HRQOL) along with control on long-term disease.[33,34] Generally, three major ways are available as treatment options for psoriasis including topical, systemic and phototherapy respectively.[35] Topical therapy is the best way to treat mild to severe disease, which should be initiated at primary level of the disease. Systemic therapy may be the alternative option where topical therapy do not elicit a satisfactory responses.[19] A detailed discussion on available antipsoriatic drug therapy along with mechanism of action and potential side effect is presented in Table 2.

CHALLENGES AND OPPORTUNITIES FOR AVAILABLE TREATMENT

Currently conventional therapy is being used for the treatment of mild to moderate psoriasis.[56] Such Patients being treated with various topical therapies such as tazarotene, corticosteroids, psoralen, Vitamin D analogues, calcineuin inhibitors, 5-aminolevulinic acid, salicylic acid, an ester of fumaric acid, anthralins (dithranol), and tacrolimus as well as some systemic medication such as cyclosporine, 6-thioguanine, mycophenolatemofetil, and methotrexate.[57] Some other biologic agent such as alefacept, adalimumab, efalizumab, infliximab, etanercept or various combination of those are also being used as a treatment option for psoriasis.[58] However, most of the available therapies are associated with problems such as decreased safety profile as well as increased side effects and limited efficacy for long term use. For example, topical corticosteroids may result in cutaneous atrophy and dyspigmentation as a major side effect which limit their long-term use.[59] Some corticosteroids are also being used with several vitamin D analogues to modulate immune system as well as to increase normalization of keratinocyte maturation.[60] Tazarotene is effective as a topical agent, which is considered to be the first-aid treatment for facial

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Table 2: Available treatment options for psoriasis.

Topical Therapy	
Calcineurin inhibitors (a) Tacrolimus[36] *Mechanism of action* This drug inhibits the activity of calcineurin phosphatase (cytoplasmic enzyme), which further inhibits the translocation of NFAT (nuclear factor of activated T cells) in the T cells. (b) Dithranol[37] *Mechanism of action* The drug accumulates inside the mitochondria and acts by impairment of energy supply to the cell by free radicals released from the oxidation of dithranol and interferes with the DNA replication, which slows down the extreme cell division that occurs in psoriatic plaques. (c) Tar[38] *Mechanism of action* Coal tar causes suppression of the hyperplastic skin occurring in some proliferative disorders.	*Side effects* Burning, itching sensation, Flu-like symptoms, headache, cough, discoloration of surrounding skin, Irritation Staining and carcinogenic risk.
Vitamin D_3 analogue: Calcipotriol[39] *Mechanism of action* It acts by reducing the production of pro-inflammatory cytokines such as interleukin (IL)-8. It also increases the production of anti-inflammatory cytokines like IL-4 and IL-10. Calcitriol[40] *Mechanism of action* It acts by increasing the calcium absorption in the intestine and kidney and leads to increase in the calcium level in the serum, consequently decreased bone desorption, decreased level of serum phosphatase and parathyroid hormone, increased resorption of renaltubule phosphate.	*Side effects* Pruritus, sharp pain, erythema, burning, and rare hypercalcemia, Anorexia, headache, thirst, sweating and polyuria.
Keratolytic agents: Salicylic acid[41] *Mechanism of action* Salicylic acid acts by reducing the intercellular bonding of corneocyte by decreasing the pH of the stratum corneum, which leads to hydration and swelling of corneocyte. Omega-3 fatty acids[42] *Mechanism ofaction* It acts by inhibition of lymph proliferation, antigen presentation and adhesion molecule presentation. Omega-3 fatty acid also inhibits the responses generated by Th1 and Th2 and inhibits the production of a pro-inflammatory cytokine. Glycolic acid[43] *Mechanism of action* It affects the lower layers of stratum corneum by disrupting adhesion of corneocytes presents within.	*Side effects* Inflammation and burning sensation on the applied area, rash, itching, wheezing, difficulty inswallowing or breathing.

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Table 2: Cont'd.

Topical Therapy	
Corticosteroids: a) Class-I (superpotent) and Class II (potent) Clobetasol propionate, Diflorasone diacetate, Halobetasol propionate, Betamethasone dipropionate, Amcinonide, Mometasone furoate, Fluocinolone acetonide[44,45] *Mechanism of action* They control the activities of cellular proteins by modifying the expression of the gene and through non-genomic mechanisms, which lead to a broad range of anti-inflammatory, vasoconstriction actions and immunosuppressant.	*Side effects* Skin infections, perioral dermatitis, skin atrophy, hypertrichosis, striae, skin thinning, telangiectasias, purpura, acneiformeruptions, burning, itching, irritation, dryness, folliculitis and secondary infection.
b) Class III (upper mid strength) and Class IV (mid strength) Triamcinolone acetonide, Betamethasone valerate, Flurandrenolide, Hydrocortisone valerate[46] *Mechanism of action* They stimulate the action of phospholipase A2 inhibitory proteins, called lipocortins, which inhibit the release of arachidonic acid and regulate the biosynthesis of inflammation-causing substances such as leukotrienes and prostaglandins.	*Side effects* Irritation, dryness, folliculitis, hypertrichosis, acneiform eruptions, hypopigmentation, perioral dermatitis, allergic contact dermatitis, polyuria, polydipsia, polyphagia, weight gain, increased susceptibility toinfection,
c) Class V (lower mid strength) Prednicarbate[47] *Mechanism of action* This drug involves the downregulation of Il-1 α, which inhibits the cytokine in keratinocytes and leads to anti-inflammatory effects.	*Side effects* gastrointestinal ulceration, pancreatitis, osteoporosis, hyperglycemia, steroid myopathy, burning sensation, itching, rash, folliculitis, reddening of skin, weeping skin lesion may occur.
Retinoids: Acitretin[48] *Mechanism of action* Acitretin modulates the mitotic activity and differentiation of the keratinocytes leads to reduced intra-epidermal movement of neutrophilic granulocytes.	*Side effects* Vitamin A toxicity (cheilitis, xerosis, nose bleeds, alopecia, increased skin fragility).

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Table 2: Cont'd.

Topical Therapy	
Systemic Therapy	
Efalizumab[49]	*Side effects*
Mechanism of action	Flu-like reactions, leukocytosis and lymphocytosis, rebound, exacerbation and arthralgia. Local reactions, infections, sore throat, headache, dizziness, fatigue, hair loss, and rash, liver fibrosis/cirrhosis, pneumonia/ alveolitis, bone marrow depression, renal damage, necrosis of soft tissue and bone, an increase of blood pressure, liver failure, nausea, anorexia, vomiting, diarrhea, hypertrichosis, gingival hyperplasia and tremor.
Efalizumab exerts its inhibitory action by reducing the movement of T-cell from the blood vessels into the tissue and leads to inhibited interaction with the endothelial cells.	
Etanercept[50]	
Mechanism of action	
Etanercept interacts with soluble pro-inflammatory cytokine called TNF-α, which leads to block the cascade of inflammation as it plays a major role in the development and regulation of inflammatory processes.	
Infliximab[51]	
Mechanism of action	
It also exerts its action by interacting with soluble pro-inflammatory cytokine called TNF-α, which leads to either neutralized pro-inflammatory activity or removal of the affected cells.	
Methotrexate[52]	
Mechanism of action	
This is a folic acid antagonist, which acts as a competitive inhibitor of the enzyme dihydrofolate reductase and prevents the conversion of dihydrofolic acid to tetrahydrofolic acid.	
Cyclosporine[53]	
Mechanism of action	
Itinhibits the activity of the calcium-calmodulin calcineurin complex and promotes the translocation of NFAT and promote the production of NFAT-dependent cytokine.	
Phototherapy	
Ultraviolet B therapy[54]	*Side effects*
Mechanism of action	Risk of skin cancer, most notably squamous cell carcinoma (SCC) and to a lesser extent basal cell carcinoma and malignant melanoma and premature skin aging.
Ultraviolet B radiation exerts its effect by binding with the nuclear DNA leads the generation of pyrimidine dimers and other photoproducts, which cause inhibition in DNA synthesis. Indirectly inhibits the proliferation of lymphocytes, which ultimately causes decreased proliferation in keratinocytes.	Pruritis, mild transient erythema, edema, headache, dizziness, acneiform eruption andsevereskin pain.
Psoralen plus ultraviolet A therapy (PUVA) and Methoxsalen[55]	
Mechanism of action	
UV activated psoralen combines with DNA to induce strong binding between the two strands of the double-helical DNA and interferes with DNA synthesis. Thus it prevents the aberrant proliferation of a cell in psoriasis plaques.	

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and flexural psoriasis that improves symptoms with less skin atrophy than other topical corticosteroids.[61] The onset of action of these agents is slow yet their adverse-effect profile is very low. Patients suffering from severe psoriasis are often treated with systemic therapies, which include methotrexate acitretin and cyclosporine in combination with phototherapy. Systemic agents and phototherapy exhibit a number of side effects such as hyperlipidemia, hypertension, renal toxicity, skin cancer and hepatotoxicity.[62] To overcome these problem, novel drug carrier are being developed that exhibit sustained release with reduced dosing frequency for improved therapeutic benefits to psoriatic patients.[63] Apart from novel drug delivery system for conventional drugs, herbal products are greatly been accepted by patients as these therapy is safer and having advantage of ease of availability, low cost, patient compliance and minimum side effect in comparison to other conventional therapeutics. Moreover, natural products have great diversity in molecular structure as well as have multidirectional mechanism of action to treat the disease. Therefore researchers are showing interest towards novel herbal products as an alternative for synthetic drugs for the treatment of psoriasis.[64]

ANTIPSORIATIC ACTIVITY OF HERBAL PLANTS

A number of herbal formulations are being used in market around the globe for treatment of psoriasis. The medicinal plants play a vital role in pharmacological research and drug development, since medicinal plants have many advantages over other medicines including various side effects, ease of availability at lower cost and patient compliance. Therefore researchers are seeking for potential herbal products as an alternative to synthetic drugs in psoriasis therapy.[65] Some of the herbal plants used in psoriasis along with their active constitute and mechanisms of action are shown in Table 3.

The efficacy of the herbal products is supported by *in vivo* studies for the treatment of psoriasis, which reveals that the inhibition of keratinocyte hyperproliferation, inhibition of hedgehog (Hh) signaling pathway, modulation of keratinocyte differentiation and suppression of phosphorylase kinase (PhK) activity are considered to be a major targets for antipsoriatic strategies.[92] The documented evidence in support of antipsoriatic herbal formulation is discussed in following text.

An ethanolic extract (95%) of Aloe vera leaf gel was evaluated as the treatment option for psoriasis using mouse tail model. The formulation shown significant differentiation in the epidermis, as measured from its degree of orthokeratosis (85.07± 3.36%) that was almost equivalent to the effect of 0.1% tazarotene gel, which was used as a standard positive control.[93]

A polysaccharide was purified from *Gynstemma pentaphyllum Makino* and investigated as antipsoriatic activity along with it's *in vitro* mechanism of action by using cultured HaCat cells as psoriasis relevant experimental model. They concluded that water-soluble polysaccharide (GP-I) can be a promising antipsoriatic agents in topical therapy.[94]

Table 3: Medicinal plant used in psoriasis.

Botanical Name: *Aloe barbadensis* Miller[66]
Family Name: Liliaceae.
Common Name: Aloe vera.
Active Constituent: Lignin.
Mechanism of action: Lignin is responsible for curing psoriasis, which majorly acts through penetration mechanism that allows AV to penetrate into inner skin layers.[67]

Botanical Name: *Azadiracta indica*[68,69]
Family Name: Meliaceae.
Common Name: Neem.
Active Constituent: Azadirachtin.
Mechanism of action: Azadirachtin penetrates in the deep layer of skin to heal the disease where vitamin E and omega 6 and 9 fatty acids of Neem oil exert a moisturizing effect on skin and help in reduction of the scales and dryness.[70]

Botanical Name: *Curcuma longa*[71]
Family Name: Zingiberaceae.
Common Name: Turmeric.
Active Constituent: Curcumin.
Mechanism of action: Curcumin inhibits nuclear factor kappa B, group of proteins, which regulates inflammation during psoriasis. It also accelerates healing of skin and increases its regenerating potential. Tumour necrosis factor alpha and interleukin are important proteins during inflammation of psoriasis. Curcumin effectively inhibits the activity of these proteins and inhibits the activation of other biochemical pathways that could lead to the advancement of the disease.[71]

Botanical Name: *Glycine max*[72]
Family Name: Fabaceae.
Common Name: Soybean.
Active Constituent: Genistein.
Mechanism of action: Genistein is treated as the main isoflavone in soybean, which exerts potent anti-inflammatory and anti-oxidant properties. Immunological evidence suggested that modulates inflammatory responses by reducing production and expression of some proinflammatory biomarkers such as TNF-α, IL-6, IL-1β, and IL-8. Genistein is also believed to exert anti-proliferative activity by inhibiting NFκB signaling.[73]

Botanical Name: *Oenothera biennis* L.[74]
Family Name: Onagraceae.
Common Name: Evening Primrose oil.
Active Constituent: Linoleic acid and Gamma-linolenic acid.
Mechanism of action: Linoleic acid and Gamma-linolenic acid help in rehydration and restoration of the skin. This essential oil also benefits psoriasis by lessening the dryness, itchiness and boosts the generation of prostaglandins naturally and helps to control skin inflammation.[75]

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Table 3: Cont'd.

Botanical Name: *Nigella sativa* Linn.[76]
Family Name: Ranunculaceae.
Common Name: Black Cumin.
Active Constituent: Thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol.
Mechanism of action: These active constituents are capable to suppress the hyperproliferation and abnormal differentiation of keratinocytes.[77]

Botanical Name: *Psoraliya corylifolia* Linn.[78]
Family Name: Fabaceae.
Common Name: Bakuchi.
Active Constituent: Psoralens, bakuchiol, bakuchalcone, isopsoralen.[79]
Mechanism of action: Psoralen is a photoactive furocoumarin, which has the capacity to absorb radiant energy at a ultra-violet range of 200-320nm to form photoproducts with pyrimidine base, which inhibits DNA synthesis to decrease proliferation of cells and therefore helpful in dermal disorders like psoriasis.

Botanical Name: *Silybum marianum*[80]
Family Name: Asteraceae.
Common Name: Milk Thistle.
Active Constituent: Silicristin, silibinin and silidianin, collectively known as Silymarin.
Mechanism of action: *Silibum marianum* inhibits the activation of human T-cell, which occurs in psoriasis.[81]

Botanical Name: *Angelica sinensis*[82]
Family Name: Apiaceae.
Common Name: Dong quay.
Active Constituent: Furocoumarin i.e. psoralen.
Mechanism of action: Exposure to UV-A causes epidermal DNA cross-linking and decreases the rate of epidermal DNA synthesis.[83]

Botanical Name: *Indigo naturalis*[84]
Family Name: Fabaceae
Common Name: Qing-Dai
Active Constituent: Indirubin
Mechanism of action: Inhibits the TNF-alpha-dependent inflammatory pathways, to down-regulate inflammatory markers, which have observed in psoriatic skin.[85]

Botanical Name: *Capsicum annum*[86]
Family Name: Solanaceae.
Common Name: Chili peppers
Active Constituent: Capsaicin
Mechanism of action: Acts by depleting the neuropeptide substance locally in the skin, which is known to elicit itching during psoriasis.[87]

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Botanical Name: *Matricaria recutita*[88]
Family Name: Asteraceae
Common Name: Chamomile.
Active Constituent: Chamazulene.
Mechanism of action: Act by the inhibition of lipoxygenase, which further forms leukotriene B4 (LTB4) and reduces inflammations.[89]

Botanical Name: *Smilax china*[90]
Family Name: Smilacaceae
Common Name: China root.
Active Constituent: Flavonoid quercetin.
Mechanism of action: Acts with reduction of leucocyte migration that significantly reduces the thickness of epidermal.[91]

The topical effect of St Johns wort (*Hypericum perforatum* L.) was evaluated in plaque psoriasis in a case study where 10 patients were treated with ointment and observed significant antipsoriatic activity within 4 weeks as they observed that modified psoriasis area severity index (PASI) score was significantly lowered at the site of application of ointment.[95]

The novel antipsoriatic activity of herbal cream containing methanolic extract of *Cassia tora* leaves was prepared to obtain a controlled drug delivery system which was claimed that *Cassia tora* leaves extract show significant anti-psoriatic activity in ultraviolet-B-induced psoriasis in rat and can be also used as a natural antioxidant.[96]

The efficacy of herbal preparation of Durr Derma was evaluated in adult patients having moderate to severe skin psoriasis, which contains black cumin as the main component along with some other active ingredients like olive oil, cocoa butter, Vitamin B_{12} and tea tree oil Vitamin A. They included 8 males and 4 females and treated them two times in a day for 12 weeks. The success rate of treatment was determined by the psoriasis area and severity index (PASI) score, which was found to be lower than 75% in 10 out of total 12 treated patients within 10 weeks while remaining patients had shown PASI reduction by 50%. The results revealed that this herbal preparation could be a promising treatment option for psoriasis.[97]

Herbal formulation of *Solanum xanthocarpum* stem was prepared and further the anti-psoriatic activity was confirmed in Imiquimod-induced psoriatic mice model, after treatment for 15 days with both oral and topical formulation. They determined the level of TNF- α, IL-1 β, IL-6 and IL17 in the animal tissues as well as psoriasis area severity index (PASI). It was concluded that topical formulation has shown better anti-psoriatic activity than oral formulation.[98]

Poly herbal aqueous formulation (SIRB-001) was developed which consist of 3 herbs including *Lonicera japonica, Rheum palmatum* L. and *Rehmannia glutinosa* L. in the ratio of 1:1:3, respectively, which demonstrated efficacious anti-psoriatic

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effects via multiple arms (anti-inflammatory, antiproliferative, proapoptotic anti-angiogenic) at the cellular level. They concluded that SIRB-001 exhibited well *in vitro* antipsoriatic properties in cell-free enzymatic assays, keratinocytes and immune cells.[99]

Double-blinded, randomized and dosage-controlled trial study was performed to determine the safety and efficacy of indirubin in the Lindi oil ointment in different concentrations to treat chronic plaque psoriasis. Lindi oil ointement 200, 100, 50 or 10 μg g^{-1} of indirubin was applied twicein a day for 8 weeks to the adult patients suffering from plaque psoriasis for more than 1 year and observed 75% to 90% reductions in PASI scores within 8 weeks. The authors finally concluded 200 μg/g of indirubin with lindi oil ointment was the most effective concentration to treat psoriasis topically.[100]

The effects of glabridin (Glab) was investigated on proinflammatory cytokines, oxidative/anti-oxidative indexes, histopathological changes and PASI scores in IMQ-induced mice and observed significantly suppressed levels of NF-κB subunit p65, nitric oxide (NO), interleukin (IL)-6, and IL-1β in HaCaT cells stimulated by lipopolysaccharide. In addition, they also observed the reduced expression of IL-17A, IL-22 and IL-23 in HaCat cells stimulated by TNF-α. The results indicated that Glab had beneficial effects on psoriasis and concluded that the underlying mechanism can be associated with the down-regulation of pro-inflammatory cytokines and the improvement of antioxidant status.[101]

HERBAL NANOTECHNOLOGY FOR PSORIASIS TREATMENT

Most of the herbal drugs from herbal origin are poorly water soluble due to presence of hydrophobic active ingredients which results in low bioavailability and increased systemic clearance. Therefore increased or repeated dose is require, that leads to limited clinical used of herbal medicines.

An alternative option is required to increase water solubility of herbal drugs as well as to localize the drug in particular site for improved patient compliance and better efficacy.[102,103] In recent years, nanoparticles and micro particles have been used widely for herbal medicines and have gained unique position for delivery of various bio actives, proteins, peptides and drugs for treatment of various skin diseases and considered to be an important one as they improve the selectivity, increase patient compliance, effectiveness and thereby reduce dose with low toxicity.[104] Several herbal active constitutes have been incorporated with different particle systems to treat various skin related immune diseases followed by biological evaluation to estimate their safety and efficacy. Table 4 states some herbal active constitutes loaded with nanoparticles for skin related immune diseases.

The number of publications relating to nanoformulation containing herbal constituents for treatment of psoriasis has been increased exponentially. For example, Laxmi *et al.* developed psoralen containing gel formulation using natural gums and polymers and further evaluated for stability, viscosity, content uniformity and pH. The perfusion study was observed using dialysis membrane in phosphate buffer with pH ranging from 6.8 to 7.0 at 37°C. The study revealed that formulation containing egg xanthan and albumin gum showed better incorporation of drug. Finally, on the basis of *in vivo* studies, the drug activity was found to be 43.3% with enhanced differentiation of orthokeratotic cell in the epidermal scales.[112]

A novel microemulsion of 5% tea tree oil was developed using Tween 80 as surfactant and Isopropyl alcohol and Isopropyl myristate as cosurfactant for treatment of psoriasis. The formulation was further tested for droplet size, PDI, pH, viscosity and surface morphology. The average droplet size was found to be between the ranges of 84-115 nm with a PDI of 0.764 which demonstrating uniformity in the emicroemulsion. The TEM image was also found to be spherical shaped having smooth boundary of the oil particles. Further *in vitro* skin diffusion studies clearly demonstrated the increased ability of microemulsions to deliver the drug through topical application.[114]

The therapeutic and clinical benefit of a topical turmeric microemulgel was evaluated on 34 patients with mild to moderated plaque psoriasis in a placebo-controlled, double blind and randomized clinical trial. The results showed improved health with lower side effects. The authors concluded on the basis of these results that microemulgel may be considered as an alternative therapeutic option for plaque psoriasis.[115]

Topical nanogel formulation of Acitretin and Aloe-emodin was prepared by using natural polymer chitin for the treatment of psoriasis. The formulated nanogel was optimized on the basis of amplitude, particle size, drug release and time of probing. Non-fickian release pattern was observed during *in-vitro* drug release at pH 5.5 suggesting prolonged release nature of the drug. It was also observed that retention of drug was high at the inner layer of skin, which is essential to treat disease like psoriasis. Biocompatibility of the drug was confirmed during the histopathological investigation after *in vivo* skin irritation study on Perry's mouse tail model.[116]

A novel nanovesicular gelof *Berberis aristata* extract was developed and evaluated for its anti-inflammatory and antipsoriatic activity. The transferosomes of modified lipid film hydration technique were prepared by using soya phosphatidyl choline and edge activator such as sodium deoxycholate, Span 80 and Tween 80. Imiquimod (IMQ) (immune modifier) was topically applied on the shaved mice to develop psoriasis-like inflammation and then studied the histopathological status of inflamed skin. Histopathological studies revelaed a marked reduction in thickness of epidermise as compared to conventional gel formulation. The results also revealed the inhibition of edema by novel formulated gel (55.76%) is greater than conventional gel along with low irritation.[117] An antipsoriatic nanovesicular gel of Acitretin (Act) was formulated for topical delivery to overcome some side effects such as skin irritation, low aqueous

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Table 4: Herbal drug containing nanoparticles:

Formulation details	Benefits of Formulation
Biological Source: *Capsicum annuum*.[105] API: Capsaicine. Polymeric nanoparticle Formulation: SLN/NLC. Encapsulation Efficiency: 88%	Enhanced skin permeability.
Biological Source: *Capsicum annuum*.[106] API: Capsaicine. Polymeric nanoparticle Formulation: Lipid-polymer hybrid nanoparticles. Encapsulation Efficiency: 92%	Nanoparticles system coated with cationic lipid contributed to the enhancement of skin permeation.
Biological Source: *Capsicum annuum*.[107] API: Capsaicine. Polymeric nanoparticle Formulation: Niosome, microemulsions. Encapsulation Efficiency: 86.71%	Better permeation.
Biological Source: *Ammianus*.[108] API: Methoxsalen. Polymeric nanoparticle Formulation: Microemulsions.	Controlled drug release.
Biological Source: *Psoralea coryfolia*.[109] API: Psoralenbabchi oil. Polymeric nanoparticle Formulation: Microemulsion gel.	Improved the penetration of psoralen and babchi oil.
Biological Source: *Curcuma longa*[110] API: Turmeric oil, Curcuminoids. Polymeric nanoparticle Formulation: Nanoemulsion,Solid lipid nanoparticles. Encapsulation Efficiency: 70%	Enhanced stability with better *in vivo* activity.
Biological Source: *Tripterygium wilfondil* Hook F.[111] API: Diterpenoid triepoxide Polymeric nanoparticle Formulation: Poly (DL-lactic acid) nanoparticles Encapsulation Efficiency: 85.7%	Enhanced solubility and reduced toxicity.
Biological Source: *Tripterygium wilfondil* Hook F.[112] API: Triptolide Polymeric nanoparticle Formulation: Solid lipid nanoparticle.	Enhanced anti-inflammatory and reduced hepatotoxicity with transdermal delivery of triptolide.

solublity, instability and serious systemic adverse effects. The optimized niosomal vesicles were incorporated in gel base matrix and further investigated for *in vivo* antipsoriatic activity using mouse tail model experiments. Finally, better skin tolerability and negligible skin irritation were revealed by histopathologic examination and primary irritation index.

A nanoemulsions of rice bran oil was developed by low energy emulsification methods, The formulation contain 10% rice bran oil, 0.05% antioxidant 10% surfactants and 0.05% preservatives in distilled water which was further and then evaluated its moisturizing activity, physical stability and irritation potential on healthy and diseased skin volunteers. The results of *in vivo* assessments and irritation potential studies indicated that newly formulated nanoemulsion had an anti-psoriatic potential.[118]

A nanoemulsion of turmeric oil composed of 42% Smix (1:1), 15% turmeric oil and 43% distilled water was prepared by using titration method and further evaluated for physical stability, irritation potential and *in vivo* anti-psoriatic activity and anti-inflammatory activity. The results reveal that formulated nanoemulsion was stable during the period of study and free form irritation in organotypic HET-CAM model. It was observed that anti-inflammatory activity in cacrageenan-induced paw edema was reduced to 70.35%.[119]

Some niosomes were prepared for liquorice extracts of ammonium glycyrrhizinate (AG), made up of surfactants (Tween 85 and Span 20) and cholesterol at various concentrations and demonstrated their utility for treatment of dermatitis, eczema and psoriasis with same efficacy as corticosteroids. The newly formed vesicles were characterized by their zeta potential (ζ), dimensions, anisotropic properties, drug entrapment efficiency, stability, cytotoxicity and skin tolerability. *In vitro* release profiles of ammonium glycyrrhizinate niosomes were evaluated in murine and human models of inflammation by using cellulose membranes. Overall result demonstrated that AG-loaded non-ionic surfactant vesicles showed good skin tolerability and anti-inflammatory activity in mice without any toxicity.[120]

Kazi and his teammates prepared various CAP-bearing systems including niosomes, liposomes and emulsomes by using film hydration method to improve topical delivery of a drug and compared various *in vitro and in vivo* parameters. TEM photographs were used to confirm the spherical shape and nanometric size of the carrier system. They found higher accumulation of drug in emul-gel formulation through skin retention studies by *in vitro* and *in vivo* experiments and concluded this formulation can be potential for topical delivery of CAP in anti psoriatic therapy.[121]

A topical formulation of psoralene ethosomes were prepared to improve entrapment, skin permeation and deposition efficiency which was further evaluated real time drug release *in vivo* in rat model by microdialysis. During *in vitro* and *in vivo* studies, the group observed 6.56 times greater skin deposition of psoralen by ethosomes as well as area under curve and peak concentration

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Table 5: Recent patents for Psoriasis and skin related therapy.

Patent publication Number(s)	Year	Authors	Brief description of patent	Reference
WO2011/042485	2013	Alexandra *et al.*	The invention provides novel pharmaceutical compositions of macrollide immunosuppressants.	[125]
US008715736B2	2014	Sachdeva *et al.*	Describe the various methods and formulation com prise active Substances encapsulated within Surface modified Nanostructured lipid carrier nanoparticles for treating a condition of the skin.	[126]
US008992994B2	2015	Roy *et al.*	Reported the nonoemulsions comprising nano size droplets of one or more anti-psoriasis agents that exhibit greater permeability, and improved bioavailability.	[127]
WO 2018/236206 Al	2018	Oi Ming *et al.*	Nanoformulation comprises of vitamin A, C and E to get synergistic effect to treat common skin disorders.	[128]
US 2019/0231897 A1	2018	Balasamy *et al.*	A platinum (II) complex loaded on a mesosilicalite nanocarrier for treatment of psoriasis	[129]

by 2.34 and 3.37 times respectively than shown by tincture. The percutaneous permeability was also found to be greater for abdomen than scapulas or chest. Overall they concluded that enhanced permeation and skin deposition by ethosomes could be helpful to reduce toxicity as well as to improve the efficacy of psoralen in long term psoralen treatment.[122,123]

Skin permeation, efficacy and localization of drug in different skin layers of nanostructured lipid carriers (NLCs) and solid lipid nanoparticles (SLNs) was investigated and their potential of improving topical delivery of capsaicin (CAP) along with toxicity was explored through *in vitro* and *in vivo* studies. The SLNs were prepared by solvent diffusion methods which were further characterized for average particle size, zeta potential and entrapment efficiency.

They observed that higher amount of CAP could be encapsulated with the NLCs as compared with SLNs. They also noted that the cumulative amounts of CAP (permeation and retention, respectively) through the skin were higher by NLCs as compared with SLNs.[124]

Apart from the reported publication patents can represents an invention in a particular field of technology therefore some patent reports are also added in the following text of this review. Table 5 represents some of the latest formulations which have been recently patented for treating psoriasis.

CONCLUSION AND SUMMARY

The increase of incidence of psoriasis has spurred several efforts to identify herbal formulation with potential therapeutic benefits. Initial efforts towards the development of herbal formulation faced many challenges with regards to efficacy, safety, poor water solubility and bioavailability with increased systemic clearance. Therefore, more scientific documentation and evidences are warranted for the promotion of herbal treatment of psoriasis using a combination of various novel colloidal carriers such as NLCs, SLNs, liposomes, niosomes and transferosomes for effective and safe delivery of various anti-psoriatic drugs. The development in the nano formulation is continuous as to be a viable strategy to target with the aim to inhibit multiple pathways responsible for the pathogenesis of psoriasis. Focused research efforts to establish the superiority of novel drug delivery systems incorporating herbal treatment for psoriasis are needed. Although this appears to be promising yet scientific proof beyond doubt must be made available. Carbon nanotubes (CNTs) and Quantum dots (QDs) based drug delivery may also be worth exploring in view of their unique properties.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

Act: Acitretin; **AD:** Atopic dermatitis; **AG:** Ammonium Glycyrrhizinate; **BSA:** Body surface area; **CAD:** Coronary artery disease; **CAMP:** Cathelicidin antimicrobial peptide; **CAP:** Capsaicin; **CNTs:** Carbon nanotubes; **DCs:** Dendritic cells; **Glab:** Glabridin; **Hh:** Hedgehog; **HRQOL:** Health-related quality of life; **IFNα:** Interferon-α; **IL:** Interleukin; **IMQ:** Imiquimod; **JAK:** Janus Kinase; **LTB4:** Leukotriene B4; **MHC:** Major histocompatibility complex; **NDDS:** Novel drug delivery systems; **NF-κB:** Nuclear factor-κB; **NLCs:** Nanostructured lipid carriers; **NO:** Nitric oxide; **PASI:** Psoriasis area severity index; **pDCs:** Plasmacytoid dendritic cells; **PhK:** Phosphorylase kinase; **PUVA:** Psoralen plus ultraviolet A therapy; **QDs:** Quantum dots; **SCC:** Squamous cell carcinoma; **SLNs:** Solid lipid nanoparticles; **TLR7:** Toll-like receptor 7; **TNF:** Tumor necrosis factor; **T-cell:** T-lymphocyte.

REFERENCES

1. Matejuk A. Skin Immunity. Archivum immunologiae et therapiae experimentalis. 2018;66(1):45-54.
2. Ayala-Fontánez N, Soler DC, McCormick TS. Current knowledge on psoriasis and autoimmune diseases. Psoriasis (Auckl). 2016;6:7-32.
3. Hemne PS, Kunghatkar RG, Dhoble SJ, Moharil SV, Singh V. Phosphor for phototherapy: Review on psoriasis. Luminescence: The journal of biological and chemical luminescence. 2017;32(3):260-70.
4. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. Cell Mol Immunol. 2012;9(4):302-9.
5. Griffiths CEM, van der Walt JM, Ashcroft DM, Flohr C, Naldi L, Nijsten T, et al. The global state of psoriasis disease epidemiology: a workshop report. The British journal of dermatology. 2017;177(1):e4-e7.
6. Thappa DM, Munisamy M. Research on psoriasis in India: Where do we stand? The Indian Journal of Medical Research. 2017;146(2):147-9.
7. Lebwohl M, Ting PT, Koo JYM. Psoriasis treatment: traditional therapy. Annals of the Rheumatic Diseases. 2005;64 Suppl 2(Suppl 2):ii83-ii6.
8. Deng S, May BH, Zhang AL, Lu C, Xue CC. Topical herbal medicine combined with pharmacotherapy for psoriasis: a systematic review and meta-analysis. Archives of dermatological research. 2013;305(3):179-89.
9. Rendon A, Schäkel K. Psoriasis Pathogenesis and Treatment. Int J Mol Sci. 2019;20(6).
10. Kim WB, Jerome D, Yeung J. Diagnosis and management of psoriasis. Can Fam Physician. 2017;63(4):278-85.
11. Papp K, Gulliver W, Lynde C, Poulin Y, Ashkenas J. Canadian guidelines for the management of plaque psoriasis: overview. Journal of cutaneous medicine and surgery. 2011;15(4):210-9.
12. Crow JM. Therapeutics: Silencing psoriasis. Nature. 2012;492(7429):S58-9.
13. Lube KM, Stulberg DL. Chronic plaque psoriasis. American family physician. 2006;73(4):636-44.
14. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. Lancet (London, England). 2007;370(9583):263-71.
15. Khosravi H, Siegel MP, Van Voorhees AS, Merola JF. Treatment of Inverse/Intertriginous Psoriasis: Updated Guidelines from the Medical Board of the National Psoriasis Foundation. Journal of drugs in dermatology : JDD. 2017;16(8):760-6.
16. Manhart R, Rich P. Nail psoriasis. Clinical and experimental rheumatology. 2015; 33(5 Suppl 93):S7-13.
17. Blakely K, Gooderham M. Management of scalp psoriasis: current perspectives. Psoriasis (Auckl). 2016;6:33-40.
18. Gianfaldoni S, Tchernev G, Wollina U, Lotti T. Pustular Palmoplantar Psoriasis Successfully Treated with Nb-UVB Monochromatic Excimer Light: A Case-Report. Open Access Maced J Med Sci. 2017;5(4):462-6.
19. Kim WB, Jerome D, Yeung J. Diagnosis and management of psoriasis. Canadian Family Physician. 2017;63(4):278-85.
20. Hoegler KM, John AM, Handler MZ, Schwartz RA. Generalized pustular psoriasis: a review and update on treatment. Journal of the European Academy of Dermatology and Venereology : JEADV. 2018;32(10):1645-51.
21. Singh RK, Lee KM, Ucmak D, Brodsky M, Atanelov Z, Farahnik B, et al. Erythrodermic psoriasis: pathophysiology and current treatment perspectives. Psoriasis (Auckl). 2016;6:93-104.
22. Liao PB, Rubinson R, Howard R, Sanchez G, Frieden IJ. Annular pustular psoriasis--most common form of pustular psoriasis in children: report of three cases and review of the literature. Pediatric dermatology. 2002;19(1):19-25.
23. Cuesta-Montero L, Belinchon I. [Connective tissue diseases and psoriasis]. Actas dermo-sifiliograficas. 2011;102(7):487-97.
24. Nickoloff BJ, Nestle FO. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. The Journal of clinical investigation. 2004;113(12):1664-75.
25. Gudjonsson JE, Johnston A, Sigmundsdottir H, Valdimarsson H. Immunopathogenic mechanisms in psoriasis. Clin Exp Immunol. 2004;135(1):1-8.
26. Johnston A, Gudjonsson JE, Sigmundsdottir H, Love TJ, Valdimarsson H. Peripheral blood T cell responses to keratin peptides that share sequences with streptococcal M proteins are largely restricted to skin-homing CD8(+) T cells. Clin Exp Immunol. 2004;138(1):83-93.
27. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. Int J Mol Sci. 2019;20(23).
28. Bugaut H, Aractingi S. Major Role of the IL17/23 Axis in Psoriasis Supports the Development of New Targeted Therapies. Front Immunol. 2021;12:621956.
29. Heidenreich R, Röcken M, Ghoreschi K. Angiogenesis drives psoriasis pathogenesis. Int J Exp Pathol. 2009;90(3):232-48.
30. Guenther L, Gulliver W. Psoriasis comorbidities. Journal of cutaneous medicine and surgery. 2009;13 Suppl 2:S77-87.
31. Halter JB, Musi N, McFarland Horne F, Crandall JP, Goldberg A, Harkless L, et al. Diabetes and cardiovascular disease in older adults: current status and future directions. Diabetes. 2014;63(8):2578-89.
32. Oliveira Mde F, Rocha Bde O, Duarte GV. Psoriasis: classical and emerging comorbidities. An Bras Dermatol. 2015;90(1):9-20.
33. Mrowietz U, Kragballe K, Reich K, Spuls P, Griffiths CEM, Nast A, et al. Definition of treatment goals for moderate to severe psoriasis: a European consensus. Archives of Dermatological Research. 2011;303(1):1-10.
34. Gisondi P, Del Giglio M, Girolomoni G. Treatment Approaches to Moderate to Severe Psoriasis. Int J Mol Sci. 2017;18(11).
35. Mohd Nordin UU, Ahmad N, Salim N, Mohd Yusof NS. Lipid-based nanoparticles for psoriasis treatment: a review on conventional treatments, recent works, and future prospects. RSC Advances. 2021;11(46):29080-101.
36. Malecic N, Young H. Tacrolimus for the management of psoriasis: clinical utility and place in therapy. Psoriasis (Auckl). 2016;6:153-63.
37. Mendonca CO, Burden AD. Current concepts in psoriasis and its treatment. Pharmacology and therapeutics. 2003;99(2):133-47.
38. Zeichner JA. Use of Topical Coal Tar Foam for the Treatment of Psoriasis in Difficult-to-treat Areas. J Clin Aesthet Dermatol. 2010;3(9):37-40.
39. Filoni A, Vestita M, Congedo M, Giudice G, Tafuri S, Bonamonte D. Association between psoriasis and vitamin D: Duration of disease correlates with decreased vitamin D serum levels: An observational case-control study. Medicine. 2018;97(25):e11185-e.
40. Kircik L. Efficacy and safety of topical calcitriol 3 microg/g ointment, a new topical therapy for chronic plaque psoriasis. J Drugs Dermatol. 2009;8(8 Suppl):s9-16.
41. Iversen L, Jakobsen HB. Patient Preferences for Topical Psoriasis Treatments are Diverse and Difficult to Predict. Dermatology and Therapy. 2016;6(2):273-85.
42. Balbás GM, Regaña MS, Millet PU. Study on the use of omega-3 fatty acids as a therapeutic supplement in treatment of psoriasis. Clinical, cosmetic and investigational dermatology. 2011;4:73-7.
43. Berardesca E, Piero Vignoli G, Distante E, Rona C. Effects of glycolic acid on psoriasis. Clinical and experimental dermatology. 1998;23(4):190-1.
44. Maccaroni E, Giovenzana GB, Palmisano G, Botta D, Volante P, Mascicchi N. Structures from powders: diflorasone diacetate. Steroids. 2009;74(1):102-11.
45. Laws PM, Young HS. Topical treatment of psoriasis. Expert opinion on pharmacotherapy. 2010;11(12):1999-2009.
46. Fowler J, Fowler L. Physician and Patient Assessment of Triamcinolone Acetonide Spray for Steroid-responsive Dermatoses. The Journal of Clinical and Aesthetic Dermatology. 2010;3(5):27-31.
47. Gupta AK, Chow M. Prednicarbate (Dermatop): profile of a corticosteroid. Journal of cutaneous medicine and surgery. 2004;8(4):244-7.
48. Spuls PI, Rozenblit M, Lebwohl M. Retrospective study of the efficacy of narrowband UVB and acitretin. The Journal of dermatological treatment. 2003;14 Suppl 2:17-20.
49. Krueger JG. The immunologic basis for the treatment of psoriasis with new biologic agents. Journal of the American Academy of Dermatology. 2002;46(1):1-23; quiz -6.
50. Thomson A. Etanercept in psoriasis: the evidence of its therapeutic impact. Core Evid. 2007;2(1):51-62.
51. Puig L. [Efficacy of treatment with infliximab in patients with moderate-severe psoriasis and high needs of therapy. A retrospective study of 43 patients]. Actas dermo-sifiliograficas. 2008;99 Suppl 4:30-5.
52. Vena GA, Cassano N, Iannone F. Update on subcutaneous methotrexate for inflammatory arthritis and psoriasis. Therapeutics and Clinical Risk Management. 2018;14:105-16.
53. Colombo MD, Cassano N, Bellia G, Vena GA. Cyclosporine regimens in plaque psoriasis: an overview with special emphasis on dose, duration, and old and new treatment approaches. Scientific World Journal. 2013;2013:805705.

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54. Mehta D, Lim HW. Ultraviolet B Phototherapy for Psoriasis: Review of Practical Guidelines. Am J Clin Dermatol. 2016;17(2):125-33.
55. Berneburg M, Herzinger T, Rampf J, Hoetzenecker W, Guenova E, Meisner C, *et al.* Efficacy of bath psoralen plus ultraviolet A (PUVA) vs. system PUVA in psoriasis: a prospective, open, randomized, multicentre study. Br J Dermatol. 2013;169(3):704-8.
56. Feldman SR, Goffe B, Rice G, Mitchell M, Kaur M, Robertson D, *et al.* The Challenge of Managing Psoriasis: Unmet Medical Needs and Stakeholder Perspectives. Am Health Drug Benefits. 2016;9(9):504-13.
57. Van de Kerkhof PC, Vissers WH. The topical treatment of psoriasis. Skin Pharmacol Appl Skin Physiol. 2003;16(2):69-83.
58. Kim N, Thiash B, Menter A. Comorbidities in psoriasis patients. Seminars in cutaneous medicine and surgery. 2010;29(1):10-5.
59. Gisondi P, Del Giglio M, Girolomoni G. Treatment Approaches to Moderate to Severe Psoriasis. International Journal of Molecular Sciences. 2017;18(11):2427.
60. Soleymani T, Hung T, Soung J. The role of vitamin D in psoriasis: a review. International Journal of Dermatology. 2015;54(4):383-92.
61. Torsekar R, Gautam MM. Topical Therapies in Psoriasis. Indian Dermatol Online J. 2017;8(4):235-45.
62. Martin G, Young M, Aldredge L. Recommendations for Initiating Systemic Therapy in Patients with Psoriasis. J Clin Aesthet Dermatol. 2019;12(4):13-26.
63. Wozel G. Psoriasis treatment in difficult locations: scalp, nails, and intertriginous areas. Clinics in dermatology. 2008;26(5):448-59.
64. Cardellina JH. Challenges and Opportunities Confronting the Botanical Dietary Supplement Industry†. Journal of Natural Products. 2002;65(7):1073-84.
65. Masood E. 'Medicinal plants threatened by over-use'. Nature. 1997;385(6617):570.
66. Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian J Dermatol. 2008;53(4):163-6.
67. Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP. Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. Molecules. 2020;25(6).
68. Alzohairy MA. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evidence-Based Complementary and Alternative Medicine. 2016;2016:11.
69. Alzohairy MA. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evidence-based Complementary and Alternative Medicine : eCAM. 2016;2016:7382506-.
70. Alzohairy MA. Therapeutics Role of Azadirachta Indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evid Based Complement Alternat Med. 2016;2016:7382506.
71. Nardo VD, Gianfaldoni S, Tchernev G, Wollina U, Barygina V, Lotti J, *et al.* Use of Curcumin in Psoriasis. Open Access Maced J Med Sci. 2018;6(1):218-20.
72. Pazyar N, Yaghoobi R. Soybean: a Potential Antipsoriasis Agent. Jundishapur Journal of Natural Pharmaceutical Products. 2015;10(1):e20924.
73. Bocheńska K, Moskot M, Smolińska-Fijołek E, Jakóbkiewicz-Banecka J, Szczerkowska-Dobosz A, Słomiński B, *et al.* Impact of isoflavone genistein on psoriasis in in vivo and in vitro investigations. Sci Rep. 2021;11(1):18297.
74. Timoszuk M, Bielawska K, Skrzydlewska E. Evening Primrose (Oenothera biennis) Biological Activity Dependent on Chemical Composition. Antioxidants. 2018;7(8):108.
75. Balić A, Vlašić D, Žužul K, Marinović B, Bukvić Mokos Z. Omega-3 Versus Omega-6 Polyunsaturated Fatty Acids in the Prevention and Treatment of Inflammatory Skin Diseases. Int J Mol Sci. 2020;21(3).
76. Dwarampudi LP, Palaniswamy D, Nithyanantham M, Raghu PS. Antipsoriatic activity and cytotoxicity of ethanolic extract of *Nigella sativa* seeds. Pharmacogn Mag. 2012;8(32):268-72.
77. Eid AM, Elmarzugi NA, Abu Ayyash LM, Sawafta MN, Daana HI. A Review on the Cosmeceutical and External Applications of *Nigella sativa*. J Trop Med. 2017;2017:7092514.
78. Li C-C, Wang T-L, Zhang Z-Q, Yang W-Q, Wang Y-F, Chai X, *et al.* Phytochemical and Pharmacological Studies on the Genus Psoralea: A Mini Review. Evidence-based Complementary and Alternative Medicine: eCAM. 2016;2016:8108643.
79. Wong T, Hsu L, Liao W. Phototherapy in psoriasis: a review of mechanisms of action. J Cutan Med Surg. 2013;17(1):6-12.
80. Milic N, Milosevic N, Suvajdzic L, Zarkov M, Abenavoli L. New therapeutic potentials of milk thistle (*Silybum marianum*). Natural product communications. 2013;8(12):1801-10.
81. Karimi G, Vahabzadeh M, Lari P, Rashedinia M, Moshiri M. "Silymarin", a promising pharmacological agent for treatment of diseases. Iran J Basic Med Sci. 2011;14(4):308-17.
82. Sundarrajan S, Lulu S, Arumugam M. Deciphering the Mechanism of Action of *Wrightia tinctoria* for Psoriasis Based on Systems Pharmacology Approach. Journal of alternative and complementary medicine (New York, NY). 2017;23(11):866-78.
83. Conforti F, Marrelli M, Menichini F, Bonesi M, Statti G, Provenzano E, *et al.* Natural and Synthetic Furanocoumarins as Treatment for Vitiligo and Psoriasis. Current Drug Therapy. 2009;4:38-58.
84. Cheng H-M, Wu Y-C, Wang Q, Song M, Wu J, Chen D, *et al.* Clinical efficacy and IL-17 targeting mechanism of Indigo naturalis as a topical agent in moderate psoriasis. BMC Complementary and Alternative Medicine. 2017;17:439.
85. Xie XJ, Di TT, Wang Y, Wang MX, Meng YJ, Lin Y, *et al.* Indirubin ameliorates imiquimod-induced psoriasis-like skin lesions in mice by inhibiting inflammatory responses mediated by IL-17A-producing γδ T cells. Mol Immunol. 2018;101:386-95.
86. Srinivasan K. Biological Activities of Red Pepper (*Capsicum annuum*) and Its Pungent Principle Capsaicin: A Review. Crit Rev Food Sci Nutr. 2016;56(9):1488-500.
87. Basharat S, Gilani SA, Iftikhar F, Murtaza M, Basharat A, Sheikh A, *et al.* Capsaicin: Plants of the Genus Capsicum and Positive Effect of Oriental Spice on Skin Health. Skin Pharmacology and Physiology. 2021;33:1-11.
88. Dawid-Pać R. Medicinal plants used in treatment of inflammatory skin diseases. Advances in Dermatology and Allergology/Postepy Dermatologii i Alergologii. 2013;30(3):170-7.
89. Pumamawati S, Indrastuti N, Danarti R, Saefudin T. The Role of Moisturizers in Addressing Various Kinds of Dermatitis: A Review. Clin Med Res. 2017;15(3-4):75-87.
90. Vijayalakshmi A, Ravichandiran V, Malarkodi V, Nirmala S, Jayakumari S. Screening of flavonoid "quercetin" from the rhizome of *Smilax china* Linn. for anti-psoriatic activity. Asian Pacific journal of tropical biomedicine. 2012;2(4):269-75.
91. Costa R, Costa Lima SA, Gameiro P, Reis S. On the Development of a Cutaneous Flavonoid Delivery System: Advances and Limitations. Antioxidants (Basel). 2021;10(9).
92. Herman A, Herman AP. Topically Used Herbal Products for the Treatment of Psoriasis - Mechanism of Action, Drug Delivery, Clinical Studies. Planta medica. 2016;82(17):1447-55.
93. Dhanabal SP, Priyanka Dwarampudi L, Murugananthan N, Vadivelan R. Evaluation of the antipsoriatic activity of Aloe vera leaf extract using a mouse tail model of psoriasis. Phytother Res. 2012;26(4):617-9.
94. Li XL, Wang ZH, Zhao YX, Luo SJ, Zhang DW, Xiao SX, *et al.* Purification of a polysaccharide from *Gynostemma pentaphyllum* Makino and its therapeutic advantages for psoriasis. Carbohydr Polym. 2012;89(4):1232-7.
95. Najafizade P, Hashemian F, Mansouri P, Farshi S, Surmaghi M, Chalangari R. The evaluation of the clinical effect of topical St Johns wort (*Hypericum perforatum* L.) in plaque type *Psoriasis vulgaris*: A pilot study. The Australasian journal of dermatology. 2012;53:131-5.
96. Singhal M, Kansara N. *Cassia tora* Linn Cream Inhibits Ultraviolet-B-Induced Psoriasis in Rats. ISRN Dermatology. 2012;2012:346510-.
97. Michalsen A, Eddin O, Salama A. A case series of the effects of a novel composition of a traditional natural preparation for the treatment of psoriasis. Journal of traditional and complementary medicine. 2016;6(4):395-8.
98. Parmar KM, Itankar PR, Joshi A, Prasad SK. Anti-psoriatic potential of *Solanum xanthocarpum* stem in Imiquimod-induced psoriatic mice model. J Ethnopharmacol. 2017;198:158-66.
99. Shraibom N, Madaan A, Joshi V, Verma R, Chaudhary A, Mishra G, *et al.* Evaluation of *In vitro* Anti-psoriatic Activity of a Novel Polyherbal Formulation by Multiparametric Analysis. Antiinflamm Antiallergy Agents Med Chem. 2017;16(2):94-111.
100. Lin YK, See LC, Huang YH, Chi CC, Hui RC. Comparison of indirubin concentrations in indigo naturalis ointment for psoriasis treatment: a randomized, double-blind, dosage-controlled trial. The British journal of dermatology. 2018;178(1):124-31.
101. Li P, Li Y, Jiang H, Xu Y, Liu X, Che B, *et al.* Glabridin, an isoflavan from licorice root, ameliorates imiquimod-induced psoriasis-like inflammation of BALB/c mice. Int Immunopharmacol. 2018;59:243-51.
102. Pandey K, Nimisha. An Overview on Promising Nanotechnological Approaches for the Treatment of Psoriasis. Recent Pat Nanotechnol. 2020;14(2):102-18.
103. Joo M, Kang J-W, Youl J, Cho, Shim B, Kim D, *et al.* Bioavailability enhancing activities of natural compounds from medicinal plants. Journal of Medicinal Plants Research. 2010;3:1204-11.
104. Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. Int J Nanomedicine. 2007;2(2):129-41.
105. Arora R, Katiyar SS, Kushwah V, Jain S. Solid lipid nanoparticles and nanostructured lipid carrier-based nanotherapeutics in treatment of psoriasis: a comparative study. Expert Opin Drug Deliv. 2017;14(2):165-77.
106. Desai PR, Marepally S, Patel AR, Voshavar C, Chaudhuri A, Singh M. Topical delivery of anti-TNFα siRNA and capsaicin via novel lipid-polymer hybrid nanoparticles efficiently inhibits skin inflammation *in vivo*. J Control Release. 2013;170(1):51-63.
107. Gupta R, Gupta M, Mangal S, Agrawal U, Vyas SP. Capsaicin-loaded vesicular systems designed for enhancing localized delivery for psoriasis therapy. Artif Cells Nanomed Biotechnol. 2016;44(3):825-34.
108. Sabale V, Vora S. Formulation and evaluation of microemulsion-based hydrogel for topical delivery. International Journal of Pharmaceutical Investigation. 2012;2(3):140-9.
109. Ali J, Akhtar N, Sultana Y, Baboota S, Ahuja A. Antipsoriatic microemulsion gel formulations for topical drug delivery of babchi oil (*Psoralea corylifolia*). Methods Find Exp Clin Pharmacol. 2008;30(4):277-85.
110. Wang X, Jiang Y, Wang YW, Huang MT, Ho CT, Huang Q. Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions. Food chemistry. 2008;108(2):419-24.

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111. Sun R, Dai J, Ling M, Yu L, Yu Z, Tang L. Delivery of triptolide: a combination of traditional Chinese medicine and nanomedicine. J Nanobiotechnology. 2022;20(1):194.
112. Mei Z, Li X, Wu Q, Hu S, Yang X. The research on the anti-inflammatory activity and hepatotoxicity of triptolide-loaded solid lipid nanoparticle. Pharmacological Research. 2005;51(4):345-51.
113. Laxmi RJ, Karthikeyan R, Babu PS, Babu RVVN. Formulation and evaluation of antipsoriatic gel using natural excipients. Journal of Acute Disease. 2013;2(2):115-21.
114. Zhao J-H, Ji L, Wang H, Chen Z-Q, Zhang Y-T, Liu Y, *et al.* Microemulsion-based novel transdermal delivery system of tetramethylpyrazine: preparation and evaluation *in vitro* and *in vivo*. International Journal of Nanomedicine. 2011;6:1611-9.
115. Sarafian G, Afshar M, Mansouri P, Asgarpanah J, Raoufinejad K, Rajabi M. Topical Turmeric Microemulgel in the Management of Plaque Psoriasis; A Clinical Evaluation. Iranian Journal of Pharmaceutical Research : IJPR. 2015;14(3):865-76.
116. Divya G, Panonnummal R, Gupta S, Jayakumar R, Sabitha M. Acitretin and aloe-emodin loaded chitin nanogel for the treatment of psoriasis. Eur J Pharm Biopharm. 2016;107:97-109.
117. Nimisha, Rizvi DA, Fatima Z, Neema, Kaur CD. Antipsoriatic and Anti-inflammatory Studies of *Berberis aristata* Extract Loaded Nanovesicular Gels. Pharmacognosy Magazine. 2017;13(Suppl 3):S587-S94.
118. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, *et al.* Formation and stability of oil-in-water nanoemulsions containing rice bran oil: *in vitro* and *in vivo* assessments. Journal of Nanobiotechnology. 2011;9:44-.
119. Ali MS, Alam MS, Imam FI, Siddiqui MR. Topical nanoemulsion of turmeric oil for psoriasis: characterization, *ex vivo* and *in vivo* assessment. International Journal of Drug Delivery; Vol 4, No 2 (2012): International Journal of Drug Delivery. 2012.
120. Marianecci C, Rinaldi F, Mastriota M, Pieretti S, Trapasso E, Paolino D, *et al.* Anti-inflammatory activity of novel ammonium glycyrrhizinate/niosomes delivery system: human and murine models. J Control Release. 2012;164(1):17-25.
121. Kazi KM, Mandal AS, Biswas N, Guha A, Chatterjee S, Behera M, *et al.* Niosome: A future of targeted drug delivery systems. J Adv Pharm Technol Res. 2010;1(4):374-80.
122. Zhang YT, Shen LN, Zhao JH, Feng NP. Evaluation of psoralen ethosomes for topical delivery in rats by using *in vivo* microdialysis. Int J Nanomedicine. 2014;9:669-78.
123. Pradhan M, Alexander A, Singh MR, Singh D, Saraf S, Saraf S, *et al.* Understanding the prospective of nano-formulations towards the treatment of psoriasis. Biomedicine and Pharmacotherapy. 2018;107:447-63.
124. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018;13(4):288-303.
125. Alexandra Sabine Baerbel Goebel, Halle (De), Microemulsion Pharmaceutical Composition Comprising A Macrolide Immunosuppressant Drug, US 8,574,562 B2, 2013
126. Mandip Singh Sachdeva, Tallahassee, Fl; Ram Patlolla, Tallahassee, Nanoparticle Formulations For Skn Delivery, US008715736B2, 2014
127. Sunilendu Bhushan Roy; Jay Shantilal Kothari; Shafiq Sheikh; Jitendra Dasharathlal Patel;Jinesh Suresh Pancholi, Topical Pharmaceutical Compositions Containing Nanodroplets For The Treatment Psoriasis, US00899299482, 2015
128. Lai, Oi Ming; My, Nicholas Khong, Mun Hoe; My, Lai, Wee Ting; My, Oil Palm-Derived Composition For Skin Treatment. WO2018/236206 Al, 2018.
129. Rabindran Jermy Balasamy, Dammam; Sadananda Acharya , Dammam; Vijaya Ravinayagam , Dammam, Hierarchical Siliceous Mesosilicalite Nanocarrier Loaded With Platinum (ii) Complex, US2019/0231897 A1, 2018.

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ANTIDIARRHOEAL ACTIVITY OF ZIZIPHUS MAURITIANA LEAF EXTRACT IN RODENTS

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Abstract

Traditional healers in India use a wide range of medicinal plants with antidiarrheal properties. Among these, Ziziphus mauritiana is one such plant claimed to have an antidiarrheal activity in Indian folklore medicine. Previous studies showed that the crude extract is endowed with the claimed property. The present study was undertaken to further the claim by screening different fractions for the said activity so that it could serve as a basis for subsequent studies. The fractions were obtained by successive extraction in soxhlet apparatus with solvents of different polarity (hydroalcoholic) followed by cold maceration of the deposit of the methanol fraction with distilled water. The antidiarrheal activity was evaluated using PEG 2 induced diarrheal model, charcoal meal test and anti-enteropooling test in mice. The test groups received various doses (300, 400, 500 mg/kg and an additional dose of 1000 mg/kg for the aqueous fraction) of the fractions, whereas positive controls received either Loperamide (3 mg/kg) or Atropine (5 mg/kg) and negative controls received vehicle (10 ml/kg). In the PEG 2 induced model, the hydro (at 400 & 500 mg/kg) fractions significantly delayed diarrheal onset, decreased stool frequency and weight of feces. The present study demonstrated that the hydroalcoholic extract possessed significant anti-diarrheal activity.

Keywords: Antidiarrheal activity, PEG 2 induced diarrhea, Gastrointestinal transit, Anti-enteropooling, Ziziphus mauritiana,

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material [1-5]. Scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many health problems (Gupta, 1994). Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts or fractions thereof or mixtures of fractions/extracts from different plants, which have been carefully standardized for their safety and efficacy [6-8]. The use of herbal drugs in the treatment of diarrhoea is a common practice in Africa [9]. Ziziphus mauritiana Lam belongs to the family Rhamnaceae. It is widely grown in mild-temperate, rather dry areas, of both hemispheres and is adapted to warm climates. It is often called merely jujube, Chinese date, Indian plump [10]. In northern Nigeria it is called magarya (Hausa) or huya (Kilba). The plant finds various uses in traditional medicine for instance; the fruits are applied on cuts and ulcers, are employed in pulmonary ailments and fevers; the dried ripe fruit is a mild laxative. The seeds are sedative and are taken sometimes with butter, to halt nausea, vomiting and abdominal pains in pregnancy. Mixed with oil, they are rubbed on rheumatic areas. The leaves are helpful in liver trouble, asthma and fever. The bitter, astringent bark decoction is taken to halt diarrhoea and dysentery and relieve gingivitis. A root decoction is given as a febrifuge, taenicide and emmenagogue, and the powdered root is dusted on wounds. Juice of the root bark is said to alleviate gout and rheumatism [11]. The root is also used in the treatment of epilepsy [12]. The dried root is also used to treat diarrhoea in Northern Parts of Nigeria (Mallam Muazzam, Herbarium Department of Medicinal Plant Research and Traditional Medicine (MPRT) NIPRD, Abuja, Nigeria, personal communication).

A search in NIPRALERT database did not yield any information on the pharmacological effect of the methanolic root extract of the plant on castor oil induced diarrhoea, small intestinal propulsion; castor

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An *In Vitro* Pharmacognostical Study on Gluconeogenesis and Glucose

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Abstract

Traditional medicine over 60% of the world's population used for health care name as Mukia maderaspatana (L.) M. Roem. (Cucurbitaceae) (Mukia) is extensively important medicine as an anti-inflammatory plant. It is rich in content of phenolics that exerts various medicinal properties. Mukia extract and its derivatives phenolics such as quercetin and phloroglucinol are investigated for their in vitro anti-inflammatory activity. Materials used was Quercetin, phloroglucinol, and methanol which extract of the dried whole plant (0.55 and 0.6 mg/ml) were studied for the inhibition of gluconeogenesis and glucose Phenolics of Mukia were analyzed by HPLC and UV-spectroscopy. Results obtained were Glucose (1.5 mg/g/h) was synthesized from pyruvate, the synthesis was whole inhibited by insulin (1 U/ml). Quercetin at 0.35 and 0.6 mg/ml caused 60% and 90% inhibition (0.43 mg/g/h and 0.12 mg/g/h glucose). Addition of insulin did not increase inhibition. Phloroglucinol inhibited 100% glucose production with or without insulin. Mukia (0.20 mg/ml) inhibited gluconeogenesis (0.65 mg/g/h) by 40%, and with insulin, inhibition increased to 50% (0.59 mg/g/h). At 0.6 mg/ml, glucose synthesis was stimulated by1.3-fold, but with insulin gets inhibited by 90% (0.12 mg/g/h glucose). Mukia possessed no effect on glucose uptake, in case it potentiated the activity of insulin mediated glucose uptake (153.82 ± 12.30 mg/dl/g/30 min) compared with insulin control (122.41 ± 9.14 mg/dl/g/30 min) ($p < 0.02$). HPLC analysis revealed the presence of phenolics. Results were concluded scientific rationale for the use of Mukia in medicine as an anti-inflammatory nutraceutical.

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Keywords: *Anti-inflammatory, nutraceutical, Glucose synthesis, Phloroglucinol, medicinal property.*

1. Introduction

The most important and has a major role in all traditional medical system especially in Ayurveda, Siddha, Unani, Homeopathy, Naturopathy and Chinese Medicine. The plants which is enough to possessing health promoting capacities and their bio active compounds or active-ingredients cure the diseases. It is important to study the pharmacology activity of individual plants with their bio active compounds deed in treating diseases. Mukiamaderaspatana (L.) M. Roem.is a species of plant many of year it possessing for cooking and medicinal remedies (Cleide de souse, 2004).

Plant profile

Kingdom: Plantae
Division: Sermatophyta
Sub-division: Angiospermae
Class: Dioctyledonae
Sub-Class: Polypetalae
Series: Calyiflorae
Order: Passiflorales
Family: Cucurbitaceae
Genus: Mukia
Species: maderaspatana

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Kap (Knowledge, Attitudes and Practices) Study on Medicine and Health Infrastructure use in Pregnant Women of Rural Areas of Ujjain Madhya Pradesh, India: A Cross-Sectional Survey

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KEYWORDS
KAP study, Pregnant, Rural areas, Asha, India

ABSTRACT
Use of medicines and also health infrastructures are important for actual benefit of the patients. So, the KAP (Knowledge, Attitudes and Practices) study need to be done to formulate the policies which will ensure rational and scientific use of national resources. It is a cross-sectional survey, observational in nature, where six hundred and fifty six pregnant women (656) participated. KAP was evaluated by using pre-designed, pre-coded and pre-tested questionnaire. 40.70 % pregnant women of the study were over the counter (OTC) medicine user and at the same time 37.5 % had knowledge about expiry date, though 66.76 % pregnant women knew about the residence of the trained females (Asha ordai) of their village, on the contrary only 17.68 % pregnant women actually used services of local qualified doctors. Present study KAP should be evaluated in beneficiaries before implementation of any intervention to improve the health.

INTRODUCTION

The process of diagnosis and treatment is a complex one. After diagnosis, treatment through prescription by advising medicines is the next step. During prescription writing, age, sex, disease condition and also other factors like pregnancy, liver and kidney functions etc. should be considered. Prescribing medicines during pregnancy is of special medical importance.[1] Use of unsafe medicine like, chloramphenicol, salvarsan, sulfanilamide (which itself not fatal) containing solvent diethyl glycol, thalidomide etc.[2,3] can be disastrous anytime. For rational use of medicine knowledge, attitude and practice on use of medicine is important. This is more relevant in India, especially in rural areas where illiteracy and ignorance is widely prevailed. Majority of the Indian population lived in rural areas but most of the health care budget is spent in the urban areas. As a result the rural populations lack access to the most basic services, even to qualified medical practitioners. The doctor patient ratio in rural areas is extremely low.[4] Only aware and compliant patients, using medicines and health infrastructures can ensure the rationality of treatment.[5] Though indicators proposed by WHO[6,7] can explain the medicine use status in any community, but other factors like patient's knowledge, attitude and practice about medicine use is also important and could not be evaluated by these indicators. Drug consumption in pregnancy were analyzed by different researchers in western world[8,9] and also in India[10,11] but there are very few studies in rural areas of Madhya Pradesh India on KAP, on use of medicine and use of health services available nearby.

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We selected rural areas of Ujjain district for analysis. Therefore, it was rational to conduct a study on this topic in rural area of Ujjain, Madhya Pradesh.

MATERIALS AND METHODS

This was an observational study. Six hundred and fifty six (656) pregnant women gave consent in writing and participated in the study. Pregnant women attending the rural clinics of Ujjain and AmaltasInstitute of Medical Science, Dewas, Madhya Pradesh, India from 1st may 2023 to 31st October 2023 were interviewed with a questionnaire and prescriptions available with the mother were also copied. The questionnaire was standardized and pretested. The study was started only after clearance from Institutional Ethics Committee.

EXCLUSION CRITERIA

Pregnant women with established high risk pregnancy or with history of medication for more than three months due to medical or pregnancy related problems were excluded from this study.

RESULTS

In the present study, out of the 656 pregnant women interviewed, 81.1 % pregnant women informed that they preserved old prescription regularly, 37.5 % had knowledge about expiry date (Figure 1), but after explanation by the investingator about the importance of expiry date, 633, i.e., 96.5 % pregnant women expressed their opinion that expiry date should be checked before consumption of any medicine. 88 % pregnant women were of the opinion that the instruction on label of commonly used medicines like iron, folic acid, paracetamol, ibuprofen etc. should be in the local languages. This would help them for better use of these medicines. They also expressed that information about the harmful effect of the medicines should also be in local language and in different colour also. 40.7 % women expressed that they use over the counter (OTC) medicines. 4.3 % also informed that they had previous signs and symptoms related to adverse drug reaction (ADR) after using medicine, but never informed this to the doctor (Table 1). Knowledge of pregnant women about health facilities available nearby was evaluated. The result showed that 50.6 % pregnant women knew about the nearest local hospital, 60.5 % had knowledge about the clinic of local doctor, 25 % knew about the availability of ambulance in the locality, 66.76 % (Figure 2) pregnant women had knowledge about the residence of trained dai of their village and 66 % had knowledge about the nearest chemist shop in the locality. Present study showed that 44.05 % pregnant women utilized the services of the local hospital at least for once, when enquired about the utilization of services of local qualified doctors, only 17.68 % used it at least for once and only 02.28 % actually used the services of ambulance available locally. 59.45 % pregnant women of this study informed that they used the service of trainedAsha/dai of that village. Regarding utilization of services of local chemist shop, 19.51 % women of the study informed that they used it at least for once (Table 2), not only for purchasing medicines but also for taking tetanus toxoid injection, dressings and for getting information on use of medicines.

Table 1: Study of knowledge of medicine and its use in pregnant women.

Knowledge and attitude of pregnant women	No. of pregnant women	Percentage (%)
Pregnant women expressed that regular checking of expiry date should be done before consumption of any medicine	633	96.5
Pregnant women expressed that use of local languages on the labelling of drugs should be done for their better understanding	577	88
Pregnant women expressed that they were OTC drug user	267	40.70
Pregnant women informed that they had previous experience (symptoms and signs) related to adverse drug reaction (ADR) after using medicine, but never informed this to the doctor	28	4.3

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The data in the above table represents the knowledge and attitude in pregnant women regarding medicine use related parameters, where total no. of pregnant women in study population (n) = 656

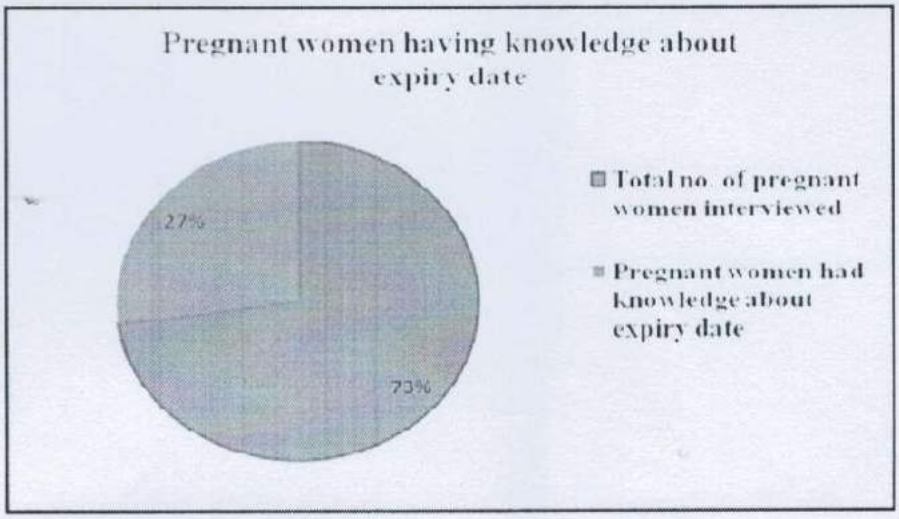

Figure 1: Knowledge of expiry date of medicine in pregnant women (n = 656)

Table 2: Study of the use of available health facilities by the pregnant women

Actual use of health facilities available near by	No. of pregnant women	Percentage (%)
Pregnant women actually used services from the local hospital at least once	289	44.05
Pregnant women actually used services from the local trained Asha/ dai at least once	390	59.45
Pregnant women actually used services from the Ambulance available in the locality at least once	15	02.28
Pregnant women actually used services from the local qualified doctors at least once	116	17.68
Pregnant women actually used services from the chemist shop at least once	128	19.50

The data of the above table represent the study result of actual use of the health facilities available in locality by the pregnant women (n=656).

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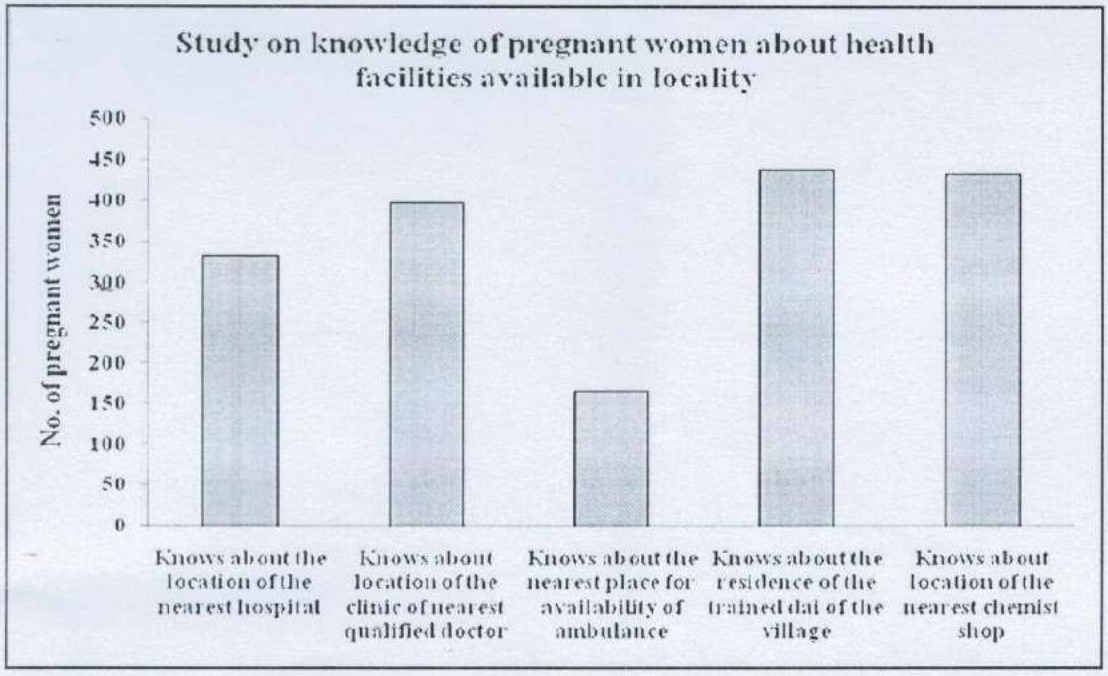

Figure 2: Knowledge of pregnant women (n = 656) about available local health facilities

DISCUSSION

As far as evaluation of the attitude, behaviour and knowledge of drug use is concerned in pregnant women, it was found that 37.5 % of pregnant women had knowledge about expiry date of drugs Though majority of study population were of opinion that expiry date should be checked before consumption of any drugs. 88 % pregnant mothers were of opinion that local languages should be used on the labels of the drugs and on the information booklet at least for the commonly used drugs like iron and folic acid, vitamins, paracetamol, ibuprofen etc. This awareness of pregnant mothers might be helpful in enhancing rational drug use ultimately. Very few studies were conducted in India on OTC drug use in pregnant women. Studies of Henry et al., 2000 from South Australia[12] and Gharro et al., 2000 from Nigeria[13] were in contradiction with the results of present study. Study of Dinesh Kumar et al., 1995 from National Institute of Nutrition, Hyderabad, India[14] was in agreement with this study, but the cross sectional study conducted at Jammu city Sharma et al., 2005[15] differed in results because of their urban study area. Study results showed that 4.3 % mothers suffered from some type of unwanted reactions due to drug use during pregnancy. Even after intensive interview, the exact cause of drugs or drug-drug interaction was not identified. This was due to the ignorance of pregnant women about ADR and poor reporting system. Present study was in agreement with observation of Dhasmana et al., 2002[16] where they observed that voluntary reporting of ADR was very low even among the physicians of a teaching hospital. The knowledge of pregnant women about emergency health service facilities available in the locality was evaluated and it was observed that they were not only in the lack of knowledge and awareness on health service

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infrastructures available nearby, but also were very poor in utilization of these resources. No relevant data was available to compare present observations on these parameters.

CONCLUSION

There were few lacunae in this study, which include the number and selection of study population, selection of villages, etc. This cross-sectional study methodology was based on interview and analysis of prescription. Knowledge, awareness and practices of rational use of medicines and health facility utilization by pregnant women or even by the general population are a neglected topic till today, though it is of immense importance. Any drug or medicine or any health infrastructure, whatever it may be, sophisticated or modern, is of no value, if not actually used by the beneficiaries. Present study showed that pregnant women were not aware about the health resources available nearby to their residence. Not only that, only a few percentages aware women among them actually used these infrastructures. From overall results of the study it may be concluded that though there was lack of knowledge about proper, rational and judicious medicine and health infrastructure use amongst pregnant women, it can be improved if they are informed properly. Efforts should be taken to disseminate the knowledge on medicine use and the use of health infrastructure available nearby, so that all health-related initiatives become successful in terms of utilization.

CONFLICT OF INTEREST: Nil
SOURCE OF FUNDING: Nil

REFERENCES

1. Tripathi KD. Essentials of Medical Pharmacology. 5thed: Jaypee Brothers New Delhi; 2003.
2. Wade OL. Prescribing of chloramphenicol and aplastic anaemia. Journal of the College of General Practioners and Research Newsletter 1996; 12: 277.
3. Billmont PS. Oxford Text Book of Clinical Pharmacology. 3rded: Oxford University Press; 1995.
4. Bhutta TI. Drug utilization data constraints in developing countries. WHO Drug Information 2002; 16(3): 237.
5. Medicines, Medical care and Drug Policy. The Independent Commission on Health in India (Report), V.H.A.I.; 2002.
6. How to investigate drug use in health facilities, selected drug use indicators. World Health Organisation, Geneva; 1999. p. 12.
7. How to investigate drug use in health facilities. Selected drug use indicators. World Health Organisation, Geneva; 1999. p. 82.
8. Durisova A, Magulova L. Drug use problem in pregnancy. BratislLekListy 2004; 105(3): 123-4.
9. Irl C, Hasford J. The PEGASUS project-a prospective cohort study for the investigation of drug use in pregnancy. Int J ClinPharmacolTher 1997; 35(12): 572-6. PMid:9455716.
10. Sharma R, Kapoor B, Verma U. Drug utilization pattern during pregnancy in North India. Indian J Med Sci 2006; 60: 277-287. http://dx.doi.org/10.4103/0019-5359.26602 PMid:16864912.
11. Srishyala MV, Krishnamurty M, Nagarani MA, Clare Sr Mary. Prescription Audit in an Indian Hospital setting using the DDD (Daily Defined Dose) Concept. Indian J Pharmacol 1994; 26: 23-28.
12. Biswas NK, Biswas RS, Pal PS, Jain SK. Pattern of Prescriptions and Drug Use in two Tertiary Hospitals in Delhi. Indian J PhysiolPharmacol 2000; 44(1): 109-112. PMid:10919106.
13. Henry A, Crowther C. Pattern of medication use during and prior to pregnancy: the MAP study. Aust N Z J ObstetGynaecol 2000; 40(2): 165-72. http://dx.doi.org/10.1111/j.1479-828X.2000.tb01140.x.
14. Dineshkumar B, Raghuram TC, Radhaiah G, Krishnaswamy K. Profile of drug use in urban and rural India. Pharmacoeconomics 1995; 7: 332-46. http://dx.doi.org/10.2165/00019053-199507040-00007 PMid:10155322.
15. Sharma R, Verma U, Sharma CL, Kapoor B. Self-medication among urban population of Jammu city, Indian J Pharmacol 2005; 37(1): 40-42. http://dx.doi.org/10.4103/0253-7613.13856.

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Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2293-2298 | ISSN:2251-6727

16. Dhasmana DC, Seth V, Mishra KC. Voluntary Adverse Drug Reaction Reporting in a Teaching Hospital. Indian J Pharmacol 2002; 34: 204-205.

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IN SILICO PHARMACOKINETIC, BIOACTIVITY AND TOXICITY STUDIES OF SEVERAL SELECTED ANTI-VIRAL DRUGS

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Abstract

As of late, new irresistible infections with huge casualty rates have emerged, including SARS-CoV, MERS-CoV, and SARS-CoV-2. To battle these pathogenic microbes, creative restorative synthetic compounds should be grown rapidly. Sadly, the traditional ways to deal with drug advancement are expensive and tedious. In this examination, virtual screening of a library of regular synthetic compounds in the ZINC data set for their liking towards SARS-CoV-2 Mpro was finished utilizing computational strategies. By keeping SARS-CoV-2 Mpro from advancing Coronavirus contamination, drugs including cinanserin, nelfinavir, baicalin, baicalein, candesartan cilexetil, chloroquine, dipyridamole, and hydroxychloroquine treat Coronavirus. Nonetheless, these drugs for the most part work to reduce the infection's side effects. The aviation routes that pass air on to and from the lungs are impacted by asthma. Different aggravations and synthetic substances that cause sensitivities (allergens) can make asthma side effects and signs show up. Due to different hereditary, ecological, and word related risk factors, the recurrence of asthma changes

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incredibly all over the planet. In this review, we utilize computational ways to deal with look at the pharmacokinetic, drug-similarity, bioactivity profile, and toxicity profile of at least one or two enemy of asthmatic drugs.

Keywords: *Silico, Pharmacokinetic, Bioactivity, Toxicity, Drugs*

1. INTRODUCTION

A worldwide illness called COVID-19 started at the end of 2019. The World Health Organization verified in January 2022 that there have been 323,610,370 confirmed cases of COVID-19 and 5,529,693 deaths overall.1 One of seven coronavirus strains that severely damage the lower respiratory system is SARS-CoV-2.2 The primary receptor for this virus is ACE2, and it spreads through the innate immune system of people. About 80% of individuals with this condition, which affects the respiratory tract, have a moderate case. 20% of patients may develop a serious condition from it. Elderly people in a study of 292 COVID-19 patients in Wuhan showed a risk increase of 15.15 percent. Congenital illnesses include hypertension, cancerous tumors, chronic obstructive pulmonary disease, coronary heart disease, and chronic renal disease become harmful as a result. If the patient is elderly and has concomitant conditions, this could result in death. Out of 145 patients with comorbidities, 51 were reported to have passed away, and 90.2% of them were 60 or older.

Proteins in viruses can be categorized as structural or non-structural based on how they operate. In order to prevent DNA from being destroyed as in the nuclear capsid, structural proteins act as a barrier against host enzymes. The main protease (Mpro), a non-structural protein, is a cysteine protease enzyme that aids in replication, like chymotrypsin. The major protease (Mpro) is a crucial enzyme that aids in the corona virus's (CoV) reproduction. It has been determined that humans lack Mpro homologues.8,9 Because Mpro inhibitor research has no negative effects on human proteases, it has a lot of potential and is very successful. At this time, vaccinations are the primary method of COVID-19 prevention. A strong immune system is necessary to combat the SARS-CoV-2 virus, though. The therapeutic treatments and vaccinations that have been promised up to this point are still being sought after by researchers and medical experts. Herbal medications as immunomodulators for the prevention and treatment of Covid-19 illness are one of the complementary and alternative therapies that have received significant development.

Wuhan was the site of the main instance of SARS-CoV-2 disease, which was accounted for in December 2019. By December 2020, a larger number of than 1.4 million individuals had died from the sickness, and more than 6.35 million individuals had been infected1 SARS-CoV-2

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has consistently represented a danger to human wellbeing, fundamentally expanding grimness and mortality on a worldwide scale. As per Patel et al. the infection can spread by various channels, like creature to-human transmission, mother-to-kid transmission, sexual contact, ophthalmic, bloodborne, waste oral, direct contact, and airborne. Regardless of the way that SARS-CoV-2 essentially causes a gentle respiratory contamination, numerous casualties have extreme sickness and ultimately die. A great deal of asymptomatic sicknesses can likewise spread the contamination to others. Patients with Coronavirus who have basic issues are bound to foster a serious sickness.

2. REVIEW OF LITREATURE

Tahir ul Qamar et al. (2021) investigated the binding mechanism of six anti-viral medications against SARS-CoV-2 and its variations using molecular docking and molecular dynamics simulations. Remdesivir, Favipiravir, and Ribavirin, which demonstrated strong binding affinity and stability inside the virus's protease and polymerase active sites, were among the possible inhibitors of the virus that their study discovered. The study also discovered that the virus's UK version was more drug-resistant than the original strain, underscoring the significance of ongoing research on novel variants.

Using molecular docking and molecular dynamics simulations, Changqing Zhang et al. (2021) conducted an in silico assessment of natural compounds' potential anti-viral activity against SARS-CoV-2. The authors discovered a number of organic compounds, including quercetin and luteolin, that have a strong affinity for the spike protein and protease active site of the virus. According to the study, natural compounds may serve as a source for future anti-viral medications.

In order to evaluate the toxicity of possible anti-COVID-19 medications, Amine El Aoufir et al. (2021) employed in silico prediction models. The quantitative structure-activity relationship (QSAR), random forest (RF), and support vector machine (SVM) algorithms were some of the computational methods used in the investigation. With a few notable outliers, such as Darunavir and Lopinavir, which showed moderate toxicity, the study indicated that the majority of the medications exhibited modest toxicity.

An in silico study was carried out by Manoj Kumar Yadav et al. in 2021 to assess the pharmacokinetics, bioactivity, and toxicity of certain anti-COVID-19 medications. The review utilized various computational procedures, including sub-atomic docking, recreations of atomic elements, and ADMET (assimilation, appropriation, digestion, discharge, and toxicity) profiling. The review found that the picked drugs had insignificant degrees of toxicity and great pharmacokinetic and bioactivity attributes.

In silico prediction models were utilized by Xuehua Zhang et al. (2021) to evaluate the toxicity of antiviral medications against SARS-CoV-2. The study used a variety of computational

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techniques, including machine learning and molecular docking. The study suggested that additional in vitro and in vivo investigations are required to confirm these predictions and indicated potential medication toxicities, such as liver damage and cardiotoxicity.

A potential anti-SARS-CoV-2 agent's pharmacokinetics and toxicity were predicted in silico by Rashid Ahmed et al. in 2021. The study made use of a variety of computational techniques, including molecular docking, ADMET profiling, and simulations of molecular dynamics. The investigation found prospective medications, such as Hydroxychloroquine, Chloroquine, and Lopinavir, with favorable pharmacokinetic and bioactivity profiles and low toxicity levels.

3. MATERIALS AND METHODS

3.1 In silico Pharmacokinetic Studies

Using computational methodologies, a few physicochemical attributes and pharmacokinetic descriptors for a couple of picked enemy of asthmatic drugs were assessed utilizing the web device Mo motivation Cheminformatics server (http://www.molinspiration.com).Molinspiratio n Cheminformatics gives a large number of devices for handling and controlling particles, like Grins and SDfile transformation, atom standardization, tautomer age, particle discontinuity, estimation of different sub-atomic properties expected in QSAR studies, atomic displaying and drug plan, excellent particle portrayal, and atomic data set devices supporting foundation. Moreover, this program offers information representation, bioactivity forecast, and part based virtual screening. Since molinspiration devices are planned in Java, they can basically run on any registering stage. Drug-resemblance is a subjective term for a characteristic that alludes to how comparable a given particle is to existing meds. It is characterized as a mind boggling difficult exercise between numerous sub-atomic qualities and underlying components. These sub-atomic qualities incorporate hydrophobicity, electronic conveyance, hydrogen holding qualities, particle size and adaptability, and obviously the presence of different pharmacophoric highlights that influence a particle's conduct in a living organic entity, including bioavailability, transport attributes, fondness to proteins, reactivity, toxicity, metabolic soundness, and numerous other factors.The Lipinski rule of five, which manages four clear physicochemical boundary ranges (MWT 500, log P 5, H-security benefactors 5, H-security acceptors 10), is utilized to evaluate the medication similarity of 90% of orally dynamic meds that have accomplished stage II clinical status. To address drug-resemblance as qualities of power, other working out procedures can be used, like ligand productivity and lipophilic effectiveness. These physicochemical qualities associated with digestive penetrability and fluid dissolvability are inside an adequate reach. Physical-synthetic elements, which make up a generally little part

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of the all out substance data about the genuine particle, have acquired prevalence as factors in examinations on sub-atomic demonstrating.

3.2 In silico Bioactivity Studies

Utilizing the instrument Molinspiration Cheminformatics server the bioactivity score of a couple of chosen compounds was likewise evaluated. Enormous compound data sets are inspected utilizing this computational science strategy to find potential novel prescription competitors. Virtual screening strategies range from clear ones that check for the presence or nonappearance of specific foundations or a match in determined sub-atomic properties to complex virtual docking methods planned to squeeze potential ligand particles into the objective receptor site. The Molinspiration bioactivity device strikes an incredible blend between screening execution, data needs for another virtual screening undertaking, and screening speed. In the Molinspiration device, the mi screen motor dissects a preparation set of dynamic mixtures (in outrageous cases, even one dynamic particle is sufficient to construct a practical model) and contrasts them and latent atoms utilizing progressed Bayesian measurements. For the preparation, just the Grins or SD document designs of dynamic mixtures are required; information on the dynamic site or it isn't expected to tie system. This is particularly useful in projects when a design based technique can't be utilized because of an absence of information in regards to the 3D receptor structure, for example, in screens planned to recognize ligands that balance G-protein coupled receptors. In light of this exploration, a part based model is made, in which a bioactivity commitment is assessed for every foundation piece. When a model has been created, the bioactivity of the particles that have gone through screening can not entirely set in stone as the amount of the movement commitments of the different atoms' pieces. This yields a sub-atomic action score, which is a number between - 3 and 3. The probability of being dynamic is higher for particles with the most noteworthy action score. Such in silico screening is very speedy; it is feasible to screen enormous assortments of particles (in excess of 100,000 atoms) in a solitary hour. Evaluating models for four critical medication classes — GPCR ligands, particle channel blockers, kinase inhibitors, and atomic receptor ligands — were made in view of the techniques recently announced. Using mi screen's implicit capacities simplifies it to make a virtual evaluating model for any objective. A further advantage of virtual screening conventions in light of Bayesian measurements is their ability to sum up, or to find the general primary requirements for bioactivity. Thus, the methodology is feasible to distinguish novel dynamic design classes notwithstanding new bioactive mixtures that are connected with the preparation set (platform jumping).

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several selected anti-viral drugs

3.3 In silico Toxicity Studies

Utilizing a computational technique and a Pentium IV processor running Pallas version 3.1 ADMETox prediction software, the toxicity of the chosen anti-asthmatic drugs was assessed. Double clicking on the symbol launched this software program. The molecule that needed to be predicted was created by double-clicking the new option, and its toxicity was then assessed by choosing the ToxAlert options. Oncogenicity, neurotoxicity, teratogenicity, immunotoxicity, and other forms of toxicities were generated, and the toxicity profile of the chemical was reported.

A few enemy of asthmatic meds were picked, and their ADME characteristics and medication similarity (as per Lipinski's standard of five) results are displayed in Table 1. Aside from ciclesonide and montelukast, every one of the picked drugs have sub-atomic loads that are inside the allowable reach (MWT 500). Instead of enormous sub-atomic weight synthetic compounds, particles with a low sub-atomic weight are all the more promptly consumed, scattered, and shipped. For certain exemptions, as atomic weight rises, the volume of the particles likewise rises relatively.

4. RESULTS AND DISCUSSION

Table 1: ADME Properties of Anti-asthmatic Agents

Name	Molecular formula	Molecular weight	LogP	TPSA	nON	nOHNH	nrotb	volume	In silico % absorption
Salbutamol	C13H21NO3	240.12	2.39	73.75	5	5	6	261.12	84.35
Terbutaline	C12H19NO3	226.30	2.08	73.75	5	5	5	264.15	83.11
Ipratropium bromide	C20H30BrNO3	341.50	-2.52	50.41	5	4	7	312.61	85.41
Theophylline	C7H8N4O2	190.18	-0.02	73.69	7	3	4	356.11	82.11
Montelukast	C35H36ClNO3S	591.30	8.91	75.49	5	5	3	245.32	86.53
Fluticasone	C22H27F3O4S	465.62	4.56	75.69	5	6	8	264.11	74.55

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Ciclesonide	C32H44O7	641.72	6.75	99.16	8	7	5	312.11	79.12
Salmeterol	C25H37NO4	513.22	4.98	82.96	6	5	2	365.23	84.32

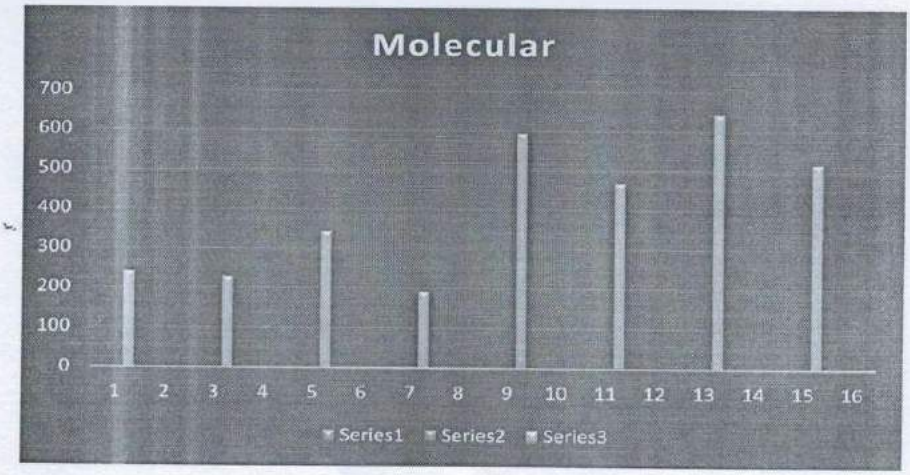

Table 2: Bioactivity of Anti-asthmatic Agents

Name	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Salbutamol	0.30	-0.04	-0.30	-0.22	0.08	0.20
Terbutaline	0.17	-0.08	-0.40	-0.36	-0.16	0.08
Ipratropium bromide	0.60	0.37	.030	-0.41	-0.09	0.19
Theophylline	-0.45	-0.80	-1.26	-2.64	-1.54	-0.24
Montelukast	0.69	-0.18	-0.19	0.19	0.35	0.31
Fluticasone	0.18	0.03	-0.70	3.01	1.08	0.95
Ciclesonide	-0.04	-0.60	-0.79	0.81	0.25	0.36
Salmeterol	0.39	0.06	0.09	0.18	0.32	0.30

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Table 3: Toxicity Profile of Anti-asthmatic Agents

Name	Toxicity	Overall toxicity	Oncogenicity	Mutagenicity	Irritation	Sensitivity	Immunotoxicity	Neurotox
Salbutamol	Probable	61	1	30	63	1	1	30
Terbutaline	Probable	61	1	30	63	1	1	30
Ipratropium bromide	High Probable	77	86	1	1	1	1	1
Theophylline	High Probable	86	86	94	1	1	1	1
Montelukast	High Probable	86	86	61	1	1	1	1
Fluticasone	High Probable	86	86	69	55	1	1	1
Ciclesonide	High Probable High Probable	85	86	41	30	1	1	1
Salmeterol	High Probable	91	1	59	41	1	1	30

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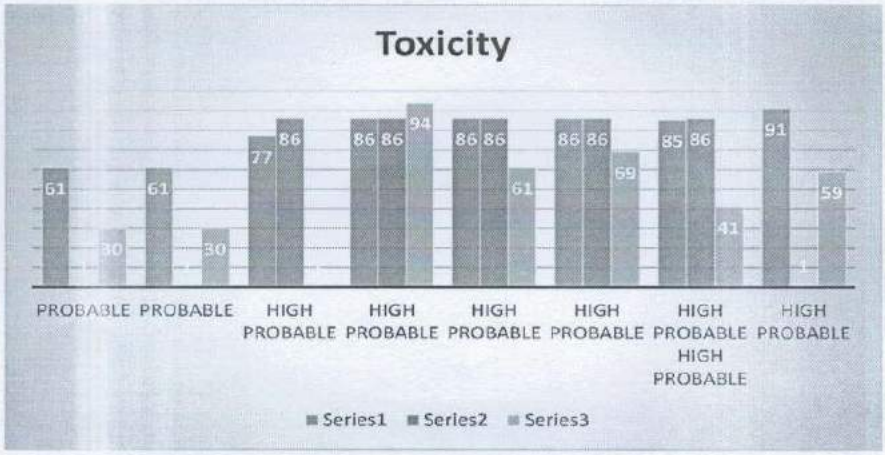

Figure 2: Toxicity Profile of Anti-asthmatic Agents

Montelukast and ciclesonide are two picked enemy of asthmatic drugs that have one infraction of Lipinski's standard of five. Montelukast has a sub-atomic load of 586.20 and a logP esteem that is more noteworthy than okay at 7.89. Except for montelukast, all specialists' MLogPs (octanol/water parcel coefficients) were figured and viewed as satisfactory per Lipinski's standards. The lipophilic productivity, which measures pharmacological power, is determined utilizing the MLogP esteem. Subsequently, the logP worth of the octanol-water parcel coefficient is urgent for QSAR exploration and objective medication plan. The hydrophobicity of the atom is assessed in the pharmacokinetic concentrate by ascertaining the logP esteem since hydrophobicity is pivotal for the conveyance of the medication in the body following retention. Topological Polar Surface Region, or TPSA, is an extremely supportive physiochemical sub-atomic trademark that uncovers data about the extremity of substances. The investigation of the medication transport properties utilized this boundary. The aggregate sum of polar iotas, principally oxygen and nitrogen with connected hydrogen, makes up polar surface region. For all of the picked enemy of asthmatic meds, percent retention was likewise evaluated utilizing the recipe %ABS = 109-(0.345 * TPSA). The sub-atomic volume assesses a particle's vehicle qualities, for example, blood-mind boundary penetrability. The quantity of rotatable bonds was not entirely set in stone to be relevant. A particle turns out to be more adaptable and has a superior restricting partiality to the limiting pocket when it has a bigger number of rotatable bonds. The bioactivity of every enemy of asthmatic medication that was picked was evaluated against six distinct protein setups. At the point when a bioactivity score is more than 0.00, it

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demonstrates that there is huge organic movement. Bioactivity scores are partitioned into three fundamental ranges.2. Assuming that the bioactivity score is somewhere in the range of 0.5 and 0.00, moderate activity.3. Idleness is available on the off chance that the bioactivity score is not exactly - 0.50. As per the review's discoveries, the picked specialists make physiological impacts and are physiologically dynamic. Table 2 contains the bioactivity score profiles for the picked specialists in general. Figure 1 shows the bioactivity score diagram of salbutamol for a few proteins. To make another utilitarian medication with a higher restricting selectivity profile and less bad secondary effects, data about the limiting fountain of the drugs is given by the bioactivity score. All chosen anti-asthmatic medications underwent toxicity profile evaluation and are included in Table 3. All of the medications, with the exception of salbutamol and terbutaline, were judged to be extremely probably to cause toxicity. The intriguing toxicological fact is that, with the exception of theophylline, all tested anti-asthmatic medications were found to exhibit teratogenicity. These research results serve as a starting point for the creation of brand-new, very effective anti-asthmatic medications. The knowledge on the pharmacokinetics of the currently available medications provided by computational analysis of all chosen anti-asthmatic pharmaceuticals serves as a guide for the development of new, more effective, and less toxic therapies.

5. CONCLUSION

Specialists have been inspired to find, reveal, and reuse the generally existing and all around described normal mixtures as possible inhibitors of SARS-CoV-2 by the worldwide test presented by the Coronavirus pandemic. Various antiviral substances and prescriptions focus on the primary and nonstructural proteins of SARS-CoV-2. One of the essential targets is the significant protease, one of the primary proteins of the infection. SARS-CoV-2 Mpro is specifically noteworthy to analysts in light of the fact that hindering its headway of Coronavirus can stop the infection's engendering. The objective protein distinguished in the ongoing examination is viral significant protease. SARS-CoV-2 Mpro inhibitors were found by pharmacophore-based virtual evaluating for normal synthetics from the ZINC data set. A painstakingly chosen assortment of financially open mixtures made explicitly for virtual screening designs is the ZINC data set.

REFERENCES

1. Anand K, Palm GJ, Mesters JR, Siddell SG, Ziebuhr J, Hilgenfeld R. Structure of coronavirus main proteinase reveals combination of a chymotrypsin fold with an extra α-helical domain. *EMBO J.* 2002;21:3213–3224. doi: 10.1093/emboj/cdf327
2. Brahmi F, Vejux A, Ghzaiel I, et al. Role of diet and nutrients in SARS-CoV-2 infection: incidence on oxidative stress, inflammatory status and viral production. *Nutrients.* 2022;14. doi: 10.3390/nu14112194

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3. Chali BU, Melaku T, Berhanu N, et al. Traditional medicine practice in the context of COVID-19 pandemic: community claim in Jimma zone, Oromia, Ethiopia. *Infect Drug Resist*. 2021;14:3773. doi: 10.2147/IDR.S331434
4. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395:507–513. doi: 10.1016/S0140-6736(20)30211-7
5. Chiang LC, Ng LT, Cheng PW, Chiang W, Lin CC. Antiviral activities of extracts and selected pure constituents of Ocimum basilicum. *Clin Exp Pharmacol Physiol*. 2005;32:811–816. doi: 10.1111/j.1440-1681.2005.04270.x
6. Chowdhury MA, Hossain N, Kashem MA, Shahid MA, Alam A. Immune response in COVID-19: a review. *J Infect Public Health*. 2020;13:1619–1629. doi: 10.1016/j.jiph.2020.07.001
7. El-Demerdash A, Metwaly AM, Hassan A, et al. Comprehensive virtual screening of the antiviral potentialities of marine polycyclic guanidine alkaloids against SARS-CoV-2 (COVID-19). *Biomolecules*. 2021;11:460. doi: 10.3390/biom11030460
8. Hu Q, Xiong Y, Zhu GH, et al. The SARS-CoV-2 main protease (Mpro): structure, function, and emerging therapies for COVID-19. *MedComm*. 2022;3:e151. doi: 10.1002/mco2.151
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497–506. doi: 10.1016/S0140-6736(20)30183-5
10. Issa SS, Sokornova SV, Zhidkin RR, Matveeva TV. The main protease of SARS-CoV-2 as a target for phytochemicals against coronavirus. *Plants*. 2022;11:1862. doi: 10.3390/plants11141862
11. Junaid K, Qasim S, Yasmeen H, et al. Potential inhibitory effect of vitamins against COVID-19. *Comput Mater Contin*. 2020;66(1):707–714. doi: 10.32604/cmc.2020.012976
12. Shahrajabian MH, Sun W, Cheng Q. Chemical components and pharmacological benefits of Basil (Ocimum basilicum): a review. *Int J Food Propert*. 2020;23:1961–1970. doi: 10.1080/10942912.2020.1828456
13. Tiwari S, Dubey N. Traditional medicinal plants as promising source of immunomodulator against covid-19. *J Exper Biol Agric Sci*. 2020;8:S126–S138. doi: 10.18006/2020.8(Spl-1-SARS-CoV-2).S126.S138
14. World Health Organization. *COVID-19 Weekly Epidemiological Update*. Geneva: World Health Organization; 2021.
15. Zhang L, Lin D, Sun X, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science*. 2020;368:409–412. doi: 10.1126/science.abb3405

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Section A-Research paper
ISSN 2063-5346

Phytoconstituent and Pharmacological screening for anti-diabetic activity of *Tephrosia villosa*

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Abstract

The *Tephrosia villosa* (L.) Pers herb extract were prepared by successive soxhlation i.e. extracting dried powder with the solvents of increasing order of polarity, and It was qualitatively observed that 70% ethanolic extract contain higher concentration of polyphenol, flavonoid, alkaloid, protein and tannin components andquantative studies of The total phenolic content of 70% EETVH was 1.08 mg/G expressed as equivalent to catechol. Similarly flavonoid content was found to be 3.21 mg/G expressed as equivalent to quercetin and total tannin content found to be 5.10 mg/G expressed as equivalent to tannic acid.

Anti-diabetic property of the 70 % EETVH was studied in *In-vitro* and *In-vivo* models, . In-vitro non enzymatic glycosylation of haemoglobin method 70% EETVH significantely inhibited the hemoglobin glycosylation which is indicated by the presence of increasing concentration of hemoglobin, the plant extract exhibited higher inhibition indicating plant extract decreases the formation of glucose hemoglobin complex. *In-vivo* anti-diabetic activity of 70% EETVH is

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carried out in experimentally induced alloxan –diabetes in albino rats and to compare activity the lower dos($1/10^{th}$ cutoff value)and higher dose ($1/5^{th}$) are selected from the toxicity studies according to OECD guidline no-423. It implies that 70% EETVH significantly reduces the elevated blood glucose .

Keywords: *Tephrosia villosa* (L.), invitro diabetic activity, invivo diabetic activity, alloxan

INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by chronic hyperglycaemia, with disturbances of carbohydrate, fat and protein metabolism resulting defects in insulin secretion, action or both[1] it is one of most serious endocrine metabolic disorder has caused significant mortality and morbidity due to micro vascular (Retinopathy ,Nephropathy)and macro vascular (Heart attack, stroke) complications[2] blood glucose is essential to life as a regulator of Homoeostasis, glucose is the obligate metabolic fuel for the brain under the physiologic condition, glucose in plasma either comes from dietary source or by the result of the breakdown of glycogen in liver or formation in the liver and kidney from other carbon compounds such as Lactate, pyruvate aminoacids and glycerol.[3] Insulin is very essential for glucose uptake into the cell, it is an hormone secreted by the beta cells of pancreas if pancreas does not produce enough insulin glucose get into the blood cells and stays in the blood this results in hyperglycaemia.[4]

DM is classified into two major subtypes: type I (insulin dependent diabetes mellitus (IDDM) and type II (non-insulin dependent diabetes mellitus (NIDDM).and Gestational diabetes. IDDM or juvenile onset diabetes results from a cellular mediated autoimmune destruction of the β-cells of the pancreas. However, NIDDM or adult-onset diabetes results from the development of insulin resistance and the affected individuals usually have insulin deficiency. Patients suffering from type I are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes can be treated with dietary changes exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population[5].

International Diabetic Federation reported that The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%). One in two (50.1%) people living with diabetes do not know that they have diabetes. The global prevalence of impaired glucose tolerance is estimated to be 7.5% (374 million) in 2019 and projected to reach 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045. high prevalence of diabetes has important social, financial and developmental implication especially In low and middle income countries,[6] The world Health organization projected that diabetes will be the 7^{th} leading cause of death.

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Plant description:
Name of the plant : *Tephrosia villosa* (L.) Pers

Synonym [a] **:**
Cracca incana Roxb.
Cracca villosa L.
Cracca villosa L. var. *incana* (Roxb.) Hiern
Galega hirta Buch.-Ham.
Galega incana Roxb.
Tephrosia ehrenbergiana Schweinf.
Tephrosia hirta (Buch.-Ham.) Benth.
Tephrosia incana (Roxb.) Wight & Arn.
Tephrosia incana (Roxb.) Wight
Tephrosia villosa (L.) Pers. var. *argentea* Thwaites
Tephrosia villosa (L.) Pers. var. *incana* (Roxb.) Baker

Taxonomy
Kingdom : Plantae
Division: Magnoliophyta
Class : Magnoliopsida
Order: Fabales
Tribe : Millettieae
Family: Leguminosae (Fabaceae)- Papilionoideae(L.) Persoon
Genus: *Tephrosia*
Species: *villosa Pers*.

The plant is known by various names in different languages as under[7]

English :	Shaggy wild indigo
Sanskrith :	Sharpunkha
Hindi :	Salunak.
Kannada:	Niligida
Tamil :	Kottukolingi
Oreia:	Kulthia, Piderkalata

Plant Form : Bushy **Herb,** shrub
Flower: In November and fruit in February in india
Distribution: *Tephrosia villosa* has a large distribution found in southern and eastern Africa, the Arabian Peninsula and across southern Asia.
Botanical Discription: Sharpunkha" an important ayurvedic drug has been in use for a long time two kinds of sharpunkha the shevet (white)and Raktha (Red) are described in some of the ayruvedic texts like Vaghbhatas "Astang Hridayam"and in Nighantus[8]. shevet sharpanakha is

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Tephrosis villosa due to its persistently villous like white part and this view receives the support also from "Shivadatta Nighantu"which mentions "Sharpunkheti vikhya shreepuspha kavachid bhavet" Genus Tephrosia Pers is a large seasonal pantropic genus of about 400species belongs to family Fabaceae, in india genus *Tephrosia* Pers is represented by 29 taxa including 27 species 1 variety and 1 sub species out of which 7 taxa are endemic to india.in india maximum taxa of Tephrosia are recorded in presidency of Madras. (Gamble,1957)[9]
The name is from greek word Tephros meaning ash-coloured, reffering to the grayish tint given to the leaves and pod by their dense hairs.
Genus *Tephrosia* Pers is characterized by herbs or shrubs
leaves stipulate,pinnate,imparipinnate ,rarely simple
Flowers generally in terminals or leaf opposed racemes.calyx lobes subequal,
petals clawed, standard sub orbicular, stamens diadelphous,
pods usually linear, flattened,many seeded,continuous or scarcety septate(Hooker 1961) some of the species are cultivated as cover crops,green manures,fish poision and ornamental.

Dignostic characters of Tephrosia are
1. Leaves cut into forks (Horn like structure).
2. Pods flat, not joined many seeded.
3. Standard petal obtuse.
4. Sepals subequal, calyx lobes connate.
5. Anthers muticous, basifixed

Tephrosia villosa is an annual or perennial bushy herb, 0.3-1.3 m tall. Stem white tomentose. Leaves imparipinnately compound with 7-19 leaflets, up to 10 cm long;stipules 2-5 mm long; leaflets obovate to elliptical, up to 21 mm x 9 mm,hairy on both sides, each side with 4-8 pairs of distinct veins. Stipulestomentose, caducous and lanceolate. Flowers in a terminal or upper axillary pseudo raceme 8-22 cm long; pedicel with densely matted hairs, 2-4 mm long; calyx densely matted hairy, tube about 2 mm long, lobes long-acuminate, to 9 mm long;standard transversely elliptical to broadly ovate, up to 7 mm x 10 mm,dorsally with dense brown hairs. Style glabrous, up to 3-5 mm long, bent sharply upward at base, twisted, penicillate. Pod strongly curved, up to 4 cm x 6 mm, densely silvery or brown tomentose,hairs to 2 mm long, 4-10-seeded. Seed 12-16, rectangular, black, smooth, with short hard excrescences, upto 4.5 mm x 2.5-2.75 mm. Flower in November and fruit in February in India. [14] The specific name 'villosa' means covered in white soft hair in Greek

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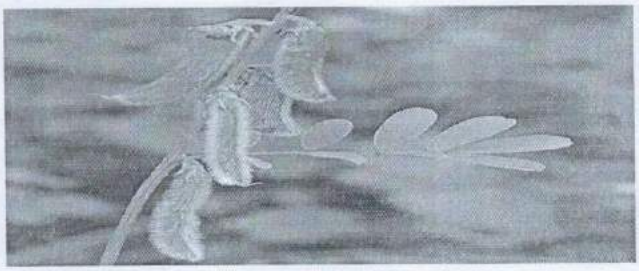
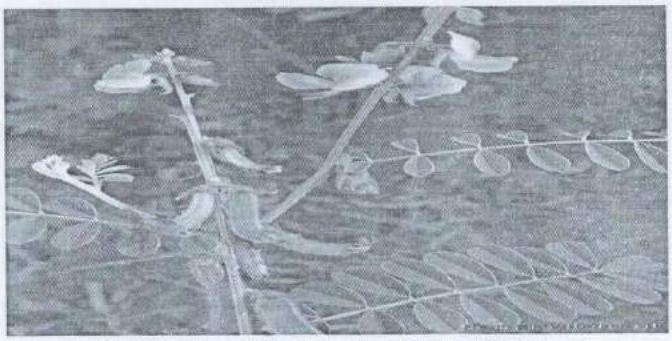


Photographs of **Tephrosia villosa Pod**



Photographs of **Tephrosia villosa Herb**

Tephrosia villosa (per) has been found to be having
Antimicrobial and Brine shrimp activity [10-11]
Antidiabetic activity [12]
Potential bio insecticide [13]
Anthelmintic activity [14]
Antioxidant activity [15]
Green corrosion inhibiter activity [16]

EXPERIMENTAL WORK

I. Preparation of extract

The *Tephrosia villosa* (L.) Pers herb extract will be prepared by successive soxhlation i.e. extracting dried powder with the solvents of increasing order of polarity i.e. Pet. ether (60-80°), Chloroform (59.5-61.5°), 70% Ethanol (64.5-65.5°) . Extracts will be concentrated under reduced pressure. and stored in airtight container in refrigerator below 10 °C.

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II. Preliminary phytochemical screening

III. Quantitative determination by Spectrophotometry

- Estimation of Total Phenolic Content
- Estimation of Total Tannin Content
- Estimation of Total Flavonoid Content

IV. Isolation of phytoconstituents

V.Structural elucidation by spectroscopic methods

VI. In vitro anti diabetic activity

1.Non-enzymatic glycosylation of haemoglobin method

2.Glucose uptake in Yeast cells

3.Alpha- Amylase inhibition assay

4. Glucose Adsorption Assay.

VII.Determination of acute toxicity

VIII. Screening of Alloxan induced anti diabetic activity and analysis of fallowing parameters

- Body weight of an animal.
- Biochemical parameters such as

a) Fasting blood glucose
b) Serum total cholesterol
c) Serum creatinine
d) Serum urea
e) Serum protein
f) Serum triglyceride
g) Serum HDL
h) Serum LDL
i) Hepatic glycogen estimation.

- Histopathological studies

II. Preliminary phytochemical screening

The obtained extract will be subjected to preliminary phytochemical screening following the standard procedures described in the practical Pharmacognosy by C.K. Kokate [17] and R.K. Khandelwal [18] results are summarized in table no.....

1. Detection of carbohydrates
2. Detection of proteins and amino acids
3. Detection of alkaloids
4. Detection of flavonoids
5. Detection of tannins
6. Detection of diterpenes.
7.Detection of steroids and triterpenoids
8. Detection of saponins
9. Detection of cardiac glycoside

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- **Keller-Kiliani's** test: A portion of dry extract is treated with 1mL of FeCl3 reagent (1 volume of 5% FeCl3 and 99 volume of glacial acetic acid). To this solution a few drops of concentrated H2SO4 is added. The presence of greenish blue color within a few minutes indicates the presence of desoxy sugar of cardiac glycosides.
- **Baljet's test**: To 1mL of the extract, 1mL of sodium picrate solution is added and the appearance of yellow to orange color reveals the presence of carbenolide

IV. Isolation of phytoconstituents and Structural elucidation by spectroscopic methods

GC-MS characterization of plants extracts

The GC-MS analyses were carried out in Shimadzu GC-MS QP2010 gas chromatograph fitted with DB1 capillary column ,carrier gas Helium with flow rate of 0.7mL\min, column oven temperature 70 °c,5 min 180 °c,5 min in 260°c and finally 5 min in 280°c, volume injected 1 microlt in n-hexane (2%) split ratio 3:0 the MS operating parameters were as fallows ionization potential 70ev; ion source temperature 200°c, solvent dealy 6.0min, scan speed 2000amu/s, the concentrated extract injected into GC-MS instrument the sample was volatilized at the injection port and eluted through a capillary column under increasing temperature

^{13}C and ^{1}H- Nuclear Magnetic Resonance (NMR)

The purified plant extract was dissolved in Chloroform-D and subjected to ^{13}C and ^{1}HNMRspectroscopic studies 500MHz (Bruker advance II). ^{13}C-spectra gives the information about various carbon functional groups while ^{1}H- NMR indicates the total number of protons associated with several groups.

In-vitro Antidiabetic activity

1.Non-enzymatic glycosylation of haemoglobin method [18]

In-vitro Antidiabetic activity of *Tephrosia villosa* (L.) Pers herb extract. Was investigated by estimating degree of non-enzymatic haemoglobin glycosylation measured colorimetrically at 520nm described by Chandrashekhar *et al*. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed.70% alcoholic extract of *Tephrosia villosa* (L.) Pers herb. was weighed and dissolved in DMSO to obtain stock solution and then 1-5 µg/ml solutions were prepared. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug for assay.

% inhibitionwas calculated as-% inhibition= A_S---A_C/00As #

Ac is Absorbance of Control

As is Absorbance of Sample

Statistical Analysis- All determinations were carried out in triplicates and data were expressed as mean +_ sem , all analysis were carried out using graph pad prisim 6 software significant at $p \leq 0.05$.

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Pharmacological activities.

TOXICITY STUDY

Acute oral toxicity study (OECD 423) [19]

Toxicity studies are carried out according to OECD guidelines 423 was followed. It is a stepwise procedure with three animals of a single sex per step. Depending on the mortality and/or, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked by morbidity of the animals a few steps may be necessary to judge the toxicity of the test substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data.

The method uses defined doses (5and classified according to the globally harmonized system. The starting dose for ethanolic extract was

2000mg/kg bodyweight (p.o). The dose was administered to the rats which were fasted overnight with water ad libitum and observed for signs of toxicity. The same dose was once again tried with another three rats and were observed for 72 hours for symptoms like change in skin colour,salivation, diarrhea, sleep, tremors, convulsions and also respiratory,autonomic and CNS effects.

Alloxan induced anti diabetic activity.[20-22]

Induction of diabetes

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6- pyrimidinetetrone) is an oxygenated pyrimidine derivative and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig (17). Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen, 2008). Alloxan monohydrate was obtained from S.D. Fine, Mumbai and all the other chemicals used were of analytical grade and were acquired from commercial sources.

The various groups used in experiment:

Group I - Served as normal control and did not receive any treatment.

Group II -Served as diabetic control and received alloxan monohydrate and vehicle Group III - Alloxan + Glibenclamide (10 mg/kg p.o.) and served as standard.

Group IV - Alloxan monohydrate + 70% EET VH(250 mg/kg, p.o.)

Group V - Alloxan monohydrate + 70% EETVH (500 mg/kg, p.o.)

Care of Diabetic Animals:

Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day and in some circumstances, more than once per day. Diabetic rats should have sufficient food and water.

Collection blood and serum samples:

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anesthesia on different occasion, i.e., 0, 4th, 7th and 10th day. The blood samples were allowed to clot

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for 30min at room temperature and then they were centrifuged at 3000 rpm for 10min. The resulting upper serum layer was collected in properly labeled, clean and dry micro-centrifuge tubes. The serum samples were stored at - 40° C and analyzed either immediately or within two weeks.

- The parameters studied were as follows:
- Body weight of an animal
- Biochemical parameters such as

j) Fasting blood glucose
k) Serum total cholesterol
l) Serum creatinine
m) Serum urea
n) Serum protein
o) Serum triglyceride
p) Serum HDL
q) Serum LDL
r) Hepatic glycogen estimation.

- Histopathological studies.

Table No.1 Phytochemical screening of *Tephrosia villosa* (L.)

Sl. No.	Solvent	Colour and Consistency	Percentage yield
1	Pet. Ether	Greenish black sticky	1.92%
2	Chloroform	Brownish black and sticky	3.95%
3	70% Ethanol	Brownish black and non sticky	18.31%
Types of Phytochemical constituents	Petroleum ether Extract	Chloroform Extract	70/ Alcoholic Extract
Alkaloids	+	+	+++
Carbohydrates	-	–	+++
Flavonoids	–	–	+++
Glycosides	-	-	+

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Tannins and Poly phenol	–	–	+++
Protein	-	-	+
Steroids	-	-	++
Saponin	-	–	+

Table No. 2 .:*In-vitro*Non-enzymatic glycosylation of haemoglobin method

S. No	Conc.n (µg/ml)	Blank	STD		EETVH	
			Abs	% inhibition	Abs	% Inhibition
1	100		0.104±0.002	69.23	0.109±0.002	70.64
2	200	0.032+0.001	0.120±0.002	73.33	0.125±0.001	74.40
3	300		0.127±0.002	74.80	0.137±0.001	76.64
4	400		0.135±0.003	76.29	0.150±0.001	78.66
5	500		0.141±0.001	77.30	0.159±0.002	79.87

STD- Standard, , Abs- Absorbance, % inh- % inhibition, Conc.n- Concentration EETVH-70% Alcoholic extract, Values are expressed as mean ± SEM.(N=3)

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Figure No.1

Fig No..... Non-enzymatic glycosylation of haemoglobin

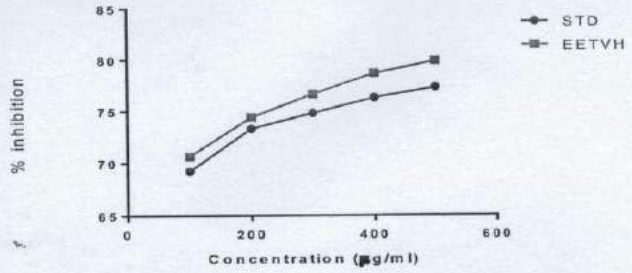

Table No. 3: Effect of 70% EETVH on body weight in alloxan induced diabetic rat

Groups	Dose (mg/kg)	Intial	*4th day*	*7th day*	*10th day*
		Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM
Control	Vehicle	199.4 ±2.017	202.0 ±1.713	203.0 ±1.183	209.5 ±2.741
Diabetic control	Alloxan(120mg/Kg)	201.5 ±3.423	181.7 ±2.076	156.8±1.222	151.8 ±2.480
standard	Alloxan(120mg/Kg) +Glibenclamide (10mg/KG)	199.0 ±1.932	198.3 ±1.978	192.2±2.372***	189.0 ±1.520**
Lower dose	Alloxan(120mg/Kg)+70%EETVH(250mg/kg)	202.3 ±2.512	194.3 ±2.011	183.3±1.783**	181.7 ±2.241**
Higher dose	Alloxan(120mg/Kg)+70%EETVH(500mg/kg)	199.7±2.155	197.7 ±2.155	188.8 ±1.783**	183.8 ±1.441**

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Figure 2

EFFECT OF 70% EETVH ON BODY WEIGHT(INITIAL BODY WT

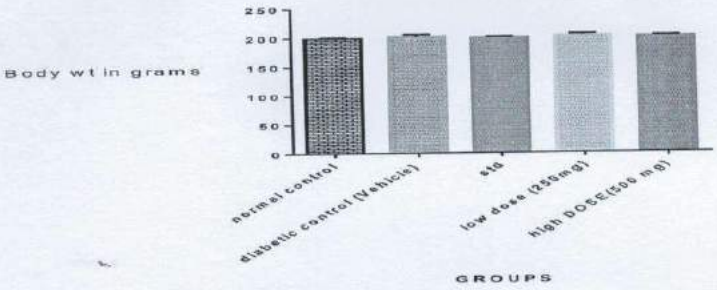

Figure 3

EFFECT OF 70% EETVH ON BODY WEIGHT (4TH DAY OF TREATMENT)

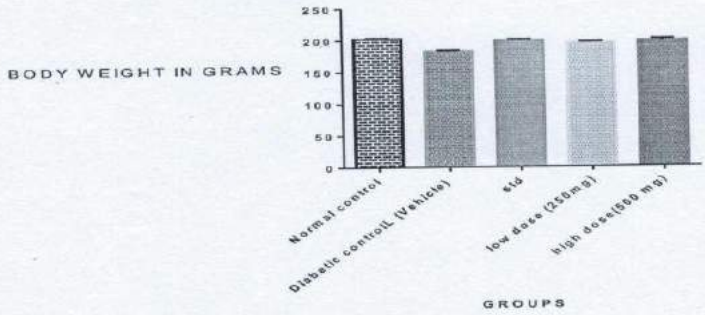

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Figure 4

EFFECT OF 70% EETVH ON BODY WEIGHT(7TH DAY OF TREATMENT)

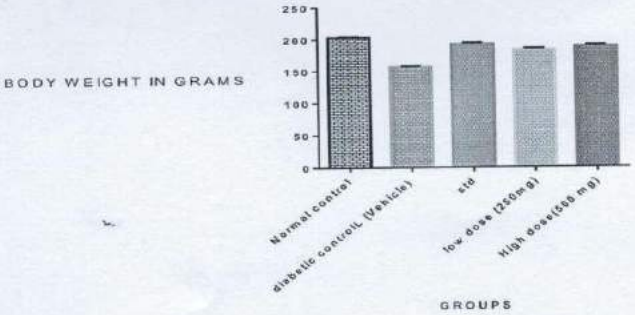
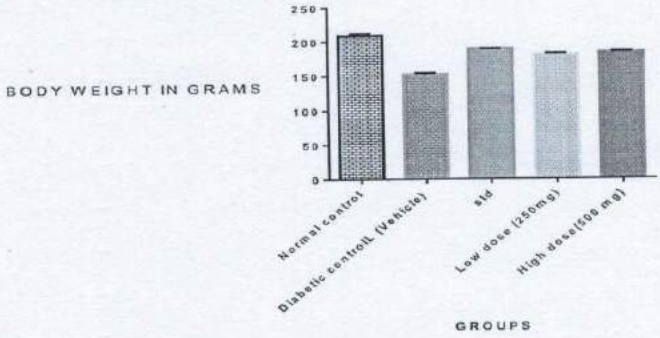


Figure 5

EEFECT OF 70% EETVH ON BODY WEIGHT(10TH DAY)



CONCLUSION

Diabetes mellitus. a major disorder in the world leading to massive economic losses. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and fed blood sugar levels in the human body. World health organization predicted that the

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developing countries will face major burden due to Diabetes. Studies conducted in India, highlighted that the prevalence of diabetes is high and also the rapid increase in the urban population.

Prolonged medications required for the treatment of diabetes mellitus, considering the limitations and side effect in the synthetic drugs, natural products derived from medicinal plants are being looked for the treatment of diabetes. Considering low toxic, ready availability and cost effective, usage of natural products for the treatment of diabetes mellitus has recommended and encouraged by WHO (World Health Organization) worldwide.

Plants have always been good source of drugs the ethnobatonical information reports many plants that may posses anti- diabetic potential *Tephrosia villosa* has a large distribution found in southern and eastern Africa, the Arabian Peninsula, across southern Asia and in south India and phytoconstituent anyless shown that the presence of polyphenols,Tannins,Alkaloids and Terpenoids in 70% alcoholic extract so it is selected.

Comp-1 was isolated from the plant extract and it was characterized and identified by structural elucidation In-vitro anti-diabetic studies shows that the plant extract posses good anti-diabetic property and its is conformed in the In-vivo anti-diabetic studies.

From the results and discussion it is concluded that the plant possess significant anti-diabetic property However, further research is required to elucidate the specific mode of action.

References

1.S.R.Paul, R.C.Gupta (1988) Pharmacobotonical studies on "Shevet Sharpunkha" – a comparative diagnostic account of *Tephrosia villosa per* and *T. Purpurea(linn).pers.*ancient science of life,vol no.VII Nos.3&4,January&April ,pages207-21.

2..Flora of the Presidency of Madras,J.S.Gamble vol.I published under the Authority of the Secretary of State for India in council London , Adlaed & Son,limited 21,Haet street, W.C.

https://doi.org/10.5962/bhl.title.21628

3. Orwa, C.; Mutua, A.; Kindt, R.; Jamnadass, R.; Anthony, S., (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya http://www.worldagroforestry.org/resources/databases/agroforestree

4.Soumith K Behra,Anima panda and Sushanth K Behra (2006) Indian jornal of Traditional knowledge vol 5(4), October, -p 519-528.

5.Prashanth p and Vidya sagar (2008) Indian journal of Traditional knowledge vol 7(2),April, 273-276.

6. Mirutse Giday, Zemede Asfaw, ZerihunWoldu (2009)Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study.J. Ethnopharmacol.

7.Raju sathiyaraju,Ariyan sarvalingam Arul balachandran,Rama koti redddy (2015) Diversity of Ethnomedical plants in Bodamali hills eastern ghats Namakkal district Tamil nadu.Journal of plant science 2015;3(2);77-84.

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INDORE INSTITUTE OF PHARMACY INDORE

Principal
Indore Institute of Pharmacy,
INDORE (M.P.)

8.Amit panday and Shwetha sing (2017) Ethnobatonical evidences of common wild medicinal herbs existing on Delhiridge a chick list Journal of medicinal plants studies 2017;5(5);46-60.
9. Jayaraman I, Ghanim A, Khan HA. (1980). A new prenylated flavanone [7-methylglabranin] from*Tephrosia villosa*. *Phytochemistry* 19.
10. Rao PP, Srimanarayana G. (1981) .Tephrinone—a new flavone from *Tephrosia villosa*. *Curr. Sci* 50,319-320.
11. Prashant A, Krupadanam GD. (1993). A new prenylated dehydrorotenoid from *Tephrosia villosa* seeds.*Journal of natural products* 56, 765-766.
12.Prashant A, Krupadanam GL. (1993). Dehydro-6-hydroxyrotenoid and lupenone from *Tephrosia villosa*. *Phytochemistry* 32, 484-486.
13. David Krupadanam GL, Sarma PN, Srimannarayana G, Subba Rao NV. (1977). New C-6 oxygenatedrotenoids from *Tephrosia villosa* -- villosin, villosone, villol and villinol *Tetrahedron Letters*18, 2125-2128.
14. Ganapaty S, Lakshminarayana K, Lakshmi P, Thomas PS. (2009). *Asian Journal of Chemistry* 21,
1007-1010.
15.Ganapaty S, Nyamathulla S, Srilakshmi GVK, Prasad KVNMR. (2008). Chemical and Antimicrobial Studies of the Roots of *Tephrosia villosa* (L) Pers.*Asian Journal of Chemistry* 20, 4498-4502.

16. Madhusudhana J, Reddy RN, Reddy BAK, Reddy MVB, Gunasekar D, Deville A, Bodo B. (2010).Two new geranyl flavanones from *Tephrosia villosa*. *Natural Product Research* 24, 743-749.
17..Ganapathy S,Nymathulla S, G V K Srilakshmi (2008) Chemical and antibacterial studies of roots of tephorsia villosa (l)per.Asian journal of chemistry vol 20,No 6 4498-4502 (2008
18.Ramadhani S O Nando, Zakario H Mbmawbo(2011) BMC Complementary and alternative medicine 2011
19.Sufiyan ahmed . B R Balakrishanan (2009),Anti diabetic activity of leaves of Tephrosia villosa pers.in alloxan induced diabetic rats Journal of pharmacy Research vol 2,Issue 3 528-532,
20.D K Patel, R Kumar, D Haloo(2012),Diabetes mellitus :An overview on its pharmacological aspects and reported medicinal plants having ant diabetic ,Asian journal of Tropical biomedicine,may;2(5) pp 411-420.
21.Varaprasad Bobbrala, Chandrashekhara K naidu(2012) Alternative approaches for the control of Sorgham pathogens using selected medicinal plants, Intech open acess book,
22..Odhong c, R G Wahane,Vaarst,Nalubwana(2014), In vitro anthelmentic effect of crude aquous extracts Tephrosia vogeli, Tephrosia villosa and carica papaya leaves and seeds African journal of Biotechnology, vol 13 (52),pp 4467-4672,20

Eur. Chem. Bull. 2023, 12(Special Issue 4), 15966-15981

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INDORE INSTITUTE OF PHARMACY INDORE

Principal
Indore Institute of Pharmacy.
INDORE (M.P.)

Section A-Research paper
ISSN 2063-5346

23..Aparna surya mani,Yejela Rajendra Prasad(2005), Phytochemical and antioxidant activity and screening of chloroform leaf and aerial parts extract of Tephorsia villosa .
24..A Samsath begum, A Jamal abdual Nasser(2017), Corrosion inhibition by aqueous extract of Tephorsia villosa leaves. World journal of Pharmaceutical research.vol 6, issue 17,1072-1100,

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RESEARCH ARTICLE

Formulation Development and Evaluation of Herbal Tablet of *Diplocyclos palmatus* (L.) Jeffry.

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ABSTRACT

Diplocyclos palmatus (L.) Jeffry. (Shivlingi), is a plant in the Cucurbitaceae family that has been utilized extensively in indigenous medicine due to its purported biological properties. The goal of the current study was to formulate herbal tablets for the treatment of inflammation that contained a hydro-alcoholic extract of *D. palmatus* (L.) Jeffry. The formulated herbal tablet underwent IP evaluation, and all the results were found satisfactory.

Keywords: *Diplocyclos palmatus* (L.) Jeffry., Flowers, Inflammation.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.3.09

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Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Diplocyclos palmatus, also referred to as shivalingi, is an annual herbaceous climber that may reach heights of 3 to 4 metres and is a member of the Cucurbitaceae family. The herb is used by different Indian tribal cultures to treat a variety of illnesses and ailments.[1] Living mammalian tissues locally react by inflaming the injured area. It is a defense mechanism used by the body to stop or slow the spread of harmful chemicals. A variety of factors can cause the symptoms and tissue damage that go along with an inflammatory response. Such elements include the creation of granulomas, leukocyte infiltration, and edoema.[2]

The tribes and local people use the plant for the treatment of inflammation, therefore it was selected.

MATERIAL AND METHODS

Plant Material

Flowers of *D. palmatus*

Extract

Hydro-alcoholic extract was taken.

Formulation Development of herbal tablet

HAEDPF and various excipients as mentioned in Table 1 were taken (all in mg) and mixed properly after passed from mesh.

Table 1: Formulation design and composition of ingredients

Components	FC							
	PHTI	PHTII	PHTIII	PHTIV	PHTV	PHTVI	PHTVII	PHTVIII
HAE	100	100	100	100	100	100	100	100
SD Lactose	117	120	123	126	117	120	123	126
Talc	15	15	15	15	0	0	0	0
Starch (Potato)	18	15	12	9	18	15	12	9
Mg Sterate	0	0	0	0	15	15	15	15

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Table 2: Assessment of prepared formulation

Specification	*HAEDPF*							
	PHTI	*PHTII*	*PHTIII*	*PHTIV*	*PHTV*	*PHTVI*	*PHTVII*	*PHTVIII*
Strength (kg/cm^2)	5.2	4.9	4.8	5.0	4.4	4.8	4.9	5.1
Friability	0.71	0.73	0.67	0.69	0.50	0.55	0.72	0.81
Weight variation	± 4.9	± 4.83	± 4.99	± 4.53	± 4.32	± 4.91	± 5.01	± 5.10
DT	22.10	25.20	30.15	45.15	12.20	18.30	22.10	30.15
DC	93.29	94.10	95.89	96.96	97.01	96.30	96.01	94.29

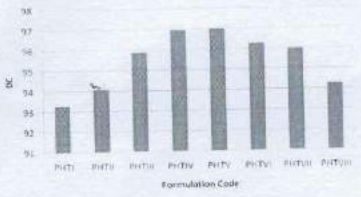

Figure 1: Drug content of herbal tablet

The powder mixtures were directly compressed using tablet punching machine using 10 mm flat shaped punch.[3-5]

Assessment parameters[6]

The formulated tablet was assessed for organoleptic properties, hardness, friability, weight variation, DT, drug content per the procedure mentioned in IP.

RESULTS AND DISCUSSION

The herbal tablet was prepared in eight batches, i.e., HT-1 to HT-8, and was assessed for its various parameters. Results showed that there were no any defects reported in all the prepared batches. The batch's color was brown, the odor was pleasant, and the taste was acceptable. The detailed results are mentioned in Table 2 and Figure 1.

CONCLUSION

In conclusion as per the results received it was revealed that the HT-5 has maximum drug content (97.01%), also the disintegration time of HT-5 is 12.20 minutes. Hence it was concluded that the HT-5 have found to be most suitable formulation and may be further investigated for anti-inflammatory activity to proven its efficacy.

REFERENCES

1. Dwivedi S. Status survey of medicinal plants wealth of Malwa region of Madhya Pradesh with special reference to conservation of vulnerable and endangered species. J. Econ. Taxon. Bot., 2009; 33(2): 443-452.
2. Mitchell RN, Cotran RS. In: Robinsons Basic Pathology, ed 7. Harcourt Pvt. Ltd., New Delhi, India, 2000; 33-42.
3. Lachman L., Liberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy, 3rd ed., Varghese publishing House Bombay, 1999; 443-453.
4. Mishra US, Murthy PN, Pasa G, Mishra D. Formulation development and evaluation of herbal tablet containing methanolic extract of Butea frondosa, Int. J. of Inst. Pharm. and Life Sci., 2011; 1(3):1-15.
5. Subrahmanyam CVS. Text Book of Physical Pharmaceutics, 2nd ed. New Delhi, Vallabh Prakashan, 2001; 253-261.
6. Aulton ME. Pharmaceutics: The Science of Dosage Form Design. 2nd ed. Churchill Livingstone, London, 2002; 322-334.

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REISHI (*GANODERMA LUCIDUM*): A POTENTIAL SOURCE OF NUTRACEUTICALS AND PREBIOTICS

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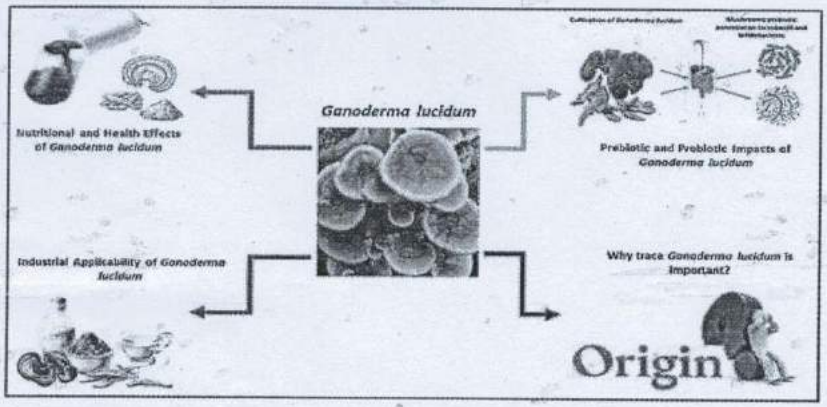
Graphical Abstract



Background:
In Asian nations, ganoderma lucidum, often known as lingzhi, has been used to generally promote health and lifespan. With high-quality proteins, amino acids, nucleosides, vitamins, critical minerals, and dietary fibres (quitin, polysaccharides), ganoderma lucidum is a highly nutritious, low-calorie food. A significant and underutilised source of nutraceuticals and prebiotics is ganoderma lucidum. The development of mushrooms is beneficial to the food, pharmaceutical, and nutraceuticals industries in developing nations like China, Japan, Korea, and India. Ganoderma lucidum has long been regarded as the best nutritious meal for obese people and for diabetics to prevent hyperglycemia due to its high fibre, low fat, and low starch content. It is mostly made up of dietary fibres and polysaccharides that encourage the growth of good bacteria. It is mostly made up of dietary fibres and polysaccharides that encourage the formation of good bacteria in the digestive tract. They are maintaining intestinal health, preventing cancer, enhancing immunity, lowering cholesterol, and preventing obesity, among other positive effects. Its potential as a nutraceutical and prebiotic is attributed to the presence of a wide range of phytoconstituents, including tocopherols, phenolics, flavonoids, carotenoids, dietary fibres, ascorbic acid, selenium, germanium, luecine, lysine, adenosine, and uracil. There is no proof of liver, kidney, or DNA toxicity with consumption of Linngzhi, according to the toxicity study on human clinical trial.

Present overview:
The current research highlights the urgent need for the scientific community to promote Ganoderma lucidum as a potential source of nutraceutical and prebiotic products. To characterise the active component (s) of this alleged medicinal mushroom, as well as to discover mechanisms of action,

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Current Medicinal Chemistry, XXXX, XX, 1-00

REVIEW ARTICLE

Nanomedicine: Innovative Strategies and Recent Advances in Targeted Cancer Therapy

Rupesh K. Gautam[1,2,#,*], Pooja Mittal[3,#], Rajat Goyal[4], Kamal Dua[5], Dinesh Kumar Mishra[2], Sanjay Sharma[6] and Rajeev Kumar Singla[1,7,*]

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Abstract: Nanomedicine's application of nanotechnology in medicine holds tremendous potential for diagnosing and treating life-threatening diseases such as cancer. Unlike conventional therapies, nanomedicine offers a promising strategy to enhance clinical outcomes while minimizing severe side effects. The principle of drug targeting enables specific delivery of therapeutic agents to their intended sites, making it a more precise and effective therapy. Combination strategies, such as the co-delivery of chemotherapeutic drugs with nucleic acids or receptor-specific molecules, are being employed to enhance therapeutic outcomes. Nanocarriers and drug delivery systems designed using these approaches offer resourceful co-delivery of therapeutic agents for anticancer therapy. Targeted drug delivery *via* nanotechnology-based techniques has become an urgent need and has shown significant improvements in therapeutic implications, pharmacokinetics, specificity, reduced toxicity, and biocompatibility. This review discusses the extrapolation of nanomaterials for developing innovative and novel drug delivery systems for effective anticancer therapy. Additionally, we explore the role of nanotechnology-based concepts in drug delivery research.

Keywords: Nanomedicine, nanotechnology, cancer, apoptosis, cell proliferation, drug delivery, nanoparticles.

1. INTRODUCTION

Cancer is the second primary root of death worldwide after cardiovascular disorders, according to the current report of WHO. It accounts for an average of 1 in 6 deaths. The number of incidences is increasing daily and is expected to rise by 70% in the next two decades. Recently, the data shared by GLOBOCAN has shown that cancer cases have risen to 19.3 million, while 10 million cancer deaths were registered worldwide in 2020. Cancer can impact any of the sites in the body through its uncontrolled growth. In men, the five most common cancer sites were identified in 2012: lungs, colorectal, prostate, liver, and stomach. The most common cancer types in females are breast, colorectal, stomach, cervix, and lungs [1, 2]. Cancer is a highly complex, multi-factorial, multigenic, and multiple-pathway disease that endangers human health and existence. It greatly strains the healthcare system and is the world's second leading cause of death. Cancer is a dangerous, life-threatening disease with one of the most formidable afflictions in the world, and its early

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Review Article

Research progress of small-molecule drugs in targeting telomerase in human cancer and aging

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Abstract

Telomeres are unique structures located at the ends of linear chromosomes, responsible for stabilizing chromosomal structures. They are synthesized by telomerase, a reverse transcriptase ribonucleoprotein complex. Telomerase activity is generally absent in human somatic cells, except in stem cells and germ cells. Every time a cell divides, the telomere sequence is shortened, eventually leading to replicative senescence and cell apoptosis when the telomeres reach a critical limit. However, most human cancer cells exhibit increased telomerase activity, allowing them to divide continuously. The importance of telomerase in cancer and aging has made developing drugs targeting telomerase a focus of research. Such drugs can inhibit cancer cell growth and delay aging by enhancing telomerase activity in telomere-related syndromes or diseases. This review provides an overview of telomeres, telomerase, and their regulation in cancer and aging, and highlights small-molecule drugs targeting telomerase in these fields.

Section snippets

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Telomeres and telomerase

Telomeres are nucleoprotein structures located at the end of chromosomes that prevent genomic instability and gene erosion. Human telomeres are made up of repeating sequences (TTAGGG) that can range in length from 2 to 15 kilobases [1,2]. Telomeric repeat binding factor 1 (TRF1), telomeric repeat binding factor 2 (TRF2), protection of telomeres 1 homolog (POT1), rhoptry-associated protein 1 (RAP1), TRF1-interacting nuclear protein 2 (TIN2), and tripeptidyl peptidase 1 (TPP1) interact to form...

Indore (Affiliated to RGPV Bhopal)
Sanjay Sharma, Assistant Professor, Pharmaceutics Department, Indore Institute of Pharmacy, (Affiliated to RGPV Bhopal)
Jhansi Kumar Mishra, , Principal , Indore Institute of Pharmacy, Indore (Affiliated to RGPV Bhopal)
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Abstract:
An organism contamination is the foremost common worldwide skin wellbeing issue. They are as a rule treated with topical or systemic antifungal treatment. Topical treatment is as a rule favored due to its focused-on treatment and less side impacts. Due to their one of kind basic and utilitarian characteristics, progressed topical carriers overcome numerous biopharmaceutical challenges related with ordinary medicate conveyance frameworks like destitute maintenance and bioavailability. Prave from writing recommends topical antifungal nanocarriers with anti-fungal specialists give predominant comes about with negligible side impacts. Distinctive sorts of nanocarriers are broadly utilized for topical antifungal pharmaceutical, counting solid-lipid nanoparticles, Microemulsions, Liposomes, Niosomes, Microsponge, Nanogel, Nanoemulsion, Micelles etc. In this article we summarize later progresses in topical carriers that are utilized to advance the helpful adequacy of hostile to parasitic drugs.

Keywords: Nanocarriers, Antifungal, Nano particles, Nanomaterials, Infectious disease, Pharmaceutical science.

1. Introduction:

One of the most common skin diseases worldwide is fungus infection. In developing and underdeveloped nations, there are an estimated 40 million people who have fungal infections. Both superficial and invasive fungal infections are possible. While invasive fungal infections are less common but have high rates of morbidity and mobility, superficial infections are more common and affect 25-30% of the population. [1-4] Initially attacking the skin's surface, fungi then spread to the deeper layers by desquamation. The fungi that cause the most superficial dermal infections include the Candida species. [5-6] Every year, about 7% of all infected patients in India pass away. An estimated 15-20% of patients have ongoing or recurrent fungal infections. [6-7] Antifungals can be divided into five major groups: polyenes, azoles, allylamines, echinocandins, and antimetabolites. Within the confines of their annular structure of macrolides, polyenes have a double bond conjugated variable. Pimaricin, amphotericin B, and nystatin are members of the anti-fungal genus. Amphotericin B alters the permeability of cell membranes by forming an ergosterol complex, which causes cell content leakage and ultimately cell demise. Azoles are made up of two or three nitrogen molecules that divide their five chemical cycles into imidazole and triazole, respectively. The 14 demethylation of lanosterol, a crucial step in the biosynthesis of ergosterol, is the mode of action of azoles. Morphine and terbinafine are the most well-liked allylamine family members. Allylamines prevent the conversion of squalene into squalene epoxide via squalene epoxidase, which is the biosynthetic route of ergosterol, a substance that makes up the fungal cell membrane. Caspofungin, micafungin, and anidulafungin are a few echinocandin examples. [8] In the area of medicine, nanotechnology is a potent tool for combating a wide range of diseases, including cancer and cardiovascular diseases. Therefore, it is not remarkable that tiny

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Current Traditional Medicine, XXXX, XX, XXXXXXX

REVIEW ARTICLE

Phytochemical, Ethnobotanical, and Global Perspectives of Genus *Echinacea*: A Panoramic Review

Disha Arora[1], Smriti Parashar[2], Rupesh K. Gautam[3,*] and Rajat Goyal[4]

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Abstract: Nature has always been a wonderful supply of therapeutic substances, providing us with a range of medicinal plants that produce helpful phytochemicals. The native species of the genus Echinacea, which are found in North America and are well-known among medicinal plants, are members of the Asteraceae family. Though there are nine different species of echinacea, only three *Echinacea angustifolia* DC, *Echinacea purpurea* (L.) Moench, and *Echinacea pallida* (Nutt.)-are utilized as medicinal herbs with a variety of therapeutic uses. Contrary to other plant families, the Asteraceae family of plants is one of the most well-known and renowned, and as a result, many of its members have been employed for therapeutic purposes. The availability of substances with a variety of medicinal characteristics is to blame for this. This review has included the investigation of the morphological traits, ethnopharmacology, and diverse pharmacological properties of the Echinacea genus. The chemistry of the genus is extensively understood, and various chemical component groups-including alkamides and caffeic acid derivatives-are believed to be crucial for activity.

Keywords: *Echinacea*, alkamides, caffeic acid derivatives, immunostimulants, coneflower and plants.

1. INTRODUCTION

Echinacea, a genus of American coneflowers (Asteraceae/Compositae) is an herbaceous perennial, native to North America are distributed throughout the eastern and central U.S. and southern Canada. The conventional taxonomic system recently supported by chloroplast genome data recognizes nine species within the genus [1-3]. North Americans used *Echinacea* species for wounds, pain, and throat infections, and in olden times (Eclectic medicine), used for septic conditions. The commercialized species of the genus have acknowledged widespread research awareness, whilst others have established roughly none [4].

1.1. Distinguishing Characteristics of Echinacea Species

The *Echinacea* genus is characterized by spiny flowering heads, with an elevated receptacle that forms the "cone". Fig. (1) and Table 1 show different species of the genus *Echinacea*.

1.1.1. E. angustifolia

Stems are 10-50 cm tall, and covered with rough thick hairs. The leaves are oblong and lanceolate with entire leaf margins; the ray flowers are very short (2-4 cm long). It is found on barren, dry prairies, and thin soils [5].

1.1.2. E. atrorubens

Stems are 30-90 cm tall, light green, hairy, and simple or rarely branched. Leaves are lanceolate and entire, often smooth. Short ray flowers (2-4 cm long) curve down to touch the stalk. The petals are dark purple, occasionally pink, or white. It grows on prairies [6].

1.1.3. E. laevigata

Stems are 50-100 cm tall and rarely branched. The leaves are ovate and sometimes serrated. The bristles of the central cone are only a quarter as long as the main part of the cone and have flexible curved tips. The ray flowers are three to ten times longer than wide. It grows in open woods and grasslands [7].

1.1.4. E. pallida

Stems are 40-90 cm tall. The leaves are lanceolate with entire margins. This is the only Echinacea with white pollen. The ray flowers are 4-9 cm, narrow, droop, and curve towards the stem. The ray flowers are usually white to pink but can very occasionally be deep purple. This plant occurs in woods, grasslands, and rocky prairies [8].

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Review on Nanosuspension as a Novel Drug Delivery System for the Treatment of Fungal Infection

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Abstract:

The current article cites the significance of promising future prospects of nanotechnology in pharmaceutical research is a novel concept. Nanosuspension, which is a part of Nanotechnology, the term "nanosuspension" refers to very small, colloid, biphasic, dispersed solid drug particles in an aqueous medium, stabilized by surfactants and polymers, manufactured according to appropriate techniques for drug delivery applications. Nanosuspension enhance the bioavailability and effective for hydrophobic drug molecules is an attractive and promising approach for dealing with the low solubility and bioavailability. The unique feature of nanosuspension incorporated for treatment of the fungal infection. In this review article we focus on preparation of nanosuspension through various techniques with their advantages and disadvantages, formulation considerations and their application in drug delivery of antifungal drug.

Keywords: Nanotechnology, Nanosuspension, Biopharmaceutical classification system, Solubility.

1. Introduction

The name itself implies fungus invading into the body, leading to serious fungal infection, which not only effect the body but also hinders the immune system. Some of the examples are; athlete's foot, jock itch, yeast infection, ringworm. Onychomycosis, tinea capitis, tinea corporis, tinea barbae, tinea pedis, and candidiasis of the skin, mucosa, and nails are examples of cutaneous fungal infections. Subcutaneous fungal infections include mycetoma, sporotrichosis, and chromoblastomycosis. Systemic fungal infections consist of North American blastomycosis and cryptococcosis. Infections with a pathogen that are limited to the stratum corneum (SC) and cause a slight to no tissue reaction are known as superficial fungal infections. Cutaneous and superficial infections are both frequently referred to as dermatophytoses [1, 2]. The most common categories of topical antifungal drugs are polyenes (nystatin, amphotericin B), azoles (clotrimazole, ketoconazole, fluconazole), alkilamines/benzilamines (naftine, butenafine), and others (griseofulvine, selenium sulphide).

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Due to their chronic and persistent character as well as the low concentrations of the medications in the SC following oral treatment, fungus infections of the skin can be challenging to treat. Because of this, systemic treatment has typically involved providing high dosages of antifungal, which enhances the probability of side effects. The commonly used systemic antifungal treatments for many years have been amphotericin B, flucytosine, itraconazole, and ketoconazole; however, medicine toxicity, the development of resistant strains, and low efficacy has limited their application clinically [3]. The formulation of pharmaceuticals successfully depends on a number of features, such as solubility, stability at room temperature, compatible with solvent, additives, and photo - stability. Roughly 40% of the novel chemical develop to date by drug discovery programmes are lipophilic or have poor water solubility [4, 5]. There are multiple formulation strategies available to address the issues of low medication solubility and bioavailability. The traditional methods include micronization, the use of fatty solutions, the use of penetration enhancers or cosolvents, the surfactant dispersion method, salt formation, precipitation, etc. However, the effectiveness of these methods in improving solubility for poorly soluble drugs is still limited. The use of vesicular systems like liposomes, dispersion of solids, emulsion and microemulsion techniques, and inclusion complexes with cyclodextrins are other ways that provide good results drug delivery system, but one of these methods' main downfalls is the fact that they don't work with all medications [6]. For pharmaceutical purposes, nanoscale design has been explored and reported over the past few decades [7]. Nanotechnology can be utilised to address the issues with the various prior techniques. Nanotechnology is the study of science and engineering at a level of 10-9 metres. Techniques like Bottom-Up Technology and Top-Down Technology are used to modify the drug microparticles/micronized drug powder into drug nanoparticles [8]. Nanosuspensions are colloidal dispersions of medication particles that have been nanoscale in size and are preserved by surfactants [9]. Nanosuspensions are suspensions made up only of the medication, which is weakly water soluble [10]. They can be used to optimize the drug's solubility if it is poorly soluble in lipid or water-based systems. A higher rate of influx of the active compound and a fast climbing to the maximum plasma level are both effects of enhanced solubility. Molecules with weak solubility, poor permeability, or both can advantage from this strategy, which creates a significant challenge for formulators. Because of the smaller particle size, it is now possible to provide poorly soluble medications locally without concerns over blood capillary blockage. The suspensions can also be turned into a solid matrix by emulsification. In addition to these benefits, liquid formulations have an advantage over others [11]. We are mainly concentrate on the various preparation methods' benefits, demerits, and pharmaceutical applications as drug delivery systems.

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1.1 Need of Nanosuspension for Solubility Enhancement

However, pharmacokinetic investigations of BCS class-II drugs showed that they have a limited oral bioavailability, which may be caused by the drug's poor water solubility. The synthesis of ß-cyclodextrin complexes, solid dispersions, and drug salt form are only a few of the traditional pharmaceutical methods used to increase drug dissolving rates. To speed up drug breakdown, a novel method that reduces drug particle size has been developed over the past 20 years [12]. The Noyes-Whitney equation implies that medications with smaller particle sizes have more surface area, which increases the rate of dissolution. The oral bioavailability of medications may be further increased by increasing the dissolution rate and the concentration gradient between the gastrointestinal lumen and systemic-circulation that results from it. A surfactant-stabilized submicron colloidal dispersion of drug particles is referred to as a nanosuspension [13]. Ultra finely dispersed solid drug particles in an aqueous medium are referred to as pharmaceutical nanosuspension and are targeted for oral, topical, parenteral, or pulmonary delivery. The solid particles in nanosuspensions usually have a particle size distribution that is less than one micron, with an average particle size around 200 and 600 nm. In nanosuspension technology, the drug is kept in the necessary crystalline state with smaller particles, resulting in a faster rate of dissolution and, consequently, better bioavailability and solubility [14].

1.2 Advantages of Nanosuspension

1. Suitable For Poorly Water Soluble Drug
2. Higher Physical Stability than Liposomes
3. Cost Efficient
4. Decreases Tissue Sensitivity
5. Improves Dosing Frequency[15]

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1.3 Classification of Antifungal

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Class Of Drug	Mechanism Of action	Example
Azoles	It prevents cytochrome P450 14a-dimethylase (P45014dm) from functioning. This is a phase in the process of sterol biosynthesis that connects lanosterol with ergosterol.	Ketoconazol
Antimetabolite	It prevents the synthesis of fungal proteins by substituting 5-flurouracil for uracil in fungal RNA. Also, it interacts with fungal DNA and hinders the synthesis of thymidine by replacing 5-flurodeoxy-uridine for it.	Flucytosin
Polyenes	It is known to act by connecting to ergosterol in the membrane of fungal cells. As a result of this interaction, the membrane depolarizes, holes form, increasing the permeability of proteins, and monovalent and divalent charges form, which results in cell death.	Amphotericin B
Allyamines	By blocking the epoxidase enzyme, it prevents the formation of ergosterol.	Terbinafin

Table 1. Classification of Antifungal

2. Method of Preparation

There are mainly two ways to make nanosuspension. The name "Bottom-up technology" means the conventional method of precipitation and the other one is Top Down technology [16]. The drug is dissolved in a solvent in bottom-up technology, and then solvent is added to a nonsolvent to precipitate the crystals. This process requires regulating the growth of the drug crystals during the precipitation process by adding surfactant to avoid the growth of microparticles.

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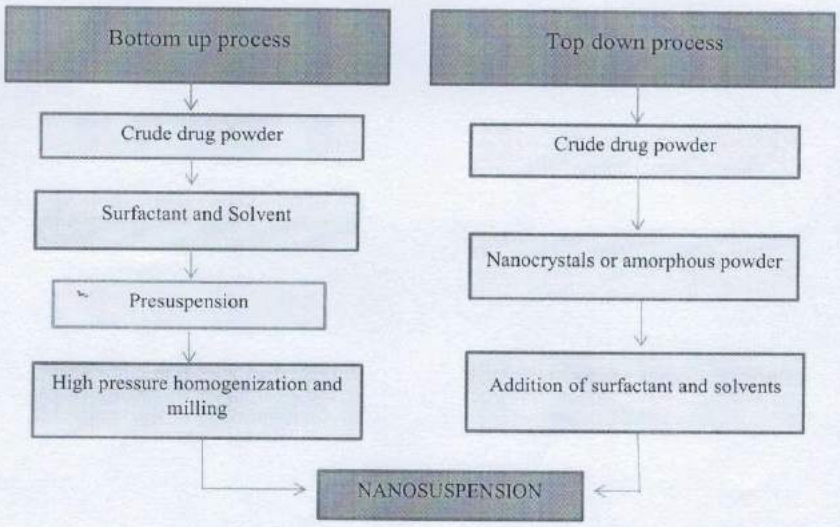

Table 2. Preparation of Nanosuspension

Media milling, high pressure homogenization in non-aqueous media, high pressure homogenization in water, and the combination of high pressure homogenization and precipitation are some of the top-down technologies.

Solvent-Antisolvent method, super critical fluid process, Emulsification Solvent evaporation technique, Lipid emulsion/Micro-emulsion template are some of the bottom up technologies [17]

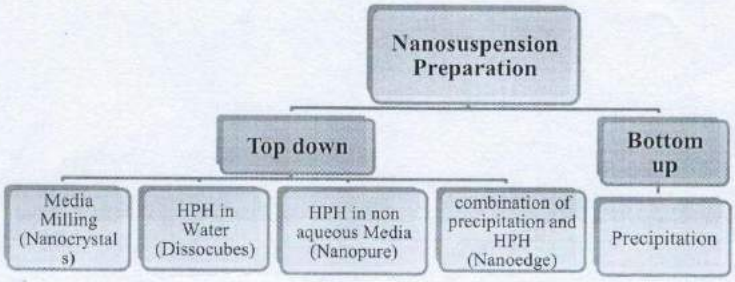

Figure 1. Method of Preparation of Nanosuspension

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2.1 Top Down

2.1.1 Media Milling:

Either pearl mills or high-shear media mills, nanosuspensions can be produced. The milling chamber, recirculation chamber, and milling shaft make up this component. Balls or pearls formed of ceramic sintered zirconium oxide or aluminium oxide are used as milling media. Charged with milling media, water, dosages, and stabilizers. The sample is impacted by the rotation of the balls at a high shear rate at constant temperature. Particle size is reduced as a result of both friction and impact forces, and Nano sized particles are produced [18].

Advantages

1) The distribution of the final nanoscale product.

2) It is simple to create nanosuspensions out of medications that are not very soluble in organic or aqueous media.

3) Simple scale up and minimum batch-to-batch variability.

Disadvantages

1) The media milling method takes a lot of time.

2) Some of the particle population comes within the micrometre range.

3) Due to the size and weight of the mill, scale up can be tough [19].

2.1.2 High Pressure Homogenization

2.1.2.1 Homogenization in aqueous media (Dissocubes):

This method involves applying high pressure to a tight valve to drive a suspension through it. The static pressure will reduce below the boiling point of water when the suspension is allowed to pass through the orifice, causing the water to boil and create gas bubbles. Bubbles will implode when the pressure returns to normal after it leaves the orifice. As result, surrounding particles will rush to the surface, which leads to a size reduction. The apvgaulin micron lab 40 homogenizer uses this technique [20].

Advantages

1) It prevents the erosion of materials that have been evaluated.

2) It is able to use for drugs that are poorly soluble in both aqueous and organic media.

Disadvantages

1) The drug must undergo pre-processing, such as micronization.

2) The price of the dosage form is increased by the need for expensive instruments [21]

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2.1.2.2 Homogenization in non-aqueous media (Nanopure):

It is homogenised in a water-free or water-mixed medium. It will be below zero or even at the freezing point. Thus, deep freeze homogenization is the name used for it. For thermolabile compounds, it is the most effective approach [22].

2.1.2.3 Nanoedge

The homogenization process or the precipitation approach will be similar to this process. It is considered that combination of these two methods delivers more stability and bioavailability.

The solution developed by this technique will be homogenised again to reduce particle size and inhibit crystal formation. There is a possibility of crystal development and issues with long-term stability in the precipitation process. Such problems will be solved through nanotechnology. The nano edge technology further includes an evaporation method for better nanosuspension development, leading to modified starting materials that are solvent-free.

2.2 Bottom Up

2.2.1 Precipitation (Solvent- antisolvent Method):

Submicron particles have been prepared using the precipitation process for many years. It is mostly used for drugs that are poorly soluble. In a suitable solvent, the first medication is dissolved. In the presence of surfactants, this solution is subsequently mixed with a miscible anti-solvent system. Rapid addition of the drug solution to the anti-solvent causes the drug to rapidly become supersaturated in the mixed solution, which leads to the formation of ultrafine drug solids. The two phases of the precipitation method are crystal growth and nuclei production. It is essential to have a high nucleation rate but a low growth rate while producing a stable suspension with the least possible particle size. The temperature affects both rates. Drug solubility in at least one solvent that is miscible with non-solvent is required for this method [23].

Advantages

1) Ease of scaling up
2) Simple procedure
3) Economic output.

Disadvantages

1) Crystal growth needs to be restricted by the addition of surfactants.
2) The material must be soluble in at least one solvent [24].

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2.2.1.1 Supercritical Fluid Process:

Through super critical fluid process, this technique uses solubilisation and Nano sizing technologies to reduce particle size. The term "super critical fluid" (SCF) refers to noncondensable dense fluids whose critical temperature (Tc) and critical pressure (Tp) are higher. Drug particles can be micronized to submicron levels using this method. Recent advances in the SCF technique allow for the production of nanoparticulate suspension with particle sizes ranging from 5 to 2000 nm. The pharmaceutical industry is unable to utilize this technique due to the low solubility of poorly water-soluble drugs and surfactants in supercritical CO2 and the high pressure needed for these procedures [25].

2.2.1.3 Melt Emulsification Method:

In this technique, the drug is mixed with the stabilizer's aqueous solution, heated above the drug's melting point, and homogenised to produce an emulsion. The sample holder was encased in a heating tape with a temperature controller during this procedure, and the temperature of the emulsion remained above the drug's melting point. The emulsion was then slowly cooled to room temperature or put in an ice bath.

Advantages

1) When using the melt emulsification method, organic solvents are completely avoided during the production process in compare to the solvent evaporation method.

Disadvantages

1) Larger particles develop and there are fewer compliant products than solvent evaporation [26].

2.2.1.4 Lipid Emulsion/ Microemulsion Template:

The majority of drugs that can be soluble by this method include those that can be dissolved in partially water miscible solvents or volatile organic solvents. This process involves dissolving the drug in a suitable organic solvent, then emulsifying it with a suitable surfactant in an aqueous phase. The organic solvent was then gradually evaporated under decreased pressure to produce drug particles that precipitated in the aqueous phase and formed the required particle size for the aqueous suspension. The produced suspension can then be suitably diluted to produce nanosuspensions. Also, nanosuspensions can be produced using microemulsion as templates. Microemulsions are isotopically transparent dispersions of two immiscible liquids, such as oil and water, which are thermodynamically stable and held up by an interfacial coating of surfactant and co-surfactant. Either the drug can be deeply mixed into the pre-formed microemulsion or it can be loaded into the internal phase. The drug nanosuspension is formed by suitably dilute the microemulsion. Lipid emulsions have the benefit of being simple to scale up and easy to produce when applied as templates for the production of nanosuspension. However, the use of organic solvents has an impact on the environment and leading to the use of large amounts of surfactant or stabilizer [27]

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Advantages

1) Superior drug solubilisation
2) It has a long shelf life
3) It is simple to make

Disadvantages

1) Using an unstable solvent
2) Using large amounts of stabilizers and surfactant [27].

2.2.1.5 Solvent Evaporation Technique:

The polymer solutions are made in emulsions and volatile solvents for the solvent evaporation process. However, dichloromethane and chloroform, which were previously utilized, have since been replaced by ethyl acetate, which has a better toxicological profile. On the evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion, the emulsion turns into a suspension of nanoparticles. The manufacture of single-emulsions, such as oil-in-water (o/w) or double-emulsions, such as (water-in-oil)-in-water, (w/o)/w, are the two basic ways of producing emulsions in conventional ways. In order to evaporate the solvent using one of these techniques, high-speed homogenization or ultrasonication must first be performed. This solvent needs to be continuously stirred magnetically at room temperature or under reduced pressure. The collected solidified nanoparticles after ultracentrifugation were lyophilized after being sterilized with distilled water to get eliminate of any additions like surfactants. The polymer concentration, stabilizer concentration, and homogenizer speed all had an impact on particle size [28].

3. Formulation Considerations

3.1 **Stabilizers:** The stabiliser plays an essential role in the formation of nanosuspensions. The high surface energy of nanosized particles can cause the drug crystals to aggregate or stick together in the absence of a suitable stabiliser. A stabilizer's main functions are properly wetting the drug particles, avoiding Ostwald's ripening and reducing nanosuspension agglomeration in order to provide a physically stable formulation by establishing steric or ionic barriers. The type and dose of the stabiliser significantly affects the in vivo behaviour and physical stability of nanosuspensions. In certain circumstances, a combination of stabilisers is required to produce a stable nanosuspension [29].

3.2 **Organic Solvents:** When nanosuspensions are to be developed using an emulsion or microemulsion as a template, organic solvents may be used in the formulation. Due to the early form of these methods, detailed information on formulation considerations is not yet available. When developing nanosuspensions using emulsions or Microemulsions as templates, it is important to take into consideration the acceptance of organic solvents in the pharmaceutical industry, their

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potential for toxicity, as well as simple it is to remove from the formulation. The formulation is preferred over the traditional hazardous solvents, such as dichloromethane, by using the pharmaceutically suitable and less hazardous liquid-miscible solvents, such as ethanol and isopropanol, and partially liquid-miscible solvents, such as ethyl acetate, ethyl format, butyl lactate, triacetin, propylene carbonate, and benzyl alcohol. Additionally, when producing nanosuspensions using a microemulsion as a template, partially liquid-miscible organic solvents can be used as the inner phase of the microemulsion [30].

3.3 **Surfactants:** Surfactants are introduced within the solution to enhance dispersion by decreasing interfacial tension. They are additionally used as deflocculating or wetting agents.

For example: Tweens and Spans - mostly used surfactants.

3.4 **Co-Surfactants:** When producing a microemulsion, the choice of cosurfactant is important. Cosurfactants have a significant impact on phase behaviour, thus it is important to look into their ability to affect drug loading and internal phase uptake for a particular microemulsion composition. As an illustration, consider bile salts, dipotassium glycerrhizinate, transcutol, glycofurol, ethanol, and isopropanol. Organic solvent is employed in formulation when nanosuspensions are produced using an emulsion or microemulsion template. Pharmaceutically accepted, less toxic solvents are used to produce the formulation. Examples include Benzyl alcohol, Methanol, Ethanol, Chloroform, Isopropanol, Ethyl Acetate, Ethyl Format, Butyl Lactate, Triacetin, and Ethyl Acetate.

3.5 **Other Additives:** According to the route of administration method or the characteristics of the drug moiety, nanosuspensions may contain additives like buffers, salts, polyols, osmogents, and cryoprotectants [31].

4. Applications of Nanosuspension

4.1 Parenteral Drug Delivery:

Patients with vasospasm caused by a subarachnoid haemorrhage are treated with Nimodipine. Nimodipine had a limited bioavailability when administered orally because of the liver's vital first-pass metabolism. An option to oral treatment that may provide a better bioavailability is intravenous injection. A promising novel drug formulation for the intravenous treatment of vasospasm associated to subarachnoid haemorrhage, Nimodipine nanosuspension produced by high-pressure homogenization showed reduce local irritation and phlebitis concerns. When the drug clofazimine is administered intravenously, the concentration in the liver, spleen, and lungs is high, exceeding the minimal inhibitory concentration for the majority of mycobacterium avium strains. In order to avoid the usage of

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surfactants and cyclodextrins to improve the bioavailability, tarazepide is produced as a nanosuspension [32].

4.2 Oral Drug Delivery:

As oral administration is comfortable and non-invasive, it is the most common approach for patients. Oral formulations also have a number of benefits for the pharmaceutical sector, including simple manufacturing, quick production, and affordable production costs. Oleanolic acid has a wide range of uses, including hepatoprotective, antitumor, antibacterial, anti-inflammatory, and antiulcer properties. However, due to its low aqueous solubility, it exhibits unpredictable pharmacokinetics when taken orally [33]. When Oleanolic acid is applied as a nanosuspension, the dissolution rate rises to roughly 90% in the first 20 minutes as opposed to about 15% for medication powder that has been micronized. The rate of drug dissolution is accelerated when drug particle size reduces to the nanoscale, and drug particle compliance to the mucosa may also be enhanced [34]. Drug intestinal absorption is increased by improved interaction with intestinal cells (bio adhesive phase) and a greater gradient of concentration between blood and GIT. Infection control is a further use for nanosuspensions. Due to its limited absorption, atovaquone and buparvaquone are effective in large doses for the treatment of leishmaniosis and opportunistic Pneumocystis carinii infections in HIV patients. Atovaquone was studied in both its micronized and nanosuspension forms, and it was discovered that the latter reduced infectivity from 40% to 15%. In another instance, buparvaquone nanosuspensions decreased infection from 2.0 to 1.02 whereas micronized particles only decreased infection to 1.47 [33].

4.3 Topical Formulations:

Drug diffusion into the skin is enhanced when the drug's saturation solubility in the topical dose form is raised through the addition of the drug's nanocrystalline form to creams and water-free ointments [34].

4.4 Pulmonary Drug Delivery:

The use of nanosuspensions can be used to produce drugs that are poorly soluble in pulmonary secretions. These drugs are administered through a dry powder inhaler technique or as suspension aerosols. Nebulization usually occurs with a mechanical or ultrasonic nebulizer.

The following are some benefits of nanosuspension over traditional pulmonary formulations:

1) An increase in the rate of diffusion and dissolution at the site of action, increasing the drug's bioavailability.

2) The drug has an affinity for mucosal membranes.

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3) As all aerosol droplets contain nanoparticles compared to the drug's macro particulate form, the drug is uniformly dispersed through the lungs.

In this case, we're using nano-preparations for drugs with poor pulmonary secretion solubility. It is nebulized using a mechanical or ultrasonic nebulizer for lung delivery. E.g. Budenoside [35]

4.5 Occular Drug Delivery:

The administration of nanosuspensions is essential for drug with poor lachrymal fluid solubility. The solubility, dissolved rate, bio adhesion, corneal penetration, resident time in a cul-de-sac, and avoidance of high tonicity produced by water-soluble drugs were all enhanced in the nano-size drug particles. It was revealed that reducing particle irritation to the eye and decreasing tearing with a particle diameter smaller than 10 m will increase the effectiveness of an ocular treatment.

For example, the high pressure homogenizer-created nanosuspensions of hydrocortisone, prednisolone, and dexamethasone demonstrated enhanced absorption rates, levels, and effects in the eye [34]

CONCLUSION AND FUTURE PROSPECTIVE

This review article highlights recent advances in therapeutic nanosuspensions developed by a variety of methods, including high pressure homogenization, emulsification and media milling. Although still in the early stages, a number of in vivo studies have shown how effective these drug delivery methods are be used for parenteral, oral, ophthalmic, and pulmonary administration, which calls for both a regulated release and a suitable level of bio adhesion. The development of novel clinical techniques for treating numerous types of diseases (such as heart disease, cancer, diabetes, Parkinson's disease, and Alzheimer's disease) as well as the flexibility and possibility these systems offer for further altering particles, surface features to improve in vivo responses. As nanoparticle absorption is size dependent, optimizing the size of drug nanosuspension are helpful in producing a formulation that will diffuse more effectively through the mucus gel layer. In addition, size reduction and the incorporation of polymers on the surface of particles can be considered the next steps in nanosuspension research.

Future research directions are summarised by the following:

- Enhancing in vivo bioavailability and relating in vitro and in vivo bioavailability data;
- Establishing controlled and sustained drug release over a prolonged period of time using biocompatible matrix polymers;
- Formulating stimuli-responsive systems, such as pH , magnetic field, light and temperature, which is important for highly toxic drugs;

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- Further research is required to better understand how nanosuspensions behave in vivo, including interactions with cells and other biological barriers such the blood-brain barrier; surface modification of nanosuspensions for either passive or active targeting to enhance the target's accessibility

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

References

[1] Hay RJ. Tropical fungal infections. Pharm Int **(1985)** 6: 97–101.

[2] Zhang AY, Camp WL, Elewski BE. Advances in topical and systemic antifungals. Dermatol Clin **(2007)** 25: 165–183.

[3] Schwartz RA. Superficial fungal infections. Lancet **(2004)** 364: 1173–1182.

[4] Sharma P, Denny WA, Garg S. Effect of wet milling process on the solid state of indomethacin and simvastatin. Int J Pharm. **(2009)** 380: 40–8.

[5] Kakrana M, Sahooa NG, Judeh LZ, Wang Y, Chong K, Loh L. Fabrication of drug nanoparticles by evaporative precipitation of nanosuspension. Int J Pharm. **(2010)** 383: 285–92.

[6] Lakshmi P, Ashwini KG. Nanosuspension technology: A review. Int J Pharm Sci. **(2010)** 2: 35–40.

[7] Verman S, Lan Y, Gokhale R, Burgessa DJ. Quality by design approach to understand the process of nanosuspension preparation. Int J Pharm. **(2009)** 377: 185–98.

[8] Nagaraju P, Krishnachaithanya K, Srinivas VD, Padma SV. Nanosuspensions: A promising drug delivery systems. Int J Pharm Sci Nano. **(2010)** 2:679–84.

[9] Barret ER. Nanosuspensions in drug delivery. Nat Rev. **(2004)** 3: 785–96.

[10] Muller RH, Gohla S, Dingler A, Schneppe T. Wise D. Handbook of pharmaceutical controlled release technology. New York: Marcel Dekker; **(2000)**. Large-scale production of solid-lipid nanoparticles (SLN) and nanosuspension (Dissocubes) pp. 359–375.

[11] Nanosuspension systems, Hamamatsu Nano technology. [Cited **(2011)** Mar 5].

INDORE INSTITUTE OF PHARMACY IQAC INDORE

Principal
Indore Institute of Pharmacy,
INDORE (M.P.)

[12] Soumya M, Gupta S, Jain R, Mazumder R; Solubility enhancement of poorly water soluble drug by using nanosuspensiontechnology. International Journal of Research and Development in Pharmacy and Life Sciences. **(2013)**; 2: 642-649.

[13] Kavitha VB, Neethu CS, Dineshkumar B, Krishnakumar K, John A; Nanosuspension Formulation: An Improved Drug Delivery System and Nanotechnology: AnInternational Journal, **(2014)**; 2: 1-5.

[14] Arunkumar N, Deecaman M, Rani C; Nanosuspension technology and its application in drug delivery. Asian Journal of Pharmaceutics. **(2009)**; 3:168-173.

[15] Muller RH, Jacobs C, Kayser O; Nanosuspensions as particulate drug formulations in drug therapy. Rationale for development and what we can expect for the future. Adv Drug Delivery, **(2001)**; 47(1): 3-19.

[16] Muller RH, Jacobs C, Kayer O; Nanosuspensions for the formulation of poorly soluble drugs. In: F Nielloud, G Marti-Mesters (ed).Pharmaceutical emulsion and suspension. New York, Marcel Dekker, **(2000)**; 383-407.

[17] Liversidge GG, Cundy KC. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int J Pharm **(1995)**; 125: 91–7.

[18] Raditkem M; Nanopure: pure drug nanoparticles for the formulation of poorly soluble drugs. New drugs, **(2001)**; 3:62-68.

[19] Bodmeier R, Ginity MC; Solvent selection in the preparation of poly (DL- lactide) microspheres prepared by solvent evaporationmethod. Int. J. Pharm., **(1998)**; 43:179-186.

[20] Kavitha VB, Neethu CS, kumar B.D, kumar K.K, John A. Nanosuspension Formulation: An Improved Drug Delivery System. Nanoscience and Nanotechnology: An International Journal, **(2014)**; 2:1-5

[21] Chen Y, Liu J, Yang X, Xu H; Oleanolic acid nanosuspensions: formulation, in-vitro characterization and enhanced hepatoprotective effect. J Pharm. Pharmacol., **(2005)**; 57:259 264.

[22] Vaneerdenbrugh B, Vandenmooter G, Augustijns P. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. International Journal of Pharmaceutics. **(2008)**; 364(1):64–75.

[23] Kayser O, Olbrich C, Yardley V, Kiderten AP, Croft SL; Formulation of amphotericin-B as nanosuspension for oral administration. Int J Pharm., **(2003)**; 254: 73-75.

[24] Peters K, Leitzke S, Diederichs JE, Borner K, Hahn H, Moller RH; Preparation of clofozimine nanosuspensions for intravenous use and evaluation of its therapeutic efficacy in murine mycobacterium avium infection. J Antimicrobe Chemother, **(2000)**; 45:77-83.

[25] Keck C, Muller R. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. European Journal of Pharmaceutics and Biopharmaceutics. **(2006)**; 62(1):3–16.

[26] Aher S.S, Malsane S.T, Saudagar R.B.Nanosuspension: An Overview.International Journal of Current Pharmaceutical Research, Vol 9, Issue 3, 19-23.

[27] Rawlins EA. **(1982)** Solutions. In: Rawlins, E. A. (ed.) Bentley's textbook of pharmaceutics. 8th edn, Bailliere Tindall, London, p 6.

[28] Muller, RH, Bohm BHL. Nanosuspensions. In: Muller RH, Benita, S, Bohm, B H L. (eds) Emulsions and nanosuspensions for the formulation of poorly soluble drugs. Medpharm Scientific Publishers, Stuttgart, **(1998)**; pp 149–174.

INDORE INSTITUTE OF PHARMACY IQAC INDORE

Principal
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INDORE (M.P.)

[29] Geetha G, Poojitha U, Khan K.A.A. Various Techniques for Preparation of Nanosuspension- A Review, International Journal of Pharma Research & Review, Sept (**2014**); 3(9):30-37.

[30] Kumar B.D, kumar K.K, John A, Paul D, Cherian J. Nanosuspension Technology in Drug Delivery System-Review Article, Nanoscience Nanotechnology-An International Journal: ISSN: 2278-1374.

[31] Sastry SV, Nyshadham JR, and Fix JA, Pharmaceutical Science and Technology Today, (**2000**); 3(4):138–145

[32] Chen Y, Liu J,Yang X, Zhao X, and Xu H, Journal of Pharmacy and Pharmacology, (**2005**);57 (20):259–264.

[33] Venkatesh T, Reddy AK, Uma Maheswari J, Deena Dalith M, and Ashok Kumar CK Der Pharmacia Lettre, (**2011**); 3 (2): 203–213.

[34] Patel H.M, Patel B.B, Shah C.N. Nanosuspension: A Novel Approach to Enhance Solubility Of poorly Water Soluble Drugs-A Review, International Journal of Advances in Pharmaceutics, Vol 5, Issue 2, (**2016**).

[35] kumar A.N, Deecarman M, Rani C. Nanosuspension Technology And Its Application in Drug Delivery, Asian Journal of Pharmaceutics. Vol 3: Jul-Sep (**2009**).

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COMPUTERIZED SYSTEM VALIDATION – A REGULATORY REQUIREMENT IN PHARMACEUTICAL INDUSTRY

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Abstract : Validation is a key requirement in terms of pharmaceutical industry as to ensure the quality which is related to the products or services being served by the pharmaceutical companies. It is an important ingredient imposed by authorities worldwide to balance the production of pharmaceutical and medical devices. In respect to this, the Food and Drug Administration (FDA) introduced Good Manufacturing Practice (GMP) to maintain and improve the quality of pharmaceutical products. Proper qualification and validation of all of the critical manufacturing equipments, utilities, and facilities in the pharmaceutical industries prior to production is one of the major GMP requirements. Computer Systems Validation (CSV) is a process used to certify and document that a computer based systems will generate information or data that meet a set of defined requirements. If a system meets these requirements, it can be assumed that it is consistently performing in the way it was intended. Computer system validation checks the effectiveness and the efficiency with which the system is meeting the purpose for which it was designed. This study aims to identify needs of computer system validation of equipment practiced in the perspective of pharmaceutical industry.

Keywords: **Computer system validation, qualification, good automated manufacturing practice, food and drug administration, validation.**

I. INTRODUCTION

Validation is an important requirement imposed by authorities worldwide to regulate the production of pharmaceutical and medical devices. Validation also ensures the quality which is related to the products or services being provided by the pharmaceutical companies, in respect to this, the Food and Drug Administration (FDA) introduced Good Manufacturing Practice to maintain and improve the quality of pharmaceutical products [1]. The US FDA was the first to initiate the definition of the principles for software validation. The goal is to protect the patient's health [2]. All new system, process and equipment must be validated before being used in the commercial production of the products. Some systems and processes whose validations is required are Water system, cleaning of equipment, manufacturing process, HVAC system, analytical method, computer system, autoclave and compressed air system [3]. A validation assessment program is a necessity in the pharmaceutical industry to ensure the adherence to pharmaceutical cGMP guidelines, and to help companies maintain consistent quality. Validation is referred to as qualification when the same approach is applied to a machine or equipment instead of a process [1].

II. GOOD MANUFACTURING PRACTICE (GMP)

It ensures that products are repeatedly produced and controlled according to the quality standards that are appropriate to the intended use and as required by the marketing authorization. The major GMP requirement is that all of the manufacturing equipment, utilities, and facilities in the pharmaceutical industries that are critical must be properly qualified and validated before proceeding for the production [4]. Inspections should be regularly performed to monitor if GMP is implemented and complied with. Computer System Validation is applied to GxP computerized system applications which are used at any point in the research, distribution, storage processes, clinical testing, manufacturing, ex;

- LIMS
- LIS
- CDS / NON CDS

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GxP computerized system means that the computer system or application is in compliance with the regulatory requirements [2].

III. COMPUTERIZED SYSTEM AND ITS OBJECTIVES

The process used to test, validate and qualify that a computer – based system does as intended, what it is designed to do in a repeatedly and accurate manner is known as Computerized System Validation (CSV). To confirm by examination and provision of objective evidence that computerized system specifications conform to user needs and intended uses, and that all requirements can be consistently fulfilled [5]. The validation process for computerized systems shall be in compliance with regulatory standards as well as corporate policies and defined quality standards. Computerized systems shall be maintained in a validated state throughout their lifecycle. The system shall be validated from the user perspective to ensure accuracy, reliability and performance. Software shall be meeting intended use and requires objective evidence that the requirements can be consistently fulfilled. A system for which the CSV is to be performed can either be a Closed System or an Open System.

3.1 Closed Systems are the systems having an environment in which system access is controlled by persons who are responsible for the content of electronic records that are on the system.

e.g.: most applications are considered to be closed systems.
Document Management Systems, Manufacturing Execution Systems, Training System.

3.2 Open Systems are the systems having an environment in which system access is not controlled by persons who are responsible for the content of electronic records that are on the system.

e.g.: Internet.

Good computer system validations have many advantages like improve quality assurance, reduce other validation cost and time, improve GMP compliance and 21 CFR part 11 regulation which impact on product quality, safety, identity or efficacy that subject to GxP rules [6].

IV. 21 CFR PART 11 REGULATIONS

The FDA provides detailed controls for Electronic Records and Electronic Signatures in the Code of Federal Regulations. Part 11 of the Code of Federal Regulations - Title 21 states that what an organization must do to implement an FDA compliant, digital QMS which includes E – Signatures and Electronic Records successfully [7].

4.1 GENERAL PROVISION

Part 11 applies to all electronic records that fall under FDA regulations. If an organization can prove to an auditor that their electronic records/signatures are as trustworthy as paper records/ink signatures, the FDA will accept electronic instead of paper.

4.2 ELECTRONIC RECORDS

Organizations using electronic records must establish and document procedures and controls that ensure the following qualities in their electronic records:

- Authenticity
- Integrity
- Confidentiality

The following topics must be addressed in documented procedures and controls:

- Computer Systems Validation (CSV)
- Record rendering
- Document storage and record retention
- System access
- Audit trails
- Workflows
- Authority checks
- Device checks
- Personnel qualifications
- Personnel accountability
- Document control [8].

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4.3 ELECTRONIC SIGNATURES

Organizations that wish to use electronic signatures must inform the FDA in writing prior to making the switch.

- Each individual who will be using an electronic signature must;
  - Have their identity confirmed and
  - Use a unique signature that has never been and will never be used by another individual.
- There are specific design requirements for electronic signatures that are biometric (e.g., fingerprint scan) and those that are not (e.g., user ID and password).
- For electronic signatures that make use of user IDs and passwords/passcodes, there are specific requirements for passwords and for passcode generating devices [9].

V. VALIDATION IN THE DAILY BUSINESS

As discussed earlier that validation is referred to as qualification when the same approach is applied to a machine or equipment instead of a process. FDA's 21 CFR Part 11 requires validation of systems to guarantee reliability, accuracy and consistent intended performance. It means defining that how the elements of their systems are supposed to work. To make sure that validation of processes is legitimate, you have to do calibration. It is a process which demonstrates that an instrument is producing results within the specified limits as compared to those produced by a traceable standard over an appropriate range of measurements. Validation of the GxP compliant systems includes all stages of the qualification and CSV is only performed when the system is GxP compliant either partially or fully, which are;

- Design Qualification (DQ)
- Installation Qualification (IQ)
- Operation Qualification (OQ)
- Performance Qualification (PQ) [3]

VI. GOOD AUTOMATED MANUFACTURING PRACTICE (GAMP)

It is a set of instructions and procedures created in 1991 by pharmaceutical professional. Pharmaceutical manufacturers and users of automated systems must obey these guidelines to attain the compliance with FDA 21 CFR part 11. It is in reference to a guidance document entitled GAMP-5: A Risk-Based Approach to Compliant GxP Computerized Systems. We now have the following four categories which are shown in the **Fig. 1**; [10]

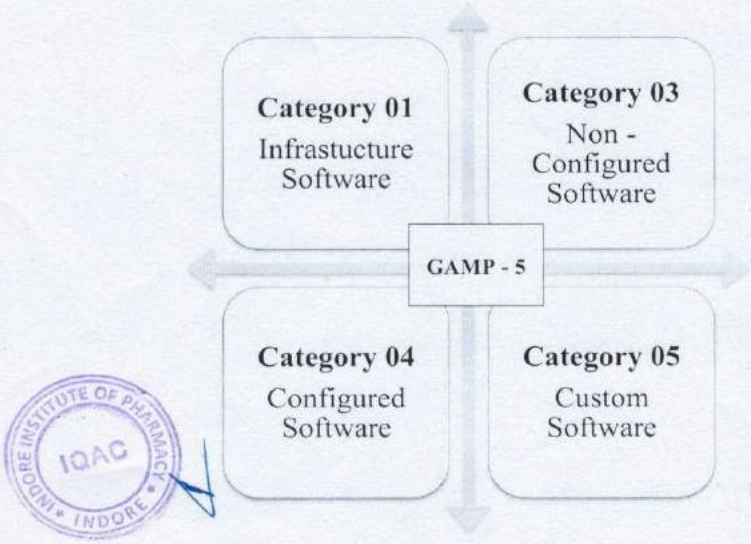

Figure 1: classification of software categories as per gamp – 5

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VII. IMPORTANCE OF SOFTWARE DEVELOPMENT LIFE CYCLE MODEL

A series of phases that helps in creating an effective software system and its management throughout the lifecycle is known as SDLC. It is the package of documentation which helps in ensuring and providing proof that an application has been implemented and in a compliant way. It is the main component of CSV [2]. Software validation happens within the environment of an established software life cycle. The software life cycle holds documentation and software engineering tasks which are necessary to support the software validation attempt. In addition, the software life cycle carries specific verification and validation functions that are appropriate for the intended use of the software [5]. The software development life cycle is a framework explaining tasks performed at each step in the software development process.
Phases of SDLC are;

- Requirements Gathering
- System Analysis and Design
- Development
- Testing
- Deployment
- Maintenance

Some of the models of SDLC in which the process executes in a sequential manner are;

- Waterfall Model
- Spiral Model
- V – Model
- Agile Model [11]

VIII. COMMON VALIDATION DELIVERABLES

A deliverable is any unique and verifiable generated results, or capability to perform a service that must be produced to complete a process, phases, or project in computer system validation. The selection of the validation deliverables depends upon the GxP assessment and Categorization of the software according to the GAMP 5. The most important thing is to define the desired project results and work through the deliverables to each of them. The more defined the deliverables are, the easier it is to forecast the timeline, the budget and the scope. Below is the list of deliverables; [12]

Table 1: system validation deliverables

Sr. No.	Validation Deliverables
1	User Requirements Specification
2	Change Control
3	Vendor Questionnaire
4	Initial Risk Assessment (GxP Determination)
5	Validation Plan
6	Functional Requirements Specification
7	Functional Specification
8	Design Specification
9	21 CFR Part 11 Compliance Assessment
10	Risk Management Protocol Cum Report
11	Security Plan and Administration
12	Coding Standard
13	Source Code Review
14	User Manual and Training SOP
15	IQ, OQ, PQ Protocol
16	IQ, OQ, PQ Test Scripts Execution
17	Deviations and Discrepancies Form
18	Requirement Traceability Matrix
19	IQ, OQ, PQ Report
20	Disaster Recovery Plan
21	Backup and Restore Plan
22	Business Continuity Plan
23	Periodic Review Questionnaire
24	Validation Summary Report

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IX. CONCLUSION

A computer system must be validated at the time of installation, before and during any project is running, any change in the software or computer system. Systematic computer system validation helps to prevent software problems from production environment. Problems occurring in the production environment of the pharmaceutical industry may lead towards the risk of patient's health and safety. To mitigate and control these risks, FDA regulation has mandated the need to perform computer software validation and these regulations has the impact of law. Failing in FDA audit can result in FDA inspectional observation and warning letters and failure to take corrective action in a particular timing can results in shutting down manufacturing facilities, consent decrees, and stiff financial penalties. So computer software validation is very important for pharmaceutical companies and laboratories. Good computer system validations have many advantages like improve quality assurance, reduce other validation cost and time, improve GMP compliance and 21 CFR part 11 regulation which impact on product quality, safety, identity or efficacy that subject to GxP rules.

X. ABBREVIATIONS

CFR: Code of Federal Regulation
CSV: Computerized System Validation
GAMP: Good Automated Manufacturing Practice
GMP: Good Manufacturing Practice
IQ: Installation Qualification
LIMS: Laboratory Information Management System
LIS: Laboratory Information Systems
NON CDS: Non Chromatography Data System
OQ: Operational Qualification
PQ: Performance Qualification
SCADA: Supervisory Control and Data Acquisition
SDLC: Software Development Life Cycle
SOP: Standard Operating Procedure
USFDA: United States Food and Drug Administration

REFERENCES

[1] Singh Asheesh, et al, "Computer system validation in the perspective of the pharmaceutical industry", Journal of Drug Delivery and Therapeutics 2018; 8(6-s): 359-365.
[2] Yves Samson and Kereon AG, "Computerized System Validation: Regulatory Compliance and Process Safety in the Pharmaceutical Industry", ERTS 2006; 1-2.
[3] Diksha Jindal, et al, "Validation – In pharmaceutical industry: Equipment validation: A brief review", Adesh University Journal of Medical Sciences & Research 2020; 2(2): 94-98.
[4] https://www.who.int/news-room/questions-and-answers/item/medicines-good-manufacturing-processes
[5] Bendale A, et al, "Computer software validation in pharmaceuticals," Asian Journal of Pharmaceutical Sciences and Clinical Research, 2011; 1(2): 27-39.
[6] https://ajaykulkarnisoftwaretesting.wordpress.com/computer-system-validationcsv/
[7] R.D. McDowall, "Effective and practical risk management options for computerised system validation", The Quality Assurance Journal 2005; 9(3-s): 196-227.
[8] https://www.perficient.com/-/media/files/guide-pdf-links/the-ultimate-guide-to-21-cfr-part-11.
[9] https://www.sharevault.com/blog/life-sciences/fda-21-cfr-part-11-explained.

[10] Shield S, "GAMP-5 A risk based approach to compliant GxP computerized systems", 2013: 1-29.
[11] https://www.leanbiologix.com/what-is-sdlc-and-how-does-it-relate-to-computer-system-validation-csv/

[12] Bhusnure, et al, "Computer validation and ethical security measures for pharmaceutical data processing," World Journal of Pharmaceutical Research, 2015; 5(1): 1092-1134.

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Evaluation of In vitro Antioxidant, Antimicrobial and Cytotoxicity Activities of Orthosiphon Pallidus Royle

DOI: https://doi.org/10.37285/ijpsn.2023.16.3.3

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ABSTRACT

Objective: The objective of present research work was to evaluate the *in vitro* antioxidant, antimicrobial and cytotoxicity activity of *Orthosiphon pallidus* Royle.

Methods: Total phenolic and flavonoid content of hydroalcoholic extract (50:50) of *Orthosiphon pallidus* Royle hydroalcoholic extract (OPRHE) was estimated and the *in vitro* antioxidant activity was determined using ABTS radical scavenging assay and metal chelating assay. Antimicrobial activity was investigated

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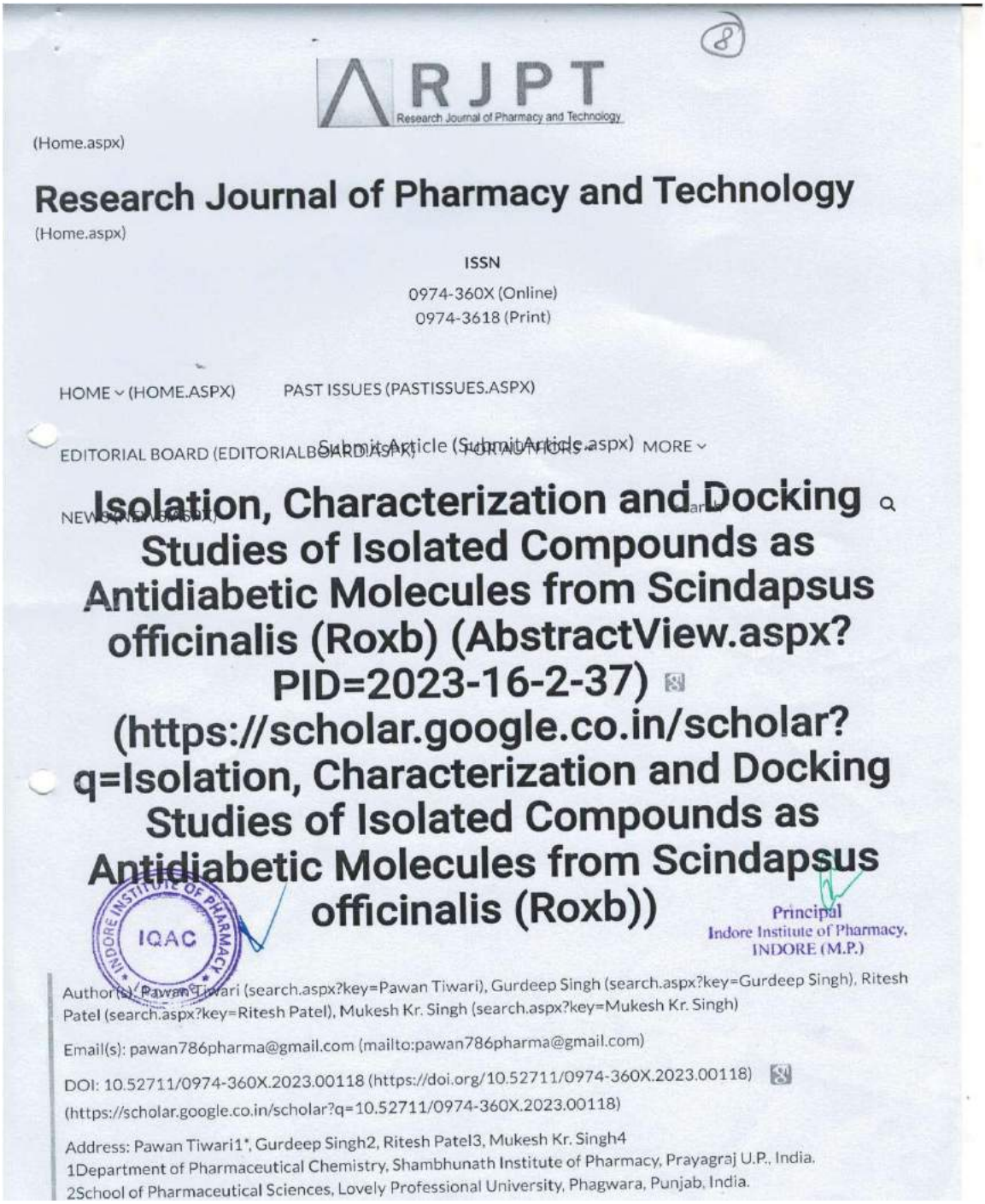

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Isolation, Characterization and Docking Studies of Isolated Compounds as Antidiabetic Molecules from Scindapsus officinalis (Roxb) (AbstractView.aspx?PID=2023-16-2-37) (https://scholar.google.co.in/scholar?q=Isolation, Characterization and Docking Studies of Isolated Compounds as Antidiabetic Molecules from Scindapsus officinalis (Roxb))

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ABSTRACT:

Natural products are essential to human life, and about half of the medications used in clinical practice today are of natural origin. The present work investigated to isolate and identify active compounds with anti-diabetic activity from Scindapsus officinalis fruits and confirm the isolated compounds' mode of action, affinity, and domain specificity relationships. Some fractions of S. officinalis ethanolic extracts were subjected to column chromatography and preparative TLC and two compounds namely 2E,4E,6E)-5-methyl-7-(2,6,6-trimethylcyclohexa-2,4-dien-1-yl)hepta-2,4,6-trien-1-ol (CN-501A) and 9-(furan-3-yl)-4-hydroxy-1,5,6,6a,9,10,10a,10b-octahydro-3H,7H-pyrano[3,4-f]isochromene-3,7-dione (CN-501B) were isolated in pure form. The structures of the isolated compounds were confirmed by UV, IR, 1H NMR and mass spectral data. The anti diabetic activity was measured using a molecular docking study and the three-dimensional structure of the target protein was downloaded from PDB. The Docking study recommended that CN – 501A and CN – 501B are existing photochemical from the plant of S. officinalis had the highest fitness docking score and hence could be a possible antidiabetic drug.

Keywords: Scindapsus officinalis () Molecular modeling () NMR () PBD () antidiabetic. ()

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REFERENCES:

1. Internal Clinical Guidelines Team. Type 2 Diabetes in Adults: Management. London: National Institute for Health and Care Excellence. 2015. 28 p.
2. Panari H, Vegunarani M. Study on Complications of Diabetes Mellitus among the Diabetic Patients. Asian Journal of Nursing Education and Research. 2016; 6(2):171-182.

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KEYWORDS: Orthosiphon Pallidus Royle, TPC, TFC, Antioxidant Activity, Antimicrobial Activity, MIC, SRB Assay

References

Choi EJ, Kim GH. Antioxidant and anticancer activity of Artemisia princeps var. orientalis extract in HepG2 and Hep3B hepatocellular carcinoma cells. Chinese Journal of Cancer Research. 2013 Oct; 25(5):536-43.

Kamran SH, Ahmad M, Shahwar D, Ajaib M. Anti-diabetic and antioxidant status of Loranthus pulverulentus obtained from two different hosts. Bangladesh Journal of Pharmacology. 2016 Jan 18; 11(1):181-9.

Sonibare MA, Aremu OT, Okorie PN. Antioxidant and antimicrobial activities of solvent fractions of Vernonia cinerea (L.) Less leaf extract. African Health Sciences. 2016 Jul 1;16(2):629-39.

Berrington D, Lall N. Anticancer activity of certain herbs and spices on the cervical epithelial carcinoma (HeLa) cell line. Evidence-Based Complementary and Alternative Medicine. 2012 Jan 1; 1-11.

Ashokan K, Muthuraman MS. Anticancer studies on Orthosiphon pallidus Royle and Peristrophe bicalyculata Nees. J Pharm Res. 2011 Aug;4(8):2654-6.

Singh MK, Gidwani B, Gupta A, Dhongade H, Kaur CD, Kashyap PP, Tripathi DK. A review of the medicinal plants of genus Orthosiphon (Lamiaceae). International Journal of Biological Chemistry. 2015;9(6):318-31.

Regina KM, Adama H, Jeanne M, Odile N. Ethnobotany and Ethnopharmacognosy of Lamiaceae

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Tunisian Melia azedarach leaves extract from their ProAntidex parameter. Bangladesh Journal of Pharmacology. 2016 Mar 15;11(2):301-7.

Sellem I, Kaaniche F, Chakchouk AM, Mellouli L. Anti-oxidant, antimicrobial and anti-acetylcholinesterase activities of organic extracts from aerial parts of three Tunisian plants and correlation with polyphenols and flavonoids contents. Bangladesh Journal of pharmacology. 2016 May 8; 11(2):531-44.

Khan WU, Khan RA, Ahmed M, Khan LU, Khan MW. Pharmacological evaluation of methanolic extract of Cyperus scariosus. Bangladesh Journal of Pharmacology. 2016 Mar 20;11(2):353-8.

Prathapan A, Singh MK, Anusree SS, Kumar DS, Sundaresan A, Raghu KG. Antiperoxidative, free radical scavenging and metal chelating activities of Boerhaavia Diffusa L. Journal of Food Biochemistry. 2011 Oct; 35(5):1548-54.

Cheruiyot KR, Olila D, Kateregga J. In-vitro antibacterial activity of selected medicinal plants from Longisa region of Bomet district, Kenya. African health sciences. 2009; 9(2): S42-6.

Perumal S, Pillai S, Cai LW, Mahmud R, Ramanathan S. Determination of minimum inhibitory concentration of Euphorbia hirta (L.) extracts by tetrazolium microplate assay. J Nat Prod. 2012; 5(2):68-76.

Nair A, Kiruthika D, Dheeba B, Tilton F. Cytotoxic Potentials Of Orthosiphon stamineus Leaf Extracts Against Pathogenic Bacteria And Colon Cancer Cells. Asian Journal of Science and Technology. 2012; 5(3):221-5.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. JNCI: Journal of the National Cancer Institute. 1990 Jul 4;82(13):1107-12.

Khan A, Khan RA, Ahmed M, Mushtaq N. In vitro antioxidant, antifungal and cytotoxic activity of methanolic extract of Calligonum polygonoides. Bangladesh Journal of Pharmacology. 2015 Apr 10; 10(2):316-20.

Ertaş A, Boğa M, Yeşil Y. Phytochemical profile and ABTS cation radical scavenging, cupric reducing antioxidant capacity and anticholinesterase activities of endemic. J. Coast. Life Med. 2014; 2:555-9.

Hakkim FL, Arivazhagan G, Boopathy R. Antioxidant property of selected Ocimum species and their secondary metabolite content. Journal of Medicinal Plants Research. 2008 Sep 1; 2(9):250-7.

Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of Spondias pinnata. BMC complementary and Alternative Medicine. 2008 Dec;8(1):1-10.

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REVIEW ARTICLE

Mechanism-based Suppression of Cancer by Targeting DNA-Replicating Enzymes

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Abstract: The human genetic structure undergoes continuous wear and tear process due to the mere presence of extrinsic as well as intrinsic factors. In normal physiological cells, DNA damage initiates various checkpoints that may activate the repair system or induce apoptosis that helps maintain cellular integrity. While in cancerous cells, due to alterations in signaling pathways and defective checkpoints, there exists a marked deviation of error-free DNA repairing/synthesis. Currently, cancer therapy targeting the DNA damage response shows significant therapeutic potential by tailoring the therapy from non-specific to tumor-specific activity. Recently, numerous drugs that target the DNA replicating enzymes have been approved or some are under clinical trial. Drugs like PARP and PARG inhibitors showed sweeping effects against cancer cells. This review highlights the mechanistic study of different drug categories that target DNA replication and thus depicts the futuristic approach of targeted therapy.

Keywords: Cancer, DNA damage response, PARP, topoisomerase, thymidylate synthase, cancer cells.

1. INTRODUCTION

Among the most deadly illnesses of our day is cancer. Tumor cells rely on the collection of gained abilities known as neoplasm hallmarks to grow uncontrollably and abnormally. These abilities include maintaining signaling, enabling immortality, avoiding growth inhibitors, refusing apoptosis, inducing angiogenesis, amplifying metastasis and invasion, reconfiguring energy metabolism, and avoiding immune system destruction [1].

Oxidative stress is the link between inflammation and genomic instability. Oxidative stress is present at high concentrations in cancer cells. Replication tension and the creation of superoxides are caused by oncogenes like MYC GTPases) [2]. By producing reactive oxygen species (ROS), inflammatory cells like white blood cells (WBCs) and macrophages can cause oxidative stress independently. Genomic instability is caused by Strand breaks of DNA and alterations (Master regulator of cell cycle entry and proliferative metabolism) and RAS (member of the Rho family of small brought on by ROS [3]. Inducing the production of inflammatory molecules, Superoxide effectively stimulates inflammatory signaling molecules [4, 5]. Maintaining genomic stability requires DNA damage response (DDR) [6]. DDR is triggered when DNA damage occurs in cells and can repair the destruction by certain deoxy nucleic acid repair mechanisms, such as recombinational restoration, - anti-terminal connecting repair, and SSBR (Single stranded break repair) [7]. In mammal cells, nucleotide mending is triggered in turn to DNA single-stranded binding (SSB) proteins, poly (ADP-ribose) polymerases (PARPs), particularly PARP1, PARP2, and PARP3 [8, 9]. These can attach damaged DNA at single-strand DNA break sites as DNA damage receptors and signal transducers, which attract DNA repair agents to the DNA break sites [9, 10]. The two primary methods for repairing DNA double-strand breaks are NHEJ (Non-homologous end joining) and HR (DSB) (Homologous recombination-DNA double-stranded breaks). The NHEJ mechanism is prone to mistakes. This method uses abrupt end joining with low resolution to repair DSB sites [11]. Significant strides in targeted therapy in cancer treatment have been made, leading to the development of numerous anticancer drugs that are cancer-type-specific. But the management of various tumor sufferers whose tumors don't react to or who have evolved

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mechanisms to tumor-specific antineoplastic agents, conventional or non-specific anticancer medications is still crucial.

Anticancer medications are made to target the full spectrum of cancer features specifically. It might be argued that a potential strategy for cancer eradication is to target genomic instability and inflammation, magnifying these "enabling qualities" to make them "disabling factors." PARPs have been a novel target in cancer therapy over the past ten years [12]. Genomic instability brought on by oxidation and replication damage and impairments in DNA healing mechanisms are exploited by PARP inhibitors. PARP and PARG (Poly(ADP-ribose) glycohydrolase) inhibition is especially effective against malignancies with elevated amounts of replication stress and chromosomal aberrations brought on by DNA healing deficiencies or oncogene-produced increases in replication origin firing. By causing more DNA damage, obstructing DNA repair, and accumulating unsettled replicated intermediaries that trigger replication and mitotic catastrophe, PARP and PARG inhibitors take advantage of and worsen these tumor vulnerabilities.

DNA topoisomerases, widely distributed enzymes that unlink DNA, are essential for many biological activities involving DNA. DNA topoisomerases come in both type I and type II varieties. One DNA strand in a duplex DNA molecule is broken by type I topoisomerases, allowing some other Strand of DNA to pass through it or the downstream DNA duplex to rotate around it, and the broken strand is then repaired. As a consequence, they change step one's linking number [13-19]. Type I topoisomerases have three subtypes: type IA, type IB, and type IC.

2. PARP AND PARG INHIBITORS

Polyadenosine diphosphate-ribose polymerase, often known as PARP, is a class of catalysts that aids in mending DNA destruction in cells. The first synthetic lethality-exploiting agent, PARPi, is approved for use in medical settings. The Poly (ADP-ribose) polymerase (PARP) family controls numerous crucial physiological activities, including the control of transcriptional, cell death, and DNA damage response. When activated by DNA damage, PARP1's poly (ADP-ribose) activity adds branched PAR chains to make it easier to engage other repair proteins to help enhance the mending of Single-stranded breaks. The first cancer medications that were particularly designed to target the DNA damage process in BRCA1/2 mutant breast and ovarian malignancies were called PARP inhibitors (PARPi). A family of 17 proteins known as PARP Poly (ADP-ribose) polymerases are involved in several cellular activities, including DNA repair, chromatin remodeling, stress response, and apoptosis [20-22]. The PARP1 protein, which was initially discovered for its function in the identification and healing of single-strand DNA breaks, is the most well-known and characterized component of the PARP glycoprotein. In mouse breast cancer cell cultures but also BRCA-deficient GEMMs, PARG deletion has already been attributed to PARPi effective resistance. It has been demonstrated that deletion of PARG transcription, irrespective of the vicinity of PARPi, permits significant PARylation to take place. The crucial process of auto-PARylation also enables PARP1 liberation to DNA. As a result, DNA defect buildup and impaired PARP1 entrapment were caused by PARG insufficiency [23-25]. Recent research reveals that PARP1 may also play a part in alternative DNA repair mechanisms, like Homologous recombination, anti-terminal linking, and DNA conflict healing (including classical and alternative) [26].

An innovative category of anti-tumor therapies known as PARP inhibitors (PARPi) fights with Nicotinamide adenine dinucleotide+ for the active position of PARP substance. The therapy of recombinational repair-deficient cancers has proven to be successful with PARPi. Targeting cancers with changes in the critical HR genes BRCA1 and BRCA2 has been done specifically using PARP inhibitors [27-29]. For the treatment of gynecological, mammary, and pancreatic cancers with BRCA mutations, various PARP inhibitors have received approval. Furthermore, 269 clinical trials are now listed on clinicaltrials.gov that look at the consumption of PARP inhibitors as a tumor treatment for chemo-resistant germline or somatic BRCA1/2 mutant mammary, ovary, pulmonary, and pancreatic cancers [30].

3. ACTION MECHANISMS OF PARP INHIBITORS

We still don't fully comprehend the fundamental route of action that causes PARP inhibitors to provide their anticancer effects. Though a consensus has not yet been established, discoveries have considerably enriched our knowledge of PARP-1 action, and various widely acknowledged ideas have arisen.

3.1. PARP and PARG Inhibitors' Molecular Mechanisms

Nicotinamide is produced as a byproduct of the process when PARPs create poly(ADP-ribose) (PAR) from NAD [31]. The primary generator of cellular PAR, PARP1, is triggered by interacting DNA lesions [32]. Multiple steps are required for PARP1 to become catalytically active, including DNA binding *via* the N-terminal Zn fingers (ZnF), helical domain unfolding (HD), NAD binding to the catalyzed pocket, and PAR catalysis [9, 10].

3.2. Inhibitors of PARP and PARG's Cellular Processes

To comprehend the action process of PARP inhibitors, it's essential to comprehend the nuclear activities of PARP1 and PARG in DNA mending, production substrate preservation, and transcription control. The DNA repair processes of SSB repair, base mending, substitute mismatch repair end-joining, and homologous recombination (HR) are all impacted by PARP1 [33]. When replication stress is present, PARP1 and PARG are essential for maintaining the stability of the replication origin [34]. On SSB restoration and replication junction stability, PARP1 or PARG reduction or suppression has the most significant effects. The mode of action of PARG and PARP inhibitors in cancer therapy [35] is illustrated in Fig. (**1**).

4. TOPOISOMERASE INHIBITORS

Topoisomerases are common enzymes that can address topological issues with DNA that are produced throughout important biological activities, including replication, recombination, transcription, and DNA assembling [36]. Such catalysts work by adding or eliminating supercoiling stresses in

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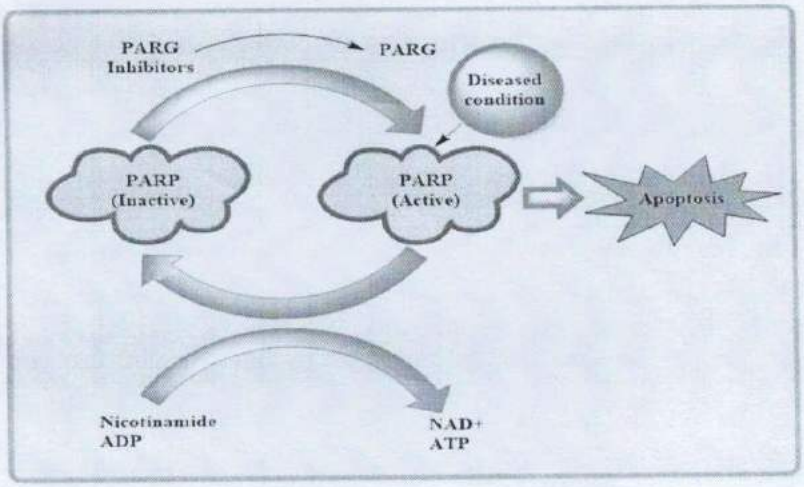

Fig. (1). Mode of action of PARG and PARP inhibitors in cancer therapy. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

DNA, weaving or unzipping DNA strands, and concatenating or decatenating spherical Plasmid DNA. DNA topoisomerases from prokaryotes and eukaryotes have been isolated in two kinds. Topoisomerase I (topo I) works by causing a transitory split in one DNA strand, while topoisomerase II (topo II) causes momentary double-strand splits [37, 38]. Because it is a type of monomeric protein, the Topo I will not necessarily require cofactors to function biologically. By separating a particular DNA strand just after the strand passing process and again ligation, it controls the geometrical configuration of the molecule. On the other hand homodimer type, Topoisomerase II needed Mg2+ as well as energy (ATP) for the enzymatic performance [39]. There is a list of topoisomerase inhibitors that possess different anticancer properties (Table **1**). These enzymes, however, were characterized as DNA recombination enzymes rather than topoisomerases.

4.1. Type-I Topoisomerase-targeting Agents

Camptothecin (CPT) is a cytotoxic alkaloid derived from the tree *Camptotheca acuminate* that preferentially inhibits topoisomerase I [40-42]. In the early 1970s, clinical studies of leukemia and lung cancer evaluated CPT's anticancer efficacy. It induced myelosuppression and dose-limiting hemorrhagic cystitis [43]. According to investigations on the relationship between structure and action, a 6-membered lactone loop is crucial for the anti-neoplastic activity of CPT. A variety of CPT congeners has been produced by altering the parent compound's A-ring. Topotecan [44] and Irinotecan [45] are good examples of it. Membranoid G, a naturally occurring substance obtained from the *Dendrilla antarctica* an Antarctic sponge, has been identified by Ottaviani *et al.* It inhibits human DNA topoisomerase 1B *in vitro*. According to research, membranoid G substantially and permanently hinders the unwinding of supercoiling when it has previously been incubated using the enzyme. By blocking the protein from attaching to the DNA, that molecule blocks its enzyme's catalytic cleavage phase. Membranoid G could be used as a lead molecule to create novel anticancer medications because it exhibits an anticancer potential on different cancer cell forms [46].

CPT11's anticancer effect is exerted by carboxylesterase, which converts it to the principle molecule 7-ethyl-10-hydroxy camptothecin (SN38)-glucuronide [4]. The medicine is beneficial in breast, colon, small-cell lung cancer (SCLC), leukemia, and stomach [44, 47, 48]. The most serious side effects of this medication include neutropenia and diarrhea. It has been demonstrated that topotecan with positively charged CPT derivative with dimethylamino side groups, is effective against malignant glioma, head and neck malignancies, and resistant colorectal cancer [45, 48]. Camptothecin-produced negative effects consist of bone marrow suppression, thrombocytopenia, and neutropenia. CPT11 and TPT both are now used in clinical trials (Table **2**), having multiple optimal dosages and oral or intravenous administration [49]. In therapeutic trials, 9-amino camptothecin, which is a hydrophobic analog of the mother alkaloid, showed encouraging results [50]. Fagaronine, a DNA-significant intercalator, and its artificial analog are two additional alkaloids that have been demonstrated to interact with topoisomerase I [51]. Topo I cannot bind to DNA when distamycin, a small DNA linker, is expressed, but it has no toxic effects [52].

4.2. Mechanism

Camptothecin was discovered in the wood of a tree called *Camptotheca acuminate*. At the same time, it had been rec-

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Table 1. Therapeutic indication of topoisomerase inhibitors.

Drug Name	Target Site	Category	Country & Year of Approval	Cancer Type
Irinotecan	TOPO-IB	Camotothecins	1994, Japan	Colon cancer
Topotecan	TOPO-IB	Camotothecins	1996, USA	Ovarian cancer, Cervical cancer
Belotecan	TOPO-IB	Epipodophyllotoxins	2003, South Korea	Bladder cancer
Teniposide	TOPO-IIA	Epipodophyllotoxins	1992, USA	Ovarian cancer, lung, gastric and breast cancer
Etoposide	TOPO-IIA	Epipodophyllotoxins	1983, USA	Testicular and ovarian cancer, SCLC
Valrubicin	TOPO-IIA	Anthracyclines	1998, USA	Patients with BCG-refractory carcinoma
Mitoxantrone	TOPO-IIA	Anthracenediones	1987, USA	Acute leukemia, prostate, breast cancer, and lymphonia
Epirubicin	TOPO-IIA	Anthracyclines	1999,USA	Advanced breast cancer
Doxorubicin	TOPO-IIA	Anthracyclines	1974, USA	Ovarian, gastric, breast cancer, thyroid cancer, and lymphoma

Table 2. Recent advances in topoisomerase inhibitors.

Drug Name	ID No.	Status	Objectives
Namitecan	NCT01748019	Phase I finished	To treat individuals with solid tumors, the compound's pharmacokinetic profile must be determined, along with the appropriate dose.
CZ-48	NCT02575638	In Phase I trial	Assessing the safety of orally given CZ-48
AR-67	NCT00389480	Phase I finished	Treatment of individuals with resistant or metastatic solid tumors with AR-67
Gimatecan	NCT04029909	In Phase I trial	Research of the drug's ADME, tolerance, and safety in patients with primary peritoneal carcinoma, metastatic ovarian epithelial carcinoma, or fallopian tube tumor.
Edotecarin	NCT00072332	Phase I finished	Evaluating edotecarin's efficacy in combination with cisplatin to treat solid tumors that have spread or are progressed
-	NCT00070031	Phase II finished	Analyzing the effectiveness of therapy for women with chemotherapy-resistant metastatic or locally advanced breast cancer
LMP400	NCT01051635	Phase I finished	The LMP400 and LMP776 are used to treat adults with lymphomas and tumor cells that have reverted.
Genz-644282	NCT00942799	Phase I finished	Evaluation of the compound's tolerance and safety
F14512	2012-005241-20(EudraCT number)	Phase II finished	Effectiveness of F14512 and cytarabine combination therapy and maximum tolerated dose in AML patients older than 60 years old
Amrubicin	NCT03253068	In Phase II trial	Treatment of SCLC with amrubicin and pembrolizumab in patients with refractory disease
Amrubicin	NCT00890955	Phase I finished	Using amrubicin and cyclophosphamide in combination therapy to treat advanced solid organ cancers
Aldoxorubicin	NCT02014844	Phase II finished	Utilizing aldoxorubicin to treat glioblastoma
Aldoxorubicin	NCT02049905	Phase III finished	Aldoxorubicin's role in the treatment of soft tissue sarcomas
Aldoxorubicin	NCT02235688	Phase I finished	Treatment of metastatic solid tumors with combination therapy (aldoxorubicin and gemcitabine)
Vosaroxin	NCT03338348	In Phase I trial	Vosaroxin and azacitidine are used to treat elderly AML patients.
Vosaroxin	NCT01913951	Phase I finished	Vosaroxin use in the treatment of myelodysplastic syndromes patients
Vosaroxin	NCT02658487	In Phase II trial	Evaluation of combination therapy for untreated AML with infusional cytarabine and vosaroxin

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ognized and established by the United States National Cancer Institute, it was researched by the same scientists who were working on paclitaxel (NCI). Midway through the 1970s, camptothecin carboxylate underwent medical studies and was beneficial against cancer, and it was discontinued due to negative effects [53]. The development of hydrophilic camptothecin congeners, topotecan, and irinotecan, was delayed until mammalian topoisomerase I (TOP1) was recognized as the drug's goal. For numerous reasons, camptothecins are pharmacologically distinct [54, 55]. The presence of single point mutations in TOP1 makes it resistant to irinotecan in eukaryotic cell lines chosen for resistance to irinotecan. Secondly, inverting the chiral center at position 20 of camptothecin will also alter its stereochemistry. Camptothecin is rendered entirely inactive. Furthermore, irinotecan swiftly enters mammalian cells and attacks TOP1 within minutes of contact. Camptothecin then reversibly attaches to TOP1cc (topoisomerase cleavage complex). Camptothecin is a cutting-edge pharmacological technique because it can be used to control drug access and TOP1ccs catching with extreme caution, this induces the breakage groups to revert shortly after the withdrawal of camptothecin. Due to all of the aforementioned factors, camptothecin is frequently utilized to investigate replication mediated [56].

Hence offering a potent method for investigating the genetic elements involved in checkpoint control and TOP1-mediated DNA repair. There are numerous restrictions on camptothecins. To begin, TOP1cc must be kept alive long enough to cause DNA damage. To sustain persistent cleavage complexes, camptothecins must be administered as a continuous infusion since they rapidly disperse from TOP1cc. Second, the maximum safe dose of camptothecins is constrained by their adverse effects, such as leucopenia and, Consequently, anti-tumor effectiveness. Irinotecan can cause severe diarrhea, and the negative impacts are likely connected to bis-piperidine which gives the drug its water-soluble quality. Third, camptothecins' -hydroxy lactone E-ring is easily changed into a carboxylate, firmly binding serum albumin but having little effect on TOP1. To address this, the camptothecin E-ring has undergone two changes. When a methylene group is added, like in the case of diflomotecan (BN80915), the E-ring is stabilized while still maintaining TOP1 inhibition. Early clinical trials for Diflomotecan are underway. As with the keto congeners, which maintain substantial anti-topoisomerase activity, removing the lactone group, which entirely prevents E-ring opening, is another method for stabilizing the E-ring. Early next year, clinical trials for the cyclobutane methylenedioxy derivative S39625 are anticipated to begin [34].

5. THYMIDYLATE SYNTHASE INHIBITORS

A novel class of drugs known as TS 2 inhibitors directly targets TS, the enzyme required for thymidylate production in the metabolic pathways of folate. These substances lessen thymidylate production, which is required for DNA production and healing, by inhibiting TS. The creation of novel TS inhibitors is primarily based upon the specific design of new compounds, in contrast to earlier empiric approaches to the identification of anticancer drugs, and drew on an in-depth understanding of the conformation, characteristics, and structure of TS as well as the action mechanism of folate analogs. It is still unknown how these substances will be used during medical practice—as sole medicines or as part of multiple drug therapies-but they are now undergoing preclinical and clinical testing [57]. Using in-silico prediction for the thymidylate synthase inhibition, Nassan *et al.* studied the anti-tumor Effect of both alpha-amino phosphonates as well as arylidine derivatives of 3-acetyl-1-aminoquinolin-2(1H)-one in albino rats for the breast cancer Given their substantial hydrophobicity, the examined compounds were predicted to impede the thymidylate synthase by molecular docking [58].

5.1. Mechanism

The formation of cytosine and thymine TMP from dUMP is a crucial stage in the process of gene synthesis. The folate co-substrate CH2THF and the enzyme TS work together to catalyze this reaction and it is the sole location where cellular thymidylate was found. During this reaction, tetrahydrofolate is changed into dihydrofolate. The cellular thymidylate nucleotides required during the repair and synthesis of DNA are produced by this process. A thymine-less condition produced by TS inhibition is harmful to cells that are actively proliferating. DNA fragmentation caused by dTTP depletion, which enhances dUTP coding errors, is likely what causes this cytotoxicity. To avoid this cytotoxicity, cells that carry the salvage enzyme TK can utilize the circulating thymidine. The amount of revolving thymidine is insufficient to substantially impact some normal tissues and tumor cells, though [59].

6. CYCLIN-DEPENDENT KINASES (CDK)

Cyclin-dependent kinases (CDK), which send signals at cell cycle checkpoints, strictly regulate all significant cell cycle changes. DNA-harming incidents based on the level of cellular damage, modify checkpoint signaling to promote DNA repair or programmed cell death. A faulty checkpoint response is common in many malignancies with proliferative mutations, which keeps tumor cells alive and passes on a survival advantage when the DNA is doubled. Many studies have been done on the processes that regulate cells and how these systems are harmed during tumor development. Changes in checkpoint regulators are a common cause of human cancers. Potential targets in cancer treatment have as a result come about an increased comprehension of these systems and checkpoint signaling events [60, 61]. A significant target for pharmacological suppression is the evolutionarily conserved enzyme named cell division cycle 7 (Cdc7) kinase, which is crucial for the smooth progression of the cell cycle as well as holds numerous connections to the CDK, both structurally and functionally.

Cdc7 holds an essential position in the control of regular cell cycle regulation. A lot was already discovered about the biological functions of Cdc7 kinase in individuals through research on lower eukaryotes, especially yeasts. This enzyme has undergone significant levels of conservation throughout evolution. Human Cdc7 interacts with and changes the kinase activity of two essential regulator proteins, Dbf4 and Drf1. Drf1 and Dbf4 have such a direct phylogenetic relationship, according to genealogical research, proving that they are members of an identical protein domain [63]. Cdc7

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has been identified as an autonomous prognostic factor and a potential therapeutic target because it is discovered to be slightly higher compared in a number of malignancies. Human Cdc7 phosphorylates several sites on Mcm2, among the 6 components of replicative DNA. Cdc7 develops into a desirable target for pharmacological suppression due to its control of DNA production, two crucial processes in the endurance of neoplasm. There are currently no Cdc7 inhibitors in human trials, there is a wealth of information on non-human studies on Cdc7 depletion as anti-cancer activity, and For this, various new compounds are being optimized [62]. Riviclib, also known as P276-00, is a cyclin-dependent kinase inhibitor that has been examined for its anticancer properties on colorectal cancer (CRC) by Manohar *et al.* This research demonstrates the potential of CDK inhibitors as single or combined therapeutics for CRC [64].

CONCLUSION

Many metal-based anti-cancer medicines have historically targeted DNA, but the limitations of current treatments have sparked a quest for novel molecular sites that might also offer distinctive chances for pharmacological utilization. The recent discovery of enzyme inhibition as a different and major target. The search for innovative candidates that target enzymes specifically is currently the focus of significant research in medical pharmacology and pharmacy. The recent significant developments in enzyme inhibition will be the main focus of this study since they represent a major goal in targeting enzymes in anticancer treatments. The huge clinical success of TOPO-I, PARG, Cdks, and TS, along with the abundance of information on enzyme inhibition that has accrued recently, unquestionably served as the driving force behind the creation of a novel anticancer medication with enzyme-inhibiting characteristics. The most recent developments in this area will be discussed, with a focus on the inhibition of telomerase, topoisomerases, protein and lipid kinases, folate synthase, and thymidylate synthase. This therapeutic advance ought to spur researchers to develop the next wave of efficient enzyme inhibitors for cutting-edge malignancy treatments. Future research will examine whether combining these derivatives with other treatments, like monoclonal antibodies or immunotherapies, might boost the antitumor effect. It is hoped that soon the arsenal will include new DNA enzyme inhibitors, combination techniques, and vulnerability chances to increase the quality of life.

LIST OF ABBREVIATIONS

CDK = Cyclin-dependent Kinases
TOP1 = Topoisomerase I
SCLC = Small-cell Lung Cancer
HR = Homologous Recombination

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Hanahan, D; Weinberg, RA Hallmarks of cancer: The next generation. *Cell.*, **2011**, *144*(5), 646-674.
[2] Vafa, O.; Wade, M.; Kern, S.; Beeche, M.; Pandita, T.K.; Hampton, G.M.; Wahl, G.M. c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: A mechanism for oncogene-induced genetic instability. *Mol. Cell.*, **2002**, *9*(5), 1031-1044. http ://dx.doi.org/10.1016/S1097-2765(02)00520-8 PMID: 12049739
[3] Tubbs, A.; Nussenzweig, A. Endogenous DNA damage as a source of genomic instability in cancer. *Cell*, **2017**, *168*(4), 644-656. http ://dx.doi.org/10.1016/j.cell.2017.01.002 PMID: 28187286
[4] Grivennikov, S.I.; Greten, F.R.; Karin, M. Immunity, inflammation, and cancer. *Cell*, **2010**, *140*(6), 883-899. http ://dx.doi.org/10.1016/j.cell.2010.01.025 PMID: 20303878
[5] Mateo, J.; Lord, C.J.; Serra, V.; Tutt, A.; Balmaña, J.; Castroviejo-Bermejo, M.; Cruz, C.; Oaknin, A.; Kaye, S.B.; de Bono, J.S. A decade of clinical development of PARP inhibitors in perspective. *Ann. Oncol.*, **2019**, *30*(9), 1437-1447. http ://dx.doi.org/10.1093/annonc/mdz192 PMID: 31218365
[6] Jeggo, P.A.; Pearl, L.H.; Carr, A.M. DNA repair, genome stability and cancer: A historical perspective. *Nat. Rev. Cancer*, **2016**, *16*(1), 35-42. http ://dx.doi.org/10.1038/nrc.2015.4 PMID: 26667849
[7] Chatterjee, N.; Walker, G.C. Mechanisms of DNA damage, repair, and mutagenesis. *Environ. Mol. Mutagen.*, **2017**, *58*(5), 235-263. http ://dx.doi.org/10.1002/em.22087 PMID: 28485537
[8] Langelier, M.F.; Riccio, A.A.; Pascal, J.M. PARP-2 and PARP-3 are selectively activated by 5' phosphorylated DNA breaks through an allosteric regulatory mechanism shared with PARP-1. *Nucleic Acids Res.*, **2014**, *42*(12), 7762-7775. http ://dx.doi.org/10.1093/nar/gku474 PMID: 24928857
[9] Eustermann, S.; Wu, W.F.; Langelier, M.F.; Yang, J.C.; Easton, L.E.; Riccio, A.A.; Pascal, J.M.; Neuhaus, D. Structural basis of detection and signaling of DNA single-strand breaks by human PARP-1. *Mol. Cell.* **2015**, *60*(5), 742-754. http ://dx.doi.org/10.1016/j.molcel.2015.10.032 PMID: 26626479
[10] Langelier, M.F.; Planck, J.L.; Roy, S.; Pascal, J.M. Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1. *Science*, **2012**, *336*(6082), 728-732. http ://dx.doi.org/10.1126/science.1216338 PMID: 22582261
[11] Bétermier, M.; Bertrand, P.; Lopez, B.S. Is non-homologous end-joining really an inherently error-prone process? *PLoS Genet.*, **2014**, *10*(1), e1004086. http ://dx.doi.org/10.1371/journal.pgen.1004086 PMID: 24453986
[12] Jiang, X.; Li, X.; Li, W.; Bai, H.; Zhang, Z. PARP inhibitors in ovarian cancer: Sensitivity prediction and resistance mechanisms. *J. Cell. Mol. Med.*, **2019**, *23*(4), 2303-2313. http ://dx.doi.org/10.1111/jcmm.14133 PMID: 30672100
[13] Champoux, J.J. DNA topoisomerases: Structure, function, and mechanism. *Annu. Rev. Biochem.*, **2001**, *70*(1), 369-413. http ://dx.doi.org/10.1146/annurev.biochem.70.1.369 PMID: 11395412
[14] Wang, J.C. Cellular roles of DNA topoisomerases: A molecular perspective. *Nat. Rev. Mol. Cell Biol.*, **2002**, *3*(6), 430-440. http ://dx.doi.org/10.1038/nrm831 PMID: 12042765
[15] Corbett, K.D.; Berger, J.M. Structure, molecular mechanisms, and evolutionary relationships in DNA topoisomerases. *Annu. Rev. Biophys. Biomol. Struct.*, **2004**, *33*(1), 95-118. http ://dx.doi.org/10.1146/annurev.biophys.33.110502.140357 PMID: 15139806

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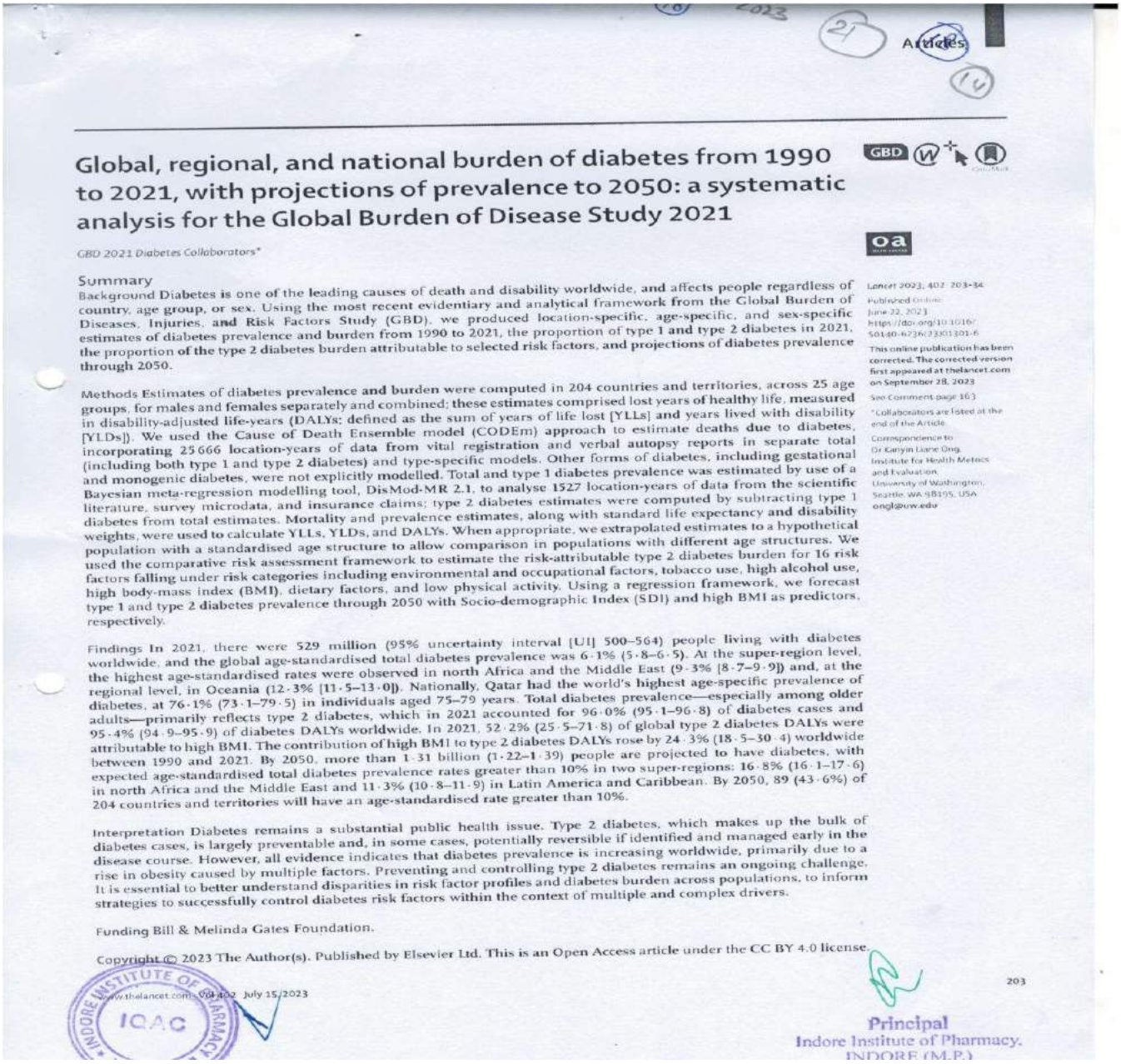

Articles

Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021

GBD 2021 Diabetes Collaborators*

Summary

Background Diabetes is one of the leading causes of death and disability worldwide, and affects people regardless of country, age group, or sex. Using the most recent evidentiary and analytical framework from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), we produced location-specific, age-specific, and sex-specific estimates of diabetes prevalence and burden from 1990 to 2021, the proportion of type 1 and type 2 diabetes in 2021, the proportion of the type 2 diabetes burden attributable to selected risk factors, and projections of diabetes prevalence through 2050.

Methods Estimates of diabetes prevalence and burden were computed in 204 countries and territories, across 25 age groups, for males and females separately and combined; these estimates comprised lost years of healthy life, measured in disability-adjusted life-years (DALYs; defined as the sum of years of life lost [YLLs] and years lived with disability [YLDs]). We used the Cause of Death Ensemble model (CODEm) approach to estimate deaths due to diabetes, incorporating 25 666 location-years of data from vital registration and verbal autopsy reports in separate total (including both type 1 and type 2 diabetes) and type-specific models. Other forms of diabetes, including gestational and monogenic diabetes, were not explicitly modelled. Total and type 1 diabetes prevalence was estimated by use of a Bayesian meta-regression modelling tool, DisMod-MR 2.1, to analyse 1527 location-years of data from the scientific literature, survey microdata, and insurance claims; type 2 diabetes estimates were computed by subtracting type 1 diabetes from total estimates. Mortality and prevalence estimates, along with standard life expectancy and disability weights, were used to calculate YLLs, YLDs, and DALYs. When appropriate, we extrapolated estimates to a hypothetical population with a standardised age structure to allow comparison in populations with different age structures. We used the comparative risk assessment framework to estimate the risk-attributable type 2 diabetes burden for 16 risk factors falling under risk categories including environmental and occupational factors, tobacco use, high alcohol use, high body-mass index (BMI), dietary factors, and low physical activity. Using a regression framework, we forecast type 1 and type 2 diabetes prevalence through 2050 with Socio-demographic Index (SDI) and high BMI as predictors, respectively.

Findings In 2021, there were 529 million (95% uncertainty interval [UI] 500–564) people living with diabetes worldwide, and the global age-standardised total diabetes prevalence was 6·1% (5·8–6·5). At the super-region level, the highest age-standardised rates were observed in north Africa and the Middle East (9·3% [8·7–9·9]) and, at the regional level, in Oceania (12·3% [11·5–13·0]). Nationally, Qatar had the world's highest age-specific prevalence of diabetes, at 76·1% (73·1–79·5) in individuals aged 75–79 years. Total diabetes prevalence—especially among older adults—primarily reflects type 2 diabetes, which in 2021 accounted for 96·0% (95·1–96·8) of diabetes cases and 95·4% (94·9–95·9) of diabetes DALYs worldwide. In 2021, 52·2% (25·5–71·8) of global type 2 diabetes DALYs were attributable to high BMI. The contribution of high BMI to type 2 diabetes DALYs rose by 24·3% (18·5–30·4) worldwide between 1990 and 2021. By 2050, more than 1·31 billion (1·22–1·39) people are projected to have diabetes, with expected age-standardised total diabetes prevalence rates greater than 10% in two super-regions: 16·8% (16·1–17·6) in north Africa and the Middle East and 11·3% (10·8–11·9) in Latin America and Caribbean. By 2050, 89 (43·6%) of 204 countries and territories will have an age-standardised rate greater than 10%.

Interpretation Diabetes remains a substantial public health issue. Type 2 diabetes, which makes up the bulk of diabetes cases, is largely preventable and, in some cases, potentially reversible if identified and managed early in the disease course. However, all evidence indicates that diabetes prevalence is increasing worldwide, primarily due to a rise in obesity caused by multiple factors. Preventing and controlling type 2 diabetes remains an ongoing challenge. It is essential to better understand disparities in risk factor profiles and diabetes burden across populations, to inform strategies to successfully control diabetes risk factors within the context of multiple and complex drivers.

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Research in context

Evidence before this study

The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) generates publicly available estimates of total (inclusive of type 1 and type 2) diabetes deaths, prevalence, years of life lost (YLLs), years lived with disability (YLDs), and disability-adjusted life-years (DALYs) at the global, super-region, region, and country and territory levels. Since GBD 2017, type-specific estimates have also been produced. The International Diabetes Federation (IDF) generates worldwide estimates of diabetes deaths and prevalence for type 1 diabetes in people aged 19 years or younger and for total diabetes in those aged 20–79 years, with the most recent estimates produced in 2021, and has projected the future prevalence of total diabetes through 2045. The NCD Risk Factor Collaboration (NCD-RisC) published global estimates in 2016 that focused on total diabetes prevalence in individuals aged 18 years and older and projected the probability that diabetes would not continue to increase by 2025. In the present study, we estimated non-fatal outcomes due to diabetes by conducting systematic reviews in PubMed from Jan 1, 1990, to Oct 16, 2018 (see appendix section 4.1.1), carrying out opportunistic searches from Jan 1, 1990, to Dec 31, 2021, and incorporating data shared by country collaborators and WHO in addition to insurance claims data. To estimate diabetes risk relative to risk factor exposure, separate systematic reviews were done for each risk factor by accessing various databases (PubMed, Embase, and Web of Science) with endpoints ranging from 2019 to 2022 (see appendix sections 5.1.1–5.1.6).

Added value of this study

Global estimates are essential to policy makers, health-care professionals, health researchers, and individuals with diabetes, but only GBD data and methods are exhaustive across diabetes type, age, and sex, for 204 countries and territories; explicitly quantify the proportion of the diabetes burden attributable to specific risk factors; predict diabetes prevalence to 2050; and are designed to capture both undiagnosed and diagnosed cases. Various research groups have made use of publicly available GBD data to report on the diabetes burden and risk factors and produce short-term forecasts. Our study, as part of the larger GBD analytical enterprise, leverages the newest available data and methods. We apply and detail the updated GBD analytical and evidentiary framework to generate comprehensive, type-specific estimates of diabetes burden for all regions of the world, across the human lifespan, for males and females separately and combined. We also quantify the proportion of type 2 diabetes attributable to 16 selected risk factors concurrently to highlight the main drivers of diabetes. The continued global spread of diabetes presents a massive public health challenge. The location-specific and population-specific data we present on the likely trajectory of diabetes in the coming decades are crucial to inform policy makers and public health professionals as they prepare to address the impending threat to the communities they serve.

Implications of all the available evidence

Policy makers and public health officials worldwide are increasingly concerned by soaring diabetes prevalence rates and their implications for health-care systems and societies. At the current pace, we project that more than 1·31 billion people will be living with diabetes by 2050, most of whom will have type 2 diabetes. Addressing escalating challenges to diabetes prevention and barriers to managing the disease and its complications will become a requisite component of health-care provision worldwide. There is an urgent need to tackle adverse trends in the prevalence of risk factors for type 2 diabetes, particularly obesity. Without new and far-reaching approaches targeting not only risk factors but also the social and logistical barriers that limit access to treatment and medical attention, diabetes will continue to exert increasingly negative effects on the quality of life of individuals, health of populations, and the strength of global economies for decades to come.

Introduction

Diabetes is a serious, chronic disease characterised by elevated blood glucose concentrations related to the effects of abnormal β-cell biology on insulin action.[1–4] According to estimates from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019, diabetes was the eighth leading cause of death and disability combined in the world, with nearly 460 million people across every country and age group living with the disease in 2019.[5] Diabetes represents a substantial burden to health-care systems,[6–9] with estimates by the International Diabetes Federation (IDF) indicating that 537 million people worldwide had diabetes in 2021, resulting in health expenditures of US$966 billion globally, forecast to reach more than $1054 billion by 2045.[10] The 2016 NCD Risk Factor Collaboration (NCD-RisC) Study projected that the probability of meeting global targets to halt the rising diabetes prevalence by 2025 was lower than 1% for women and even lower for men.[11] Diabetes is also a major risk factor for ischaemic heart disease and stroke,[12] which were estimated by GBD 2019 to be the first and second leading causes, respectively, of the global disease burden.[5]

Type 1 and type 2 diabetes are the most common forms of the disease and are diagnosed through well established criteria.[13,14] Type 1 diabetes often develops during childhood, while type 2 diabetes has a strong genetic component and a robust association with obesity and a sedentary lifestyle.[15,16] Although prevention and management approaches differ between diabetes types, there are well established strategies to reduce the disease burden, including limiting risk factors for type 2 diabetes,[17] increasing access to treatment such as insulin,[18] and enhancing the health-system infrastructure.[19,20] However, social determinants of health have led to considerable disparities across populations in risk factor profiles,

For estimates from GBD 2019 see https://vizhub.healthdata.org/gbd-compare

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access to screening and treatment, and available health services.[16–19] Hence, the burden of diabetes-related deaths and disability, as well as their drivers, varies widely.[14,20–24] The *Lancet* Commission on diabetes published in 2020 highlights the unequal burden of the disease on people in low-income and middle-income countries (LMICs), reporting that 80% of diabetes cases occur in LMICs.[1] The *Lancet* Commission noted that, in addition to underfunded and ill-prepared health-care systems, LMICs are beset by socioeconomic challenges such as poor nutrition, poverty, and physical inactivity, and emphasised the pressing need for accurate, focused data to guide the development of effective programmes targeting these factors. It was further argued as imperative to accurately identify and characterise the populations at highest risk—defined by their demographic features and exposure to key risk factors—in addition to forecasting how the diabetes burden is expected to increase along these dimensions in the future.

In response to this need and in support of recent calls to action sounded by the global community, as embodied in initiatives such as the 2020 *Lancet* Commission on diabetes and the 2021 WHO Global Diabetes Compact, our work applies and explicates the newly updated methodological framework of GBD to generate estimates of total diabetes and type-specific (type 1 and type 2) diabetes prevalence and burden from 1990 to 2021. This approach allows us to break down these estimates with a high degree of granularity by location, age, and sex, and to present a more holistic picture of the landscape of diabetes—including drivers of the disease and how they have changed over time, as well as forecasting global and location-specific diabetes prevalence through 2050.

This manuscript was produced as part of the GBD Collaborator Network and in accordance with the GBD Protocol.[25]

Methods

Overview

To obtain the data used in models, GBD conducts systematic reviews and opportunistic searches, and utilises data shared by country collaborators and WHO. Data seeking is iterative and continuously in process in order to identify new sources. Information on data seeking efforts conducted for GBD iterations through GBD 2019 has been published previously[4,8,17] and is provided in the appendix (section 4.1.1). For this study, we identified 27 193 data sources to which we applied the methodological and evidentiary framework provided by GBD. The present analysis does not reflect the potential impact of the COVID-19 pandemic on diabetes prevalence and burden since these data were not available at the time of the analysis.

We report primarily on diabetes prevalence and burden because these metrics are particularly salient for characterising type 2 diabetes and capturing aspects of the rapid global rise of diabetes; however, we also provide mortality estimates in the appendix (table S24). Moreover, mortality data were included in the calculation of our principal metrics: prevalence (via cause-specific mortality rates used in the compartmental disease modelling process) and years of life lost (YLLs; via measures of expected age of mortality), and, by extension, disability-adjusted life-years (DALYs), which are the sum of YLLs and years lived with disability (YLDs).

We report many estimates generated as age-standardised results (ie, extrapolated to a hypothetical population with a standardised age structure) to allow comparison of estimates made in populations with different age structures. The standard population was calculated with the non-weighted mean of the age-specific population proportional distributions for all national locations with populations greater than 5 million in 2019 from GBD 2019.

This study complies with the Guidelines for Accurate and Transparent Health Estimates Reporting (GATHER) statement (appendix table S1).[26]

Mortality

There were 25 666 location-years of death data included in the fatal model (appendix section 3.1). We used vital registration data and verbal autopsy[27] data coded as diabetes since 1980 (appendix section 3.2.1). Codes for causes that either did not lead to death or were intermediate causes, but for which diabetes could have been the underlying cause—referred to as garbage codes[13]—were eligible for inclusion in the diabetes model[4] (appendix section 3.2.1). Approximately 11·1% of deaths coded to diabetes were reassigned from garbage codes.

More than 50% of deaths coded to diabetes did not specify a type. We developed a log-linear regression model to predict the type-specific proportion of deaths among those coded to unspecified diabetes. The model was informed by data that specified the diabetes type and, for a given country, included two parameters: first, country-years in which more than 50% of deaths due to diabetes were coded as being due to type 1 or type 2 diabetes; and second, for country-years with type-specific coding, those in which 70% or more of type-specific deaths for people older than 25 years were coded as type 2 diabetes. We included prevalence of obesity as a covariate and redistributed deaths accordingly to type 1 or type 2 diabetes. More details are provided in the appendix (section 3.2.3).

We ran separate mortality models for type 1 diabetes, type 2 diabetes, and total diabetes. We assumed that all deaths due to diabetes in people younger than 15 years were from type 1 diabetes. We used the Cause of Death Ensemble model (CODEm),[28] a highly automated analytical tool that selects an ensemble of mixed-effects or spatiotemporal Gaussian regression models of mortality rates or cause fractions with varying combinations of predictive covariates. Ensembles were

For more on the **WHO Global Diabetes Compact** see https://www.who.int/initiatives/the-who-global-diabetes-compact

See Online for appendix

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selected on the basis of out-of-sample predictive validity testing. We included 19 covariates, six associated with type 1 diabetes and 13 associated with type 2 diabetes, which were selected on the basis of known or postulated relationships with development or management of diabetes (appendix section 3.3).

Non-fatal outcomes

The reference case definition for diabetes was a fasting plasma glucose (FPG) concentration of 7 mmol/L (126 mg/dL) or greater, or a person using insulin or diabetes medication. We included any population-representative source that provided individual-level data or reported the prevalence or incidence of diabetes defined by the source's glucose threshold from tests of FPG, glycated haemoglobin (HbA_1c), oral glucose tolerance (OGTT), or post-prandial glucose (PPG), or any population-representative source that reported mean FPG and uncertainty around the estimate.[11] We also used insurance claims data from the USA and Taiwan (province of China), since these were locations for which we had access to insurance claims. We included studies reporting the incidence of type 1 diabetes. We incorporated data found through systematic reviews conducted from Jan 1, 1990, to Oct 16, 2018 (appendix section 4.1.1),[12–17] and carried out opportunistic literature searches from Jan 1, 1990, to Dec 31, 2021. Between 2020 and 2021, we reviewed all data provided by GBD collaborators through the Global Health Data Exchange (GHDx) and prospectively sought individual-level data from the WHO STEPwise Approach to NCD Risk Factor Surveillance (STEPS) surveys (appendix section 4.1.1). There were 1527 location-years of data from 172 countries (84·3% of the 204 countries and territories included in GBD) used in the diabetes modelling process (appendix section 4.1.1).

For more on the Global Health Data Exchange see http://ghdx.healthdata.org/

For more on the WHO STEPwise Approach to NCD Risk Factor Surveillance see https://www.who.int/teams/noncommunicable-diseases/surveillance/systems-tools/steps

Where possible, we used individual-level data from surveys that collected glucose measurements to calculate age-sex-year-location-specific prevalence estimates and used the information included in the survey metadata to inform how the sampling strategy, sampling frame, and sampling weights were incorporated into the estimates and uncertainty.

We used the meta-regression Bayesian, regularised, trimmed (MR-BRT)[11] tool to generate coefficients that were used to adjust estimates from studies that did not define diabetes with the reference definition (appendix section 4.2.2). For data from people aged younger than 15 years, we assumed that all diabetes cases were type 1 diabetes and that all patients had sought hospital care due to their insulin dependence. We also converted population-level mean FPG estimates to diabetes prevalence estimates (appendix section 4.2.2).

We ran separate non-fatal models for type 1 diabetes and total diabetes. We used a hierarchical Bayesian meta-regression modelling tool, DisMod-MR 2.1,[9] to estimate prevalence due to diabetes from 1990 to 2021. Differential equations in DisMod-MR 2.1 produce a consistent set of estimates based on data on prevalence, incidence, and cause-specific mortality rates generated from the fatal modelling process. In the type 1 diabetes model, we included three predictive covariates: proportion of livebirths in women aged 35 years and older and maternal education (years per capita) as predictors of type 1 diabetes incidence; and the Healthcare Access and Quality Index (HAQ Index)[18] as a predictor of type 1 diabetes excess mortality rate. We assumed there was no remission (ie, no cure). In the total diabetes model, we included two predictive covariates—prevalence of obesity and year as predictors of diabetes prevalence—and assumed that annual remission could be no more than 1% (appendix sections 4.3.1 and 4.3.2). Because most data sources in adults reported prevalence of total diabetes or did not use a robust strategy to exclude people with type 1 diabetes, we were not confident in the accuracy of the data available that were labelled as type 2 diabetes. As an alternative, we subtracted the year-age-sex-location-specific estimates of type 1 diabetes from total diabetes to produce estimates of type 2 diabetes (appendix section 4.3.3).

YLLs, YLDs, and DALYs

The methods for calculating YLLs, YLDs, and DALYs have been described elsewhere,[1] but in brief, YLLs were the product of the number of deaths and standard life expectancy at each age of death,[19] and YLDs were the product of the prevalence of each sequela and its corresponding disability weight.[11] We included estimates for four diabetic sequelae for each type of diabetes: neuropathy, diabetic foot, lower limb amputation, and vision loss due to retinopathy. Each sequela had separate disability weights that were used to calculate YLDs (appendix sections 4.2.4, 4.3.4, and 4.4). YLDs were corrected for comorbidities with all other causes of ill health, assuming independence and a multiplicative function. DALYs were the sum of YLLs and YLDs.

Risk-attributable burden

We modelled 16 detailed risk factors for diabetes: ambient particulate matter pollution, household air pollution from solid fuels, smoking, second-hand smoke, high alcohol use, high body-mass index (BMI), diet low in fruits, diet low in vegetables, diet low in whole grains, diet high in red meat, diet high in processed meat, diet high in sugar-sweetened beverages, diet low in fibre, low physical activity, high air temperature, and low air temperature (appendix section 5.1). These risk factors fall into six categories: environmental or occupational, tobacco use, high alcohol use, high BMI, dietary risks, and low physical activity. All risk factors have been shown to be associated with type 2 diabetes, but high air temperature and low air temperature are the only risk factors associated with type 1 diabetes.[20]

To quantify the relationship between each risk factor and diabetes, we carried out meta-analyses following the

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comparative risk assessment approach, a framework used by GBD since 2002 that is predicated on a causal web of hierarchically organised, potentially overlapping health risks.[14,15] For each risk factor analysed here, we estimated the relative risk of diabetes as a function of risk exposure, using the following methods, which have been extensively detailed elsewhere.[11,16] In brief, we did a literature review of studies that estimated diabetes risk relative to risk factor exposure and extracted data to input into a set of flexible meta-regression procedures using regularised splines to estimate risk functions, as an alternative to imposing a log-linear risk–outcome relationship. Accuracy was further improved by using a robust likelihood-based approach—least-trimmed squares—to detect and trim 10% of the outlying data, testing and adjusting for bias related to study design, and integrating over exposure ranges to account for inconsistency in exposure levels between data sources.

Following methods established previously,[16] we used DisMod-MR 2.1 or spatiotemporal Gaussian process regression to estimate exposure distributions for each risk factor by age, sex, year, and location, and further determined the theoretical minimum risk exposure level (TMREL), the counterfactual level of exposure that would minimise the risk of diabetes, on the basis of epidemiological evidence. Exposure, relative risk estimates, and TMRELs were used to calculate population attributable fractions (PAFs) for each risk factor by location, age, sex, and year. PAFs quantify the proportional reduction in diabetes that would occur if exposure to the given risk factor was reduced to the TMREL. PAFs were multiplied by metrics of disease burden—in this instance, DALYs—to estimate the risk-attributable burden.

Forecasting

We used forecasted Socio-demographic Index (SDI)[17] as a predictor in a regression model to estimate the prevalence of type 1 diabetes and forecasted BMI as the predictor for estimating the prevalence of type 2 diabetes. These metrics were forecast through 2050, by age, sex, year, and location.[15] For each location (l), age (a), sex (s), and year (y), we logit-transformed the GBD 2019 diabetes prevalence estimates $logit(Y_{l,a,s,y})$ and used a fixed coefficient (β_1) on SDI only for type 1 diabetes (equation 1)

$$E[logit(Y_{l,a,s,y})] = \beta_1 SDI + \alpha_{l,a,s}$$

and BMI for type 2 diabetes (equation 2) over time, and a random intercept (α).

$$E[logit(Y_{l,a,s,y})] = \beta_1 BMI + \alpha_{l,a,s}$$

We computed the difference between the GBD estimates in rate space and the forecasted estimates for 2021 and shifted the forecasting trend through 2050 to align with that of GBD. To calculate the number of cases, we used the forecasted population multiplied by the corresponding predicted prevalence. Population forecasts are described by Vollset and colleagues.[18]

Uncertainty and presentation of results

At each modelling step described above, parameter uncertainty was incorporated by randomly drawing 100 samples from each age-sex-location-year-specific parameter distribution and propagating this uncertainty forward through each subsequent step of the analysis. Likewise, 95% uncertainty intervals (UIs) for final estimates were calculated by generating 100 random draws from the estimate distribution and taking the 2·5th and 97·5th percentile values across the 100 draws.

All count data reported are presented to three significant figures, while rates and percentages are presented to one decimal place.

Geographical locations reported

Diabetes estimates were generated for 204 countries and territories that are grouped on the basis of epidemiological patterns into seven super-regions, with these super-regions further grouped into 21 regions based on geographical and epidemiological similarity (see appendix section 7, table S18, for the full GBD location hierarchy).

Code availability

All codes used for these analyses are publicly available online. Analyses were carried out with R (version 4.2.2).

For the codes see https://github.com/ihmeuw/bindu/tree/diabetes_lancet_2023

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or the writing of the report.

Results

Total diabetes prevalence

In 2021, there were 529 million (95% UI 500–564) people of all ages, worldwide, living with diabetes, yielding a global age-standardised prevalence of 6·1% (5·8–6·5; figure 1). To facilitate comparison, we re-stratified our results into age groups reported by IDF and NCD-RisC.[4,11] We estimated there were 485 million (456–517) adults aged 20–79 years with diabetes in 2021 (for comparison with the 2021 IDF estimate of 537 million in the same age group), and 321 million (304–341) people aged 18 years and older with diabetes in 2010 (for rough comparison with the 2014 NCD-RisC estimate of 422 million in the same age group; appendix table S20). Age-standardised total diabetes prevalence rates varied at the super-region level; north Africa and the Middle East had an age-standardised total diabetes prevalence rate of 9·3% (8·7–9·9), with country-specific rates of more than 10% in 11 countries in this region: Iraq (15·3%; 14·3–16·2), Kuwait (15·2%; 14·1–16·3), Qatar

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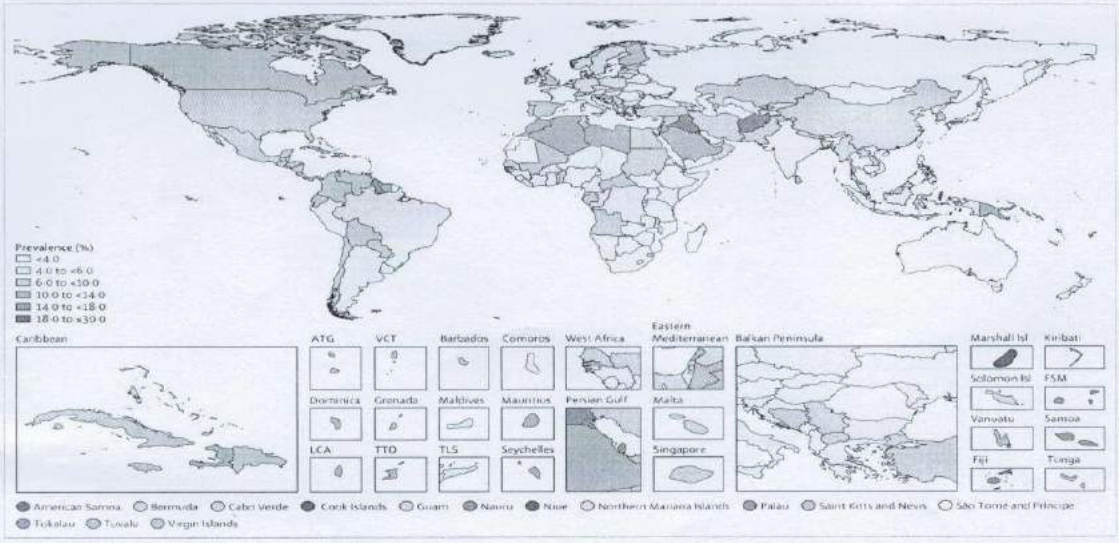

Figure 1: Age-standardised total diabetes prevalence rates in 2021
ATG=Antigua and Barbuda. VCT=Saint Vincent and the Grenadines. LCA=Saint Lucia. TTO=Trinidad and Tobago. Isl=Islands. FSM=Federated States of Micronesia. TLS=Timor-Leste

(15·1%; 14·0–16·2), Bahrain (15·0%; 14·1–15·8), Afghanistan (14·6%; 13·5–15·5), Morocco (13·8%; 12·7–14·7), Jordan (13·5%; 12·6–14·5), Saudi Arabia (11·3%; 10·6–12·0), Lebanon (11·1%; 10·3–11·8), Libya (10·6%; 9·9–11·6), and Algeria (10·0%; 9·3–10·7). Oceania had the highest regional age-standardised prevalence, at 12·3% (11·5–13·0), where 15 of 18 countries and territories had a prevalence greater than 10%; age-standardised prevalence rates were greater than 20% in the Marshall Islands, at 22·2% (20·7–23·9), and American Samoa, at 21·4% (19·9–22·7). Eastern sub-Saharan Africa had the lowest regional diabetes prevalence, at 2·9% (2·7–3·1). The age-standardised diabetes prevalence exceeded 10% in 43 countries and territories in 2021 (figure 1).

Sex-specific total diabetes prevalence

The global age-standardised total diabetes prevalence was higher in males than in females (6·5% [95% UI 6·2–7·0] vs 5·8% [5·4–6·1]), with a male-to-female sex ratio of 1·14 (1·13–1·15). The ratio varied geographically, from 1·26 (1·24–1·28) in the high-income super-region to 0·96 (0·94–0·97) in the Latin America and Caribbean super-region. At the regional level, the age-standardised diabetes prevalence in males was 1·40 (1·30–1·48) times higher than in females in central sub-Saharan Africa, but prevalence among females was more than 10% higher than in males in central Latin America, southern sub-Saharan Africa, and the Caribbean. In 64 (31·4%) countries and territories, age-standardised diabetes prevalence was lower in males than in females, and in six countries—Azerbaijan, Haiti, Laos, Mauritania, Zimbabwe, and Belize—prevalence in males was more than 20% lower than in females. Of the 140 (68·6%) countries and territories where diabetes was more prevalent in males than in females, in three countries—Angola, Uganda, and Gabon—the male prevalence was more than 50% higher than the female prevalence (appendix figure S24).

Age-specific total diabetes prevalence

Globally, total diabetes prevalence exceeded 20% in every age group between 65–95 years but was less than 1% in age groups younger than 20 years. Global diabetes prevalence

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peaked between ages 75–79 years, at 24·4% (95% UI 22·3–26·2). In this age group, prevalence was highest in the north Africa and Middle East super-region, at 39·4% (36·3–42·3), and lowest in central Europe, eastern Europe, and central Asia, at 19·8% (18·3–21·6; figure 2). At the regional level, Oceania had the highest age-specific total diabetes prevalence in the world, at 43·0% (40·7–45·9) in people aged 75–79 years. The highest country-level prevalence was found in Qatar, at 76·1% (73·1–79·5) in people aged 75–79 years. In people aged 30–34 years, ten countries and territories—all in Oceania—had an age-specific prevalence that exceeded 10%: Marshall Islands (19·5% [16·8–23·1]), American Samoa (17·3% [14·8–20·3]), Cook Islands (15·1% [12·9–17·9]), Niue (14·9% [12·8–17·4]), Palau (13·6% [11·9–15·9]), Tokelau (13·1% [11·4–15·2]), Samoa (12·9% [11·0–15·1]), Nauru (12·1% [10·5–13·8]), Federated States of Micronesia (10·6% [9·2–12·2]), and Kiribati (10·4% [9·0–11·8]; appendix figure S23).

Type-specific diabetes prevalence

Type 2 diabetes cases made up 96·0% (95% UI 95·1–96·8) of all diabetes cases. More than 90% of the age-standardised diabetes prevalence rate in every super-region was due to type 2 diabetes. In two regions, less than 90% of diabetes cases were due to type 2 diabetes: 86·4% (83·3–89·4) in Australasia and 89·3% (87·0–91·5) in western Europe. Type 2 diabetes prevalence made up more than 80% of total diabetes cases in all 204 countries and territories and more than 90% of total diabetes cases in 183 (89·7%) countries and territories. There was no difference in type-specific breakdown by sex.

Total diabetes burden: YLLs, YLDs, and DALYs

Globally, there were 37·8 million (95% UI 35·4–40·2) total diabetes-related YLLs and 41·4 million (29·5–55·4) YLDs, yielding 79·2 million (67·8–92·5) DALYs due to diabetes in 2021 (table; appendix table S21). Type 2 diabetes made up 94·0% (93·6–94·6) of diabetes YLLs, 96·6% (96·0–97·3) of YLDs, and 95·4% (94·9–95·9) of DALYs. The global age-standardised diabetes DALY rate was 915·0 (782·6–1067·5) per 100 000, the YLL rate was 437·4 (409·2–464·1) per 100 000, and the YLD rate was 477·6 (340·7–637·4) per 100 000.

The age-standardised DALY rate was more than 1000 per 100 000 in four GBD super-regions: Latin America and Caribbean, sub-Saharan Africa, north Africa and the Middle East, and south Asia. Regionally, the age-standardised DALY rate varied from a high of 3577·0 (95% UI 3157·0–4120·5) per 100 000 in Oceania to a low of 511·8 (402·0–648·3) per 100 000 in western Europe. At the country level, Fiji had the highest age-standardised DALY rate, at 7333·9 (6066·7–8776·7) per 100 000. In 47 (23·0%) countries and territories, age-standardised DALY rates were greater than 2000 per 100 000 (table).

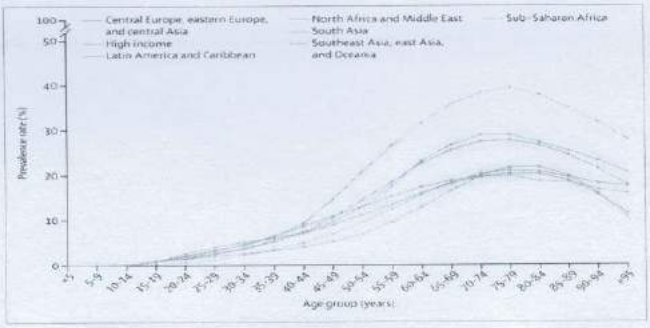

Figure 2: Prevalence of total diabetes by age and GBD super-region in 2021
The shaded areas represent 95% uncertainty intervals. GBD=Global Burden of Diseases, Injuries, and Risk Factors Study

Type 2 diabetes risk factors

In 2021, 58·9 million (95% UI 44·2–73·9) DALYs or 76·5% (58·0–87·5) of DALYs due to type 2 diabetes were attributable to risk factors. Of the 16 risk factors we analysed, high BMI was the primary risk factor for type 2 diabetes worldwide, accounting for 52·2% (25·5–71·8) of global type 2 diabetes DALYs. Among the other risk factor groups, dietary risks combined accounted for 25·7% (8·6–40·7), environmental or occupational risks combined accounted for 19·6% (12·7–26·5), tobacco use accounted for 12·1% (4·5–20·9), low physical activity accounted for 7·4% (3·0–11·2), and alcohol use accounted for 1·8% (0·3–3·9) of type 2 diabetes DALYs.

High BMI contributed more than 60% of type 2 diabetes DALYs in three super-regions: north Africa and the Middle East; Latin America and Caribbean; and central Europe, eastern Europe, and central Asia. Among the 21 regions analysed, the proportion of type 2 diabetes DALYs due to high BMI ranged from 68·0% (95% UI 37·8–85·8) in north Africa and the Middle East to 39·5% (17·1–58·4) in south Asia. High BMI contributed more than 60% of DALYs in 11 other regions: central Latin America, central Asia, southern Latin America, eastern Europe, southern sub-Saharan Africa, high-income North America, Australasia, tropical Latin America, central Europe, Andean Latin America, and Oceania. In south Asia, high BMI contributed less than 40% of type 2 diabetes DALYs. High BMI contributed more than 50% of DALYs in 167 (81·9%) countries and territories.

The proportion of global type 2 diabetes DALYs attributable to high BMI increased by 24·3% (95% UI 18·5 to 30·4), from 42·2% (19·8 to 59·9) in 1990 to 52·2% (25·5 to 71·8) in 2021. Although there were increases in

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	DALY count in 2021 (thousands)	Percentage change in DALY count, 1990–2021 (%)	Age-standardised DALY rate in 2021 (per 100 000)	Percentage change in age-standardised DALY rate, 1990–2021 (%)
Global	**79 200 (67 800 to 92 500)**	**189·8% (171·1 to 203·4)**	**915·0 (782·6 to 1067·4)**	**38·6% (29·7 to 45·3)**
Central Europe, eastern Europe, and central Asia	**4370 (3670 to 5230)**	**126·9% (119·3 to 132·4)**	**700·0 (588·3 to 840·2)**	**70·8% (65·4 to 75·1)**
Central Asia	800 (625 to 970)	236·0% (211·0 to 257·1)	923·6 (780·8 to 1119·9)	96·6% (82·6 to 108·9)
Armenia	32·3 (27·0 to 39·3)	52·3% (38·8 to 67·0)	771·2 (646·7 to 942·3)	4·5% (−4·7 to 14·9)
Azerbaijan	97·7 (77·0 to 122)	249·3% (196·6 to 319·7)	870·6 (689·0 to 1090·4)	72·0% (45·0 to 108·0)
Georgia	49·4 (40·1 to 60·8)	57·6% (42·9 to 78·0)	903·5 (732·2 to 1121·7)	81·4% (65·6 to 104·6)
Kazakhstan	144 (111 to 180)	142·5% (118·1 to 164·1)	750·2 (581·8 to 932·6)	70·4% (53·5 to 85·2)
Kyrgyzstan	30·8 (23·7 to 38·0)	199·3% (172·8 to 226·4)	560·7 (436·3 to 696·9)	76·5% (61·8 to 91·6)
Mongolia	17·2 (13·6 to 21·1)	337·2% (268·5 to 419·9)	564·3 (448·0 to 688·7)	76·7% (48·4 to 112·3)
Tajikistan	52·6 (43·1 to 64·1)	237·8% (182·8 to 307·7)	801·9 (659·3 to 955·8)	63·2% (36·7 to 96·7)
Turkmenistan	44·3 (35·8 to 53·2)	357·2% (286·9 to 446·2)	929·3 (757·9 to 1110·5)	112·3% (80·2 to 153·5)
Uzbekistan	341 (292 to 411)	479·8% (418·0 to 538·7)	1147·1 (980·2 to 1375·1)	150·9% (124·3 to 175·8)
Central Europe	1550 (1250 to 1890)	73·7% (63·6 to 82·0)	748·1 (598·1 to 913·7)	23·0% (15·3 to 29·4)
Albania	17·8 (13·7 to 23·2)	147·8% (119·1 to 175·6)	417·5 (323·5 to 544·8)	26·9% (12·3 to 40·5)
Bosnia and Herzegovina	75·2 (61·2 to 91·0)	171·1% (134·0 to 204·2)	1253·6 (1022·6 to 1527·9)	88·3% (62·4 to 111·5)
Bulgaria	115 (89·8 to 138)	35·5% (23·2 to 48·7)	868·1 (675·6 to 1051·7)	24·6% (13·5 to 36·7)
Croatia	62·7 (49·6 to 75·9)	73·0% (58·1 to 84·2)	743·1 (580·3 to 903·3)	29·7% (18·3 to 37·8)
Czechia	171 (136 to 211)	106·7% (87·4 to 125·6)	829·8 (659·6 to 1029·7)	35·7% (22·9 to 47·6)
Hungary	136 (109 to 170)	55·8% (43·0 to 69·8)	747·5 (594·9 to 932·2)	22·5% (12·9 to 32·3)
Montenegro	8·79 (7·12 to 10·7)	118·1% (95·9 to 139·0)	896·2 (728·1 to 1091·2)	39·7% (26·3 to 52·0)
North Macedonia	41·7 (32·6 to 51·1)	153·4% (116·8 to 190·3)	1268·1 (1000·4 to 1554·4)	46·4% (26·1 to 68·5)
Poland	520 (427 to 632)	26·6% (66·0 to 86·6)	764·5 (626·8 to 926·1)	13·5% (6·0 to 20·7)
Romania	167 (130 to 208)	47·7% (33·0 to 61·6)	482·9 (374·8 to 602·4)	18·8% (5·3 to 30·8)
Serbia	161 (127 to 197)	73·7% (56·3 to 95·3)	1020·2 (794·1 to 1249·1)	24·1% (11·2 to 40·2)
Slovakia	52·0 (40·1 to 64·2)	62·0% (43·5 to 79·3)	565·3 (438·2 to 700·8)	4·8% (−7·5 to 15·4)
Slovenia	22·1 (17·5 to 27·6)	68·7% (54·6 to 82·3)	540·8 (423·5 to 678·6)	0·0% (−9·2 to 8·5)
Eastern Europe	2020 (1730 to 2370)	153·9% (144·0 to 165·1)	596·8 (508·3 to 706·5)	104·6% (97·4 to 112·7)
Belarus	52·3 (41·0 to 65·9)	59·8% (44·9 to 73·7)	353·4 (281·1 to 444·3)	36·7% (25·0 to 48·6)
Estonia	14·5 (11·9 to 18·0)	130·0% (115·0 to 147·2)	635·2 (517·4 to 798·5)	96·5% (84·4 to 110·4)
Latvia	23·8 (19·8 to 28·8)	105·3% (90·9 to 119·9)	702·4 (588·1 to 852·5)	104·7% (89·8 to 120·0)
Lithuania	26·9 (22·3 to 33·3)	132·9% (111·9 to 149·3)	551·1 (454·0 to 687·3)	107·7% (90·0 to 123·5)
Moldova	36·6 (29·3 to 46·3)	95·9% (81·0 to 112·7)	668·0 (537·5 to 847·3)	62·0% (49·9 to 75·0)
Russia	1580 (1370 to 1830)	206·7% (191·9 to 225·4)	671·2 (583·8 to 780·4)	131·4% (121·6 to 145·0)
Ukraine	285 (217 to 354)	42·8% (29·1 to 56·9)	409·4 (312·0 to 504·9)	38·4% (24·3 to 52·3)
High income	**12 800 (10 200 to 15 700)**	**114·7% (94·6 to 132·5)**	**676·9 (536·0 to 839·8)**	**31·6% (19·1 to 43·4)**
Australasia	226 (183 to 288)	140·8% (122·7 to 164·8)	469·2 (378·1 to 602·4)	15·3% (5·5 to 27·1)
Australia	188 (152 to 240)	148·1% (126·5 to 175·4)	482·3 (373·1 to 597·4)	17·8% (7·1 to 32·0)
New Zealand	38·6 (30·8 to 46·9)	111·0% (91·4 to 126·4)	503·2 (399·3 to 616·2)	4·6% (−5·1 to 13·6)
High-income Asia Pacific	2340 (1780 to 2980)	133·5% (110·4 to 154·9)	642·5 (487·3 to 829·0)	29·3% (14·9 to 42·3)
Brunei	8·37 (7·00 to 9·97)	213·7% (155·8 to 259·6)	2279·7 (1946·8 to 2686·7)	−7·2% (−23·2 to 5·8)
Japan	1400 (1050 to 1800)	99·3% (83·1 to 118·5)	512·8 (381·4 to 665·9)	21·4% (8·4 to 34·5)
Singapore	56·7 (40·2 to 78·0)	179·9% (133·2 to 221·4)	661·1 (467·9 to 910·6)	−21·2% (−34·5 to −9·3)
South Korea	879 (670 to 1130)	215·6% (172·8 to 257·4)	966·4 (737·7 to 1251·9)	16·4% (0·0 to 33·5)
High-income North America	5470 (4400 to 6580)	170·0% (147·7 to 188·3)	928·6 (744·8 to 1172·5)	53·6% (40·6 to 63·6)
Canada	435 (335 to 562)	205·2% (168·7 to 246·4)	668·1 (519·4 to 859·5)	49·9% (32·1 to 69·8)
Greenland	0·361 (0·282 to 0·435)	116·5% (91·6 to 180·6)	492·5 (386·3 to 591·1)	21·3% (−0·3 to 44·5)
USA	5040 (4060 to 6010)	168·1% (145·4 to 185·2)	958·5 (770·8 to 1150·9)	53·9% (41·0 to 63·6)
Southern Latin America	648 (524 to 802)	105·9% (86·1 to 126·9)	762·3 (617·3 to 946·0)	12·7% (1·6 to 24·4)
Argentina	426 (350 to 522)	81·8% (62·6 to 99·7)	780·1 (639·2 to 957·3)	8·0% (−3·5 to 18·7)
Chile	183 (146 to 233)	205·6% (172·5 to 248·0)	725·1 (577·6 to 923·6)	24·1% (10·7 to 41·0)
Uruguay	38·7 (32·3 to 47·0)	90·9% (76·4 to 111·7)	753·0 (623·6 to 919·4)	42·7% (31·5 to 58·3)

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	DALY count in 2021 (thousands)	Percentage change in DALY count, 1990–2021 (%)	Age-standardised DALY rate in 2021 (per 100 000)	Percentage change in age-standardised DALY rate, 1990–2021 (%)
(Continued from previous page)				
Western Europe	4020 (3280 to 5030)	62·6% (47·1 to 77·4)	511·8 (402·0 to 648·3)	13·2% (1·6 to 25·9)
Andorra	0·724 (0·579 to 0·941)	212·5% (148·7 to 282·8)	510·6 (404·9 to 665·7)	22·8% (–2·3 to 50·4)
Austria	66·6 (54·3 to 79·9)	49·2% (33·0 to 65·7)	402·0 (322·2 to 492·3)	3·5% (–9·2 to 16·9)
Belgium	96·7 (73·1 to 129)	61·1% (41·8 to 80·6)	494·3 (372·4 to 669·1)	19·6% (4·3 to 35·0)
Cyprus	17·2 (14·2 to 21·5)	58·3% (36·0 to 79·9)	823·5 (722·7 to 1091·7)	–39·6% (–48·3 to –31·4)
Denmark	46·1 (38·3 to 55·6)	76·9% (59·3 to 94·3)	440·8 (359·4 to 538·1)	24·0% (11·3 to 37·0)
Finland	55·7 (41·6 to 72·8)	101·8% (87·3 to 116·7)	527·7 (427·5 to 756·2)	39·3% (28·1 to 50·6)
France	426 (345 to 524)	90·3% (74·4 to 108·1)	351·7 (278·2 to 445·7)	25·0% (13·1 to 37·8)
Germany	804 (662 to 966)	56·8% (40·0 to 73·5)	482·1 (390·3 to 593·3)	35·1% (2·3 to 28·9)
Greece	102 (77·2 to 132)	88·6% (77·0 to 101·5)	534·8 (359·5 to 703·1)	42·6% (32·1 to 52·7)
Iceland	2·10 (1·57 to 2·72)	188·7% (156·8 to 211·8)	408·8 (301·2 to 540·4)	55·7% (38·0 to 68·4)
Ireland	27·7 (21·4 to 36·3)	81·2% (58·9 to 104·1)	386·6 (295·5 to 505·8)	0·8% (–11·6 to 13·9)
Israel	81·9 (67·3 to 98·8)	159·1% (141·9 to 176·2)	690·7 (567·3 to 839·3)	4·7% (–2·4 to 11·7)
Italy	665 (557 to 792)	31·0% (20·5 to 42·4)	521·1 (422·4 to 637·8)	–11·5% (–20·3 to –2·5)
Luxembourg	4·30 (3·31 to 5·48)	109·4% (81·7 to 139·5)	440·0 (336·1 to 565·0)	11·9% (–3·5 to 23·1)
Malta	6·28 (5·03 to 7·94)	127·8% (96·4 to 163·9)	738·0 (585·7 to 953·5)	13·0% (–3·7 to 32·2)
Monaco	0·283 (0·214 to 0·359)	129·5% (104·8 to 154·5)	375·5 (275·0 to 478·4)	72·1% (61·5 to 94·7)
Netherlands	137 (111 to 171)	32·3% (17·1 to 51·7)	445·2 (355·9 to 563·0)	–16·5% (–26·3 to –3·8)
Norway	36·8 (29·4 to 45·5)	54·1% (46·2 to 61·4)	433·2 (339·1 to 545·4)	7·0% (1·0 to 12·8)
Portugal	157 (124 to 199)	68·2% (48·4 to 90·8)	736·1 (573·1 to 952·9)	6·9% (–7·6 to 22·3)
San Marino	0·232 (0·179 to 0·300)	167·6% (132·4 to 204·5)	413·3 (313·5 to 540·1)	47·9% (27·4 to 65·2)
Spain	554 (423 to 729)	62·8% (43·1 to 82·2)	690·1 (491·1 to 868·0)	1·3% (–12·3 to 14·6)
Sweden	84·0 (68·2 to 102)	58·2% (45·1 to 70·5)	465·0 (365·4 to 577·8)	15·9% (5·9 to 25·1)
Switzerland	89·2 (68·0 to 118)	75·4% (53·4 to 97·7)	578·8 (435·7 to 770·7)	12·7% (–1·5 to 26·7)
UK	601 (458 to 764)	92·4% (70·3 to 111·6)	580·3 (431·2 to 751·1)	53·5% (34·0 to 70·1)
Latin America and Caribbean	**9160 (7850 to 10 600)**	**193·1% (177·6 to 205·7)**	**1446·1 (1240·9 to 1673·7)**	**10·1% (4·1 to 15·0)**
Andean Latin America	582 (473 to 707)	290·7% (245·4 to 344·7)	962·1 (782·4 to 1166·8)	40·5% (24·1 to 60·0)
Bolivia	142 (115 to 177)	257·4% (198·9 to 343·6)	1482·2 (1205·0 to 1828·0)	27·0% (6·5 to 57·4)
Ecuador	206 (168 to 252)	359·9% (300·1 to 413·6)	1257·7 (1027·0 to 1532·8)	57·6% (37·0 to 76·0)
Peru	233 (183 to 290)	264·7% (208·3 to 330·1)	678·6 (532·1 to 844·7)	34·8% (13·5 to 58·7)
Caribbean	924 (774 to 1140)	124·6% (105·5 to 146·5)	1732·1 (1442·8 to 2116·7)	12·9% (3·7 to 23·9)
Antigua and Barbuda	2·36 (2·01 to 2·82)	122·3% (104·1 to 142·7)	2202·5 (1875·0 to 2613·0)	6·6% (–1·9 to 16·3)
The Bahamas	7·60 (6·26 to 9·55)	166·7% (136·4 to 206·8)	1759·3 (1452·3 to 2185·9)	2·8% (–10·7 to 17·8)
Barbados	9·94 (8·16 to 12·1)	61·1% (38·7 to 86·2)	2015·7 (1652·9 to 2424·1)	–7·9% (–20·6 to 6·8)
Belize	6·79 (5·71 to 8·08)	343·1% (303·1 to 388·5)	2082·4 (1767·0 to 2459·3)	35·5% (24·4 to 48·1)
Bermuda	1·15 (0·906 to 1·41)	68·5% (45·8 to 89·5)	928·4 (732·2 to 1144·6)	–14·4% (–26·1 to –3·1)
Cuba	149 (116 to 196)	68·8% (50·4 to 88·4)	806·9 (626·1 to 1061·3)	–5·0% (–15·6 to 6·4)
Dominica	2·35 (1·95 to 2·77)	60·0% (42·9 to 77·2)	2592·0 (2151·2 to 3058·7)	23·4% (10·5 to 37·1)
Dominican Republic	159 (126 to 192)	304·1% (244·9 to 357·3)	1566·3 (1244·2 to 1882·6)	67·1% (42·6 to 89·0)
Grenada	3·40 (2·91 to 4·01)	94·6% (74·3 to 115·1)	2908·0 (2508·7 to 3409·0)	16·6% (5·2 to 27·8)
Guyana	24·4 (19·2 to 29·3)	108·5% (79·0 to 139·9)	3427·6 (2755·8 to 4160·9)	25·6% (8·3 to 43·2)
Haiti	242 (196 to 320)	142·2% (91·1 to 198·8)	2931·0 (2369·9 to 3870·4)	6·9% (–15·6 to 30·8)
Jamaica	65·4 (53·5 to 77·9)	90·0% (58·7 to 123·0)	2115·9 (1729·3 to 2520·6)	9·5% (–8·7 to 28·4)
Puerto Rico	123 (99·5 to 154)	94·1% (76·1 to 112·1)	1934·2 (1541·0 to 2440·5)	9·9% (–0·2 to 22·0)
Saint Kitts and Nevis	1·49 (1·22 to 1·81)	78·1% (50·3 to 103·0)	2031·5 (1681·0 to 2430·3)	–11·9% (–23·4 to –1·9)
Saint Lucia	5·27 (4·28 to 6·35)	107·4% (85·5 to 132·6)	2309·0 (1874·8 to 2774·3)	–19·1% (–27·7 to –9·0)
Saint Vincent and the Grenadines	3·86 (3·24 to 4·57)	85·5% (63·5 to 107·5)	2712·3 (2301·1 to 3218·6)	–4·2% (–15·2 to 6·8)
Suriname	14·0 (11·0 to 16·7)	243·1% (194·0 to 289·5)	2140·5 (1695·3 to 2537·5)	44·4% (23·5 to 64·1)
Trinidad and Tobago	67·3 (54·7 to 80·9)	100·5% (73·5 to 132·4)	3468·0 (2824·5 to 4171·5)	–10·8% (–27·8 to 3·3)
Virgin Islands	3·59 (2·76 to 4·41)	145·7% (107·8 to 182·0)	2082·7 (1591·3 to 2557·8)	27·3% (5·9 to 44·1)

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	DALY count in 2021 (thousands)	Percentage change in DALY count, 1990–2021 (%)	Age-standardised DALY rate in 2021 (per 100 000)	Percentage change in age-standardised DALY rate, 1990–2021 (%)
(Continued from previous page)				
Central Latin America	4810 (4120 to 5540)	222·5% (202·5 to 239·1)	1865·9 (1601·9 to 2146·4)	13·9% (6·7 to 20·1)
Colombia	470 (362 to 590)	180·0% (148·7 to 207·6)	841·3 (647·5 to 1057·6)	−2·6% (−14·5 to 7·4)
Costa Rica	59·1 (46·2 to 74·5)	334·4% (301·0 to 362·0)	1074·4 (841·0 to 1353·2)	47·4% (36·0 to 58·1)
El Salvador	99·5 (81·8 to 119)	269·1% (224·2 to 328·5)	1625·6 (1333·0 to 1942·8)	93·2% (70·3 to 125·1)
Guatemala	277 (233 to 323)	737·3% (662·0 to 836·9)	2377·2 (2006·3 to 2777·2)	212·5% (182·1 to 250·2)
Honduras	100 (80·4 to 126)	454·8% (386·5 to 533·2)	1434·0 (1156·8 to 1787·3)	85·0% (63·4 to 111·4)
Mexico	3160 (2720 to 3530)	192·5% (173·7 to 206·9)	2451·3 (2122·5 to 2733·0)	−5·3% (−14 to 10·5)
Nicaragua	76·5 (63·0 to 95·4)	329·5% (283·2 to 379·5)	1498·1 (1244·5 to 1854·1)	47·1% (30·3 to 64·3)
Panama	56·7 (45·0 to 67·2)	337·9% (289·3 to 388·2)	1265·1 (1010·5 to 1510·6)	56·3% (38·3 to 74·7)
Venezuela	502 (402 to 605)	316·5% (256·3 to 384·2)	1597·3 (1280·8 to 1922·2)	39·1% (19·0 to 62·0)
Tropical Latin America	2850 (2460 to 3290)	165·0% (153·9 to 177·1)	1092·4 (945·9 to 1261·3)	−0·6% (−5·1 to 3·9)
Brazil	2740 (2370 to 3160)	159·7% (149·2 to 172·1)	1075·2 (931·4 to 1239·0)	−2·7% (−7·1 to 2·1)
Paraguay	110 (89·2 to 136)	429·0% (348·8 to 553·8)	1831·6 (1487·5 to 2246·3)	107·8% (75·9 to 157·6)
North Africa and Middle East	**6650 (5330 to 8120)**	**348·3% (296·1 to 389·1)**	**1338·3 (1087·5 to 1632·2)**	**67·5% (48·0 to 82·5)**
North Africa and Middle East	6650 (5330 to 8120)	348·3% (296·1 to 389·1)	1338·3 (1087·5 to 1632·2)	67·5% (48·0 to 82·5)
Afghanistan	366 (282 to 462)	327·2% (250·3 to 393·8)	2099·1 (1634·4 to 2633·7)	93·7% (59·7 to 122·1)
Algeria	417 (320 to 545)	481·2% (417·5 to 544·4)	1148·4 (855·6 to 1413·8)	101·2% (80·0 to 121·4)
Bahrain	37·5 (30·6 to 45·2)	693·4% (565·3 to 819·2)	3125·4 (2614·7 to 3660·0)	21·9% (4·0 to 42·8)
Egypt	1220 (993 to 1440)	386·2% (306·5 to 462·3)	1713·4 (1406·1 to 2009·2)	122·4% (86·5 to 156·0)
Iran	780 (631 to 947)	426·2% (363·8 to 464·6)	961·3 (786·6 to 1158·7)	82·1% (58·8 to 95·7)
Iraq	608 (453 to 754)	369·3% (286·7 to 449·5)	2193·8 (1688·9 to 2691·6)	45·9% (21·7 to 70·0)
Jordan	148 (117 to 184)	491·2% (391·1 to 606·2)	1792·3 (1459·9 to 2220·5)	1·6% (−16·3 to 22·0)
Kuwait	62·7 (46·2 to 80·4)	682·3% (600·7 to 758·0)	1666·7 (1268·4 to 2159·2)	60·8% (44·7 to 77·9)
Lebanon	80·8 (64·1 to 101)	177·6% (132·3 to 219·9)	1481·5 (1174·1 to 1856·7)	18·5% (−0·5 to 37·6)
Libya	84·9 (67·4 to 108)	560·4% (464·1 to 655·3)	1392·5 (1122·5 to 1767·4)	123·5% (88·7 to 154·7)
Morocco	559 (438 to 693)	451·5% (389·5 to 516·7)	1592·8 (1247·8 to 1970·3)	132·6% (110·1 to 161·2)
Oman	39·6 (32·9 to 47·6)	302·2% (205·7 to 377·3)	1656·2 (1410·2 to 1957·2)	30·2% (−1·7 to 57·6)
Palestine	47·1 (40·0 to 55·8)	278·8% (210·9 to 343·0)	1782·5 (1530·8 to 2084·5)	29·4% (5·3 to 51·9)
Qatar	34·2 (26·3 to 44·2)	1235·6% (946·6 to 1505·7)	2217·1 (1280·0 to 2815·6)	6·4% (−18·6 to 27·2)
Saudi Arabia	391 (306 to 489)	541·8% (375·2 to 679·2)	1456·8 (1179·8 to 1781·1)	64·3% (21·1 to 98·2)
Sudan	225 (172 to 279)	281·2% (216·0 to 341·0)	989·8 (784·3 to 1227·1)	81·0% (52·5 to 106·7)
Syria	147 (116 to 185)	239·1% (179·9 to 299·4)	1090·0 (864·6 to 1369·0)	48·9% (23·0 to 75·6)
Tunisia	152 (114 to 192)	451·4% (329·7 to 525·9)	1111·2 (838·2 to 1392·4)	116·2% (86·2 to 145·3)
Türkiye	1010 (833 to 1250)	184·1% (136·4 to 236·1)	1074·0 (888·7 to 1319·3)	10·8% (−2·9 to 31·1)
United Arab Emirates	82·2 (62·2 to 107)	1161·8% (863·5 to 1360·7)	1486·3 (1176·6 to 1815·6)	10·8% (−15·9 to 31·8)
Yemen	132 (101 to 173)	337·6% (264·5 to 424·4)	800·4 (616·8 to 1059·2)	59·1% (31·5 to 89·3)
South Asia	**18 000 (15 500 to 20 500)**	**267·0% (230·1 to 299·6)**	**1153·4 (999·6 to 1306·6)**	**44·6% (30·0 to 58·2)**
South Asia	18 000 (15 500 to 20 500)	267·0% (230·1 to 299·6)	1153·4 (999·6 to 1306·6)	44·6% (30·0 to 58·2)
Bangladesh	1650 (1350 to 2060)	282·6% (226·1 to 342·0)	1148·7 (939·1 to 1425·3)	37·8% (18·1 to 61·4)
Bhutan	6·50 (5·16 to 7·83)	210·3% (152·9 to 284·2)	1061·5 (851·2 to 1278·8)	41·6% (17·3 to 75·5)
India	13 900 (11 900 to 15 800)	262·9% (225·9 to 304·6)	1106·2 (952·1 to 1250·3)	44·0% (28·6 to 61·8)
Nepal	304 (245 to 377)	285·3% (224·2 to 373·6)	1240·2 (1009·1 to 1516·9)	63·5% (37·0 to 102·0)
Pakistan	2070 (1650 to 2420)	283·3% (232·6 to 347·4)	1604·6 (1301·4 to 1859·8)	80·1% (57·6 to 110·9)
Southeast Asia, east Asia, and Oceania	**20 800 (17 600 to 24 300)**	**187·7% (165·6 to 206·9)**	**735·6 (621·3 to 861·9)**	**25·3% (15·2 to 33·8)**
East Asia	12 400 (9900 to 15 000)	171·1% (144·8 to 190·5)	592·5 (472·1 to 720·7)	24·0% (9·3 to 35·3)
China	11 700 (9310 to 14 200)	172·9% (145·5 to 194·1)	581·5 (460·5 to 707·6)	25·2% (9·7 to 37·0)
North Korea	257 (205 to 326)	176·8% (127·3 to 229·0)	764·9 (611·9 to 962·9)	41·4% (16·1 to 66·3)
Taiwan (province of China)	406 (337 to 489)	124·7% (104·9 to 143·5)	1002·2 (832·0 to 1210·2)	−8·4% (−17·1 to −0·1)

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(Continued from previous page)				
Oceania	308 (269 to 355)	213·9% (151·7 to 270·2)	3577·0 (3157·0 to 4120·5)	22·5% (−1·4 to 48·4)
American Samoa	2·24 (1·92 to 2·62)	208·3% (159·3 to 263·7)	4307·8 (3692·1 to 4989·9)	49·8% (26·4 to 75·9)
Cook Islands	1·03 (0·868 to 1·18)	91·1% (56·2 to 120·5)	4029·3 (3361·3 to 4643·7)	−2·0% (−19·6 to 12·9)
Federated States of Micronesia	3·19 (2·55 to 3·85)	138·3% (93·8 to 194·0)	3933·7 (3207·9 to 4681·1)	50·1% (22·6 to 85·2)
Fiji	59·9 (49·1 to 72·5)	182·8% (118·8 to 252·7)	7333·9 (6066·7 to 8776·7)	38·5% (7·5 to 71·5)
Guam	2·54 (2·06 to 3·03)	137·3% (110·1 to 163·5)	1289·1 (1045·8 to 1545·5)	0·1% (−12·1 to 11·4)
Kiribati	4·42 (3·61 to 5·50)	166·2% (112·0 to 238·9)	5510·6 (4508·6 to 6709·3)	36·3% (6·5 to 69·5)
Marshall Islands	2·45 (1·89 to 3·17)	293·0% (206·7 to 367·4)	5750·8 (4384·5 to 7411·2)	59·8% (32·2 to 101·0)
Nauru	0·272 (0·220 to 0·341)	68·7% (35·2 to 117·5)	4870·4 (4039·5 to 5855·2)	38·6% (12·5 to 78·5)
Niue	0·0887 (0·0723 to 0·105)	54·6% (29·8 to 92·5)	4095·0 (3321·0 to 4823·2)	62·3% (27·9 to 89·6)
Northern Mariana Islands	1·38 (1·17 to 1·70)	228·5% (159·0 to 283·2)	2199·4 (1871·3 to 2680·6)	14·1% (−8·5 to 34·0)
Palau	0·891 (0·763 to 1·08)	227·0% (169·0 to 309·2)	3726·9 (3210·3 to 4536·0)	43·4% (19·2 to 78·6)
Papua New Guinea	187 (157 to 225)	239·8% (143·5 to 337·0)	3062·0 (2597·2 to 3685·0)	18·6% (−14·6 to 52·3)
Samoa	5·48 (4·61 to 6·52)	152·3% (109·8 to 200·3)	3390·8 (2876·6 to 4057·4)	44·0% (20·0 to 70·2)
Solomon Islands	13·6 (11·0 to 16·9)	268·7% (154·5 to 421·5)	3473·1 (2878·9 to 4232·1)	46·6% (2·6 to 97·3)
Tokelau	0·0503 (0·0410 to 0·0600)	49·1% (25·5 to 79·2)	3345·0 (2747·6 to 3948·8)	31·9% (11·1 to 56·3)
Tonga	2·99 (2·44 to 3·46)	90·0% (55·1 to 130·2)	3640·0 (2966·9 to 4203·8)	35·5% (10·8 to 63·6)
Tuvalu	0·357 (0·300 to 0·422)	93·5% (65·7 to 128·5)	3259·8 (2755·1 to 3847·4)	27·5% (9·7 to 49·6)
Vanuatu	6·01 (5·05 to 6·97)	301·3% (216·9 to 400·6)	3006·9 (2550·6 to 3504·3)	45·7% (16·0 to 80·1)
Southeast Asia	8100 (7220 to 9290)	216·6% (185·8 to 244·3)	1220·7 (1084·5 to 1393·3)	31·8% (19·3 to 43·3)
Cambodia	160 (130 to 199)	252·3% (173·7 to 337·9)	1205·1 (982·9 to 1497·6)	35·1% (5·7 to 66·9)
Indonesia	3570 (2190 to 2960)	224·0% (181·4 to 258·5)	1067·0 (913·7 to 1215·4)	48·7% (29·7 to 64·4)
Laos	69·4 (56·8 to 86·4)	159·1% (105·5 to 232·1)	1199·1 (1145·8 to 1723·4)	20·1% (−3·5 to 52·0)
Malaysia	318 (258 to 382)	224·1% (186·5 to 257·5)	1073·7 (879·7 to 1284·2)	7·3% (−5·2 to 19·7)
Maldives	3·22 (2·67 to 3·91)	188·6% (135·5 to 215·2)	867·6 (730·0 to 1043·3)	−21·9% (−35·1 to −9·8)
Mauritius	65·1 (59·2 to 72·6)	340·4% (317·6 to 360·7)	3480·5 (3163·3 to 3879·7)	87·5% (78·0 to 96·5)
Myanmar	1000 (832 to 1200)	119·9% (66·5 to 179·8)	1936·5 (1650·3 to 2388·4)	11·0% (−14·7 to 40·8)
Philippines	1190 (1080 to 1310)	268·2% (244·3 to 298·0)	1357·9 (1234·4 to 1488·3)	39·9% (31·5 to 50·7)
Seychelles	1·82 (1·44 to 2·31)	347·5% (295·4 to 398·0)	1524·8 (1215·2 to 1928·2)	114·8% (91·9 to 138·8)
Sri Lanka	529 (416 to 646)	301·7% (230·8 to 378·2)	1952·5 (1540·5 to 2378·8)	62·1% (34·1 to 91·5)
Thailand	1070 (847 to 1290)	248·5% (187·6 to 317·9)	996·4 (790·8 to 1194·2)	23·8% (2·0 to 49·2)
Timor-Leste	9·40 (7·69 to 11·5)	350·7% (255·7 to 461·0)	1051·4 (861·6 to 1284·4)	69·5% (35·9 to 110·2)
Viet Nam	1090 (913 to 1300)	217·6% (154·4 to 281·3)	1118·3 (935·5 to 1329·5)	33·1% (6·4 to 58·8)
Sub-Saharan Africa	**7560 (6720 to 8730)**	**175·6% (145·4 to 200·4)**	**1387·6 (1247·6 to 1589·2)**	**21·4% (7·9 to 31·6)**
Central sub-Saharan Africa	1060 (887 to 1270)	195·0% (138·8 to 260·6)	1631·3 (1376·3 to 1914·5)	14·7% (−6·8 to 39·8)
Angola	235 (191 to 292)	262·9% (175·0 to 356·7)	1650·6 (1379·4 to 2022·0)	15·8% (−11·8 to 45·6)
Central African Republic	57·6 (45·8 to 73·2)	137·2% (97·2 to 194·9)	2120·9 (1666·3 to 2574·1)	16·4% (−3·3 to 40·9)
Congo (Brazzaville)	61·8 (51·3 to 74·7)	194·8% (132·9 to 269·1)	1954·0 (1656·2 to 2322·6)	9·1% (−10·8 to 31·9)
Democratic Republic of the Congo	670 (554 to 803)	184·1% (115·7 to 259·5)	1548·2 (1287·0 to 1836·5)	14·7% (−11·5 to 45·7)
Equatorial Guinea	11·7 (9·02 to 15·7)	239·8% (153·6 to 347·4)	1903·0 (1507·2 to 2541·0)	21·1% (−6·2 to 57·5)
Gabon	26·8 (21·2 to 34·4)	166·8% (115·3 to 242·9)	2245·1 (1805·7 to 2846·3)	31·1% (5·9 to 65·6)
Eastern sub-Saharan Africa	2390 (2170 to 2720)	112·9% (93·2 to 140·7)	1197·0 (1080·8 to 1351·0)	−5·4% (−14·4 to 6·3)
Burundi	69·9 (54·8 to 96·1)	87·2% (49·5 to 135·8)	1248·1 (972·5 to 1719·7)	−10·7% (−29·9 to 15·4)
Comoros	7·38 (5·70 to 9·02)	143·7% (79·3 to 203·7)	1369·6 (1049·2 to 1675·2)	10·9% (−18·4 to 39·6)
Djibouti	9·15 (7·24 to 12·2)	445·9% (327·7 to 596·2)	1289·2 (1051·9 to 1683·3)	35·7% (5·1 to 71·2)
Eritrea	54·0 (41·8 to 68·4)	218·7% (159·8 to 288·4)	1606·5 (1256·2 to 2033·8)	16·5% (−3·3 to 36·7)
Ethiopia	573 (502 to 639)	31·3% (10·8 to 58·7)	1125·9 (989·3 to 1258·3)	−38·8% (−48·0 to −27·5)
Kenya	254 (221 to 302)	284·1% (224·3 to 367·9)	987·6 (865·7 to 1167·0)	38·8% (16·7 to 67·8)

(Table continues on next page)

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	DALY count in 2021 (thousands)	Percentage change in DALY count, 1990–2021 (%)	Age-standardised DALY rate in 2021 (per 100 000)	Percentage change in age-standardised DALY rate, 1990–2021 (%)
(Continued from previous page)				
Madagascar	144 (116 to 179)	159·9% (110·5 to 221·8)	1051·9 (849·2 to 1309·9)	16·4% (−4·5 to 44·4)
Malawi	113 (92·0 to 135)	118·1% (80·9 to 162·7)	1284·3 (1038·3 to 1531·5)	12·7% (−5·6 to 35·9)
Mozambique	204 (160 to 249)	175·0% (113·9 to 240·1)	1476·8 (1183·7 to 1765·7)	41·2% (11·5 to 73·7)
Rwanda	79·1 (57·6 to 106)	54·4% (19·1 to 90·6)	1126·8 (801·4 to 1517·9)	−26·1% (−42·4 to −10·2)
Somalia	142 (111 to 179)	225·8% (162·6 to 304·4)	1631·2 (1318·2 to 2027·8)	15·1% (−5·9 to 40·9)
South Sudan	71·7 (57·2 to 95·4)	125·8% (72·3 to 209·8)	1553·8 (1246·4 to 2083·2)	33·3% (1·3 to 82·9)
Tanzania	322 (270 to 382)	157·5% (115·3 to 210·6)	1090·2 (923·7 to 1291·3)	12·6% (−4·8 to 37·5)
Uganda	214 (167 to 286)	185·4% (105·5 to 261·7)	1239·5 (959·8 to 1659·6)	21·4% (−11·9 to 53·1)
Zambia	131 (105 to 162)	127·0% (110·8 to 250·6)	1499·3 (1194·8 to 1858·4)	10·5% (−13·5 to 36·6)
Southern sub-Saharan Africa	1290 (1190 to 1410)	260·9% (231·1 to 288·7)	2128·5 (1978·7 to 2333·3)	73·3% (59·6 to 86·1)
Botswana	25·0 (21·4 to 28·9)	191·9% (119·0 to 273·4)	1690·0 (1443·9 to 1932·7)	16·8% (−10·8 to 49·3)
Eswatini	20·8 (16·4 to 27·4)	244·5% (170·7 to 326·3)	3134·1 (2669·6 to 4250·2)	62·0% (32·3 to 129·0)
Lesotho	36·2 (28·3 to 44·8)	205·6% (134·9 to 330·9)	2711·4 (2145·2 to 3314·6)	131·9% (80·9 to 220·4)
Namibia	28·3 (22·2 to 35·6)	153·7% (99·6 to 217·1)	1901·2 (1501·7 to 2375·0)	22·3% (0·7 to 57·1)
South Africa	1030 (937 to 1140)	271·1% (239·4 to 298·5)	2150·8 (1962·4 to 2362·3)	71·5% (57·6 to 84·8)
Zimbabwe	145 (120 to 179)	258·5% (180·1 to 353·4)	1899·2 (1570·7 to 2329·5)	96·8% (55·1 to 145·3)
Western sub-Saharan Africa	2820 (2340 to 3340)	213·3% (166·3 to 259·3)	1245·7 (1058·9 to 1460·3)	33·8% (14·6 to 51·2)
Benin	82·6 (67·5 to 101)	311·2% (234·0 to 382·9)	1344·0 (1096·9 to 1632·3)	53·0% (25·9 to 76·3)
Burkina Faso	128 (105 to 157)	176·5% (111·4 to 239·5)	1119·2 (915·3 to 1359·2)	16·3% (−9·8 to 39·2)
Cabo Verde	6·20 (4·80 to 7·34)	418·7% (360·6 to 489·5)	1316·5 (1029·0 to 1552·2)	160·2% (132·2 to 194·7)
Cameroon	223 (168 to 285)	326·9% (242·2 to 462·6)	1533·2 (1181·5 to 1947·8)	44·5% (14·1 to 89·2)
Chad	87·3 (70·0 to 109)	282·3% (217·7 to 353·9)	1327·8 (977·1 to 1518·7)	69·1% (39·8 to 101·6)
Côte d'Ivoire	179 (145 to 219)	295·2% (214·4 to 377·1)	1306·9 (1127·7 to 1673·7)	45·6% (17·0 to 78·5)
The Gambia	16·0 (13·1 to 20·2)	376·0% (282·7 to 471·2)	1407·0 (1130·9 to 1778·1)	73·5% (39·4 to 105·8)
Ghana	283 (229 to 351)	380·0% (278·8 to 499·8)	1502·0 (1222·6 to 1838·6)	82·2% (43·4 to 129·8)
Guinea	80·5 (65·3 to 98·3)	164·9% (110·2 to 232·3)	1267·2 (1043·5 to 1556·9)	50·7% (20·2 to 88·7)
Guinea-Bissau	15·6 (13·0 to 19·0)	152·0% (111·1 to 218·8)	1247·0 (1463·8 to 2118·2)	36·7% (11·8 to 69·5)
Liberia	36·3 (27·8 to 46·2)	228·6% (162·6 to 289·8)	1427·7 (1112·3 to 1804·9)	54·0% (24·9 to 86·3)
Mali	176 (147 to 213)	241·1% (182·5 to 795·4)	1629·9 (1415·5 to 2038·6)	48·7% (24·1 to 71·6)
Mauritania	24·8 (19·2 to 31·6)	170·0% (119·1 to 249·5)	1065·7 (839·4 to 1351·9)	25·5% (1·9 to 61·6)
Niger	100 (79·4 to 131)	294·3% (224·2 to 375·0)	1015·3 (812·7 to 1335·0)	40·7% (18·9 to 68·3)
Nigeria	1140 (917 to 1380)	154·5% (106·4 to 213·1)	1103·6 (914·0 to 1298·9)	16·0% (−6·0 to 40·9)
São Tomé and Príncipe	1·17 (0·885 to 1·46)	201·2% (151·9 to 252·6)	911·6 (711·8 to 1136·5)	65·2% (48·7 to 89·5)
Senegal	138 (113 to 167)	270·8% (212·3 to 337·6)	1618·7 (1325·9 to 1973·4)	59·3% (33·4 to 87·1)
Sierra Leone	50·6 (41·3 to 63·4)	222·9% (164·9 to 291·0)	1151·4 (950·3 to 1419·0)	55·6% (29·9 to 93·5)
Togo	47·9 (38·1 to 61·5)	343·6% (270·5 to 446·9)	1085·9 (870·3 to 1387·9)	51·7% (25·6 to 83·5)

Data in parentheses are 95% uncertainty intervals. Count data are presented to three significant figures, and percentages and rates are presented to 1 decimal place. GBD=Global Burden of Diseases, Injuries, and Risk Factors Study. DALY=disability-adjusted life-year.

Table: **DALY counts and age-standardised DALY rates per 100 000 population and the corresponding percentage change in DALY counts and age-standardised DALY rates between 1990 and 2021 for diabetes globally, in 21 GBD regions and 204 countries**

every super-region, the largest change occurred in south Asia, with an increase of 58·0% (44·0 to 75·4). At the regional level, the increase in type 2 diabetes DALYs attributable to high BMI between 1990 and 2021 was greater than 45% in south Asia (58·0%; 44·0 to 75·4), central sub-Saharan Africa (48·8%; 35·8 to 61·2), and east Asia (45·7%; 33·5 to 57·3). Over this period, the proportion of DALYS due to high BMI increased in every country and territory, ranging from an increase of 77·2% (52·2 to 107·9) in Viet Nam to 1·3% (−1·5 to 4·1) in Czechia (figure 3).

Diabetes prevalence over time: 1990 to 2021, and forecasts to 2050

Between 1990 and 2021, the global age-standardised prevalence of diabetes increased by 90·5% (95% UI 85·8–93·6), from 3·2% (3·0–3·5) to 6·1% (5·8–6·5; appendix table S22). This increase exceeded 100% in two super-regions: north Africa and the Middle East (161·5%; 154·3–168·7) and the high-income super-region (114·8%; 109·6–119·7). Six regions (north Africa and the Middle East, high-income North America,

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central Asia, Oceania, Andean Latin America, and southern Latin America) showed a prevalence increase of more than 100% from 1990 to 2021, while six additional regions (western Europe, southern sub-Saharan Africa, eastern Europe, south Asia, high-income Asia Pacific, and central sub-Saharan Africa) showed an increase of more than 90%. The age-standardised diabetes prevalence increased by more than 100% in 97 (47·5%) of 204 countries and territories and by more than 200% in three countries and one territory: Egypt (284·3%; 262·7–305·9), Greenland (263·6%; 236·8–296·3), Timor-Leste (225·3%; 206·7–243·7), and Seychelles (211·5%; 193·5–230·7). The age-standardised diabetes prevalence increased by less than 30% in only two countries: Mexico (19·7% [16·7–22·4]) and the Philippines (29·1% [24·3–33·8]; appendix table S22).

Between 2021 and 2050, the global age-standardised total diabetes prevalence is expected to increase by 59·7% (95% UI 54·7–66·0), from 6·1% (5·8–6·5) to 9·8% (9·4–10·2), resulting in 1·31 billion (1·22–1·39) people living with diabetes in 2050, or an annualised rate of change of 3·31%. Of this increase, 49·6% is driven by trends in obesity, and the remaining 50·4% is driven by demographic shifts. The age-standardised diabetes prevalence is projected to be higher than 10% in two super-regions: north Africa and the Middle East (16·8% [16·1–17·6]) and Latin America and Caribbean (11·3% [10·8–11·9]). The age-standardised diabetes prevalence rate is projected to exceed 10% in 89 (43·6%) countries and territories and to surpass 20% in 24 (11·8%) countries and territories. Every country and territory in three regions—Oceania, north Africa and the Middle East, and central Latin America—is projected to have a diabetes prevalence rate exceeding 10% by 2050. In 13 of 18 countries and territories in Oceania, ten of 21 countries in north Africa and the Middle East, and one country in the Caribbean, the diabetes prevalence will be greater than 20% by 2050. There are no countries and territories where diabetes prevalence rates are expected to decrease (appendix table S23).

The projected increase in total diabetes prevalence is expected to be driven by type 2 diabetes. The age-standardised global prevalence of type 2 diabetes is projected to rise by 61·2% (95% UI 56·2–68·1), from 5·9% (5·5–6·3) in 2021 to 9·5% (9·0–9·9) in 2050, affecting more than 1·27 billion (1·19–1·35) people. This varies by super-region, from 82·7% (76·8–90·5) in north Africa and the Middle East to 30·3% (27·3–33·0) in the high-income region. Age-standardised type 2 diabetes prevalence will increase by more than 70% in six regions: north Africa and the Middle East (82·7%; 76·8–90·5), east Asia (80·1%; 72·1–89·2), central sub-Saharan Africa (79·9%; 72·4–89·9), southern sub-Saharan Africa (74·7%; 67·9–83·7), central Latin America (74·7%; 68·5–80·2), and Australasia (71·9%; 63·6–81·8). The age-standardised type 2 diabetes prevalence is projected to increase by more than 100% in 11 countries in three regions: seven countries (Oman, United Arab Emirates, Syria, Iran, Libya, Sudan, and Saudi Arabia) in north Africa and the Middle East, two countries (Kenya and Tanzania) in eastern sub-Saharan Africa, and two countries (Zimbabwe and Botswana) in southern sub-Saharan Africa. The age-standardised global prevalence of type 1 diabetes is expected to increase by 23·9% (95% UI 17·8–32·4), from 0·2% (0·2–0·3) in

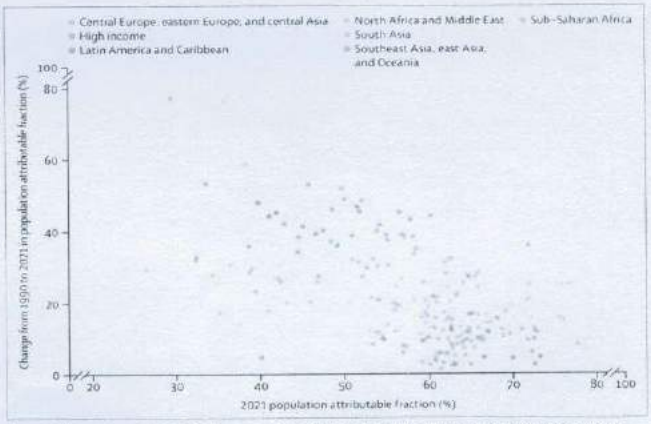

Figure 3: **Change from 1990 to 2021 in population attributable fraction for high BMI in relation to type 2 diabetes, by GBD super-region**
BMI=body-mass index. GBD=Global Burden of Diseases, Injuries, and Risk Factors Study.

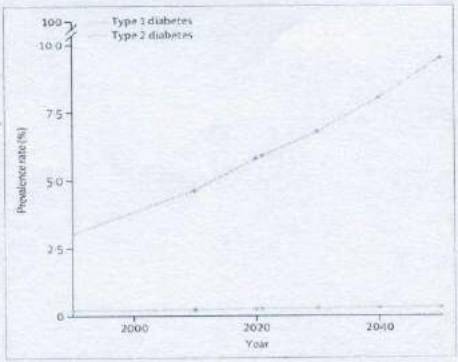

Figure 4: **Global age-standardised prevalence of type 1 and type 2 diabetes from 1990 through 2050 forecasts**
The shaded area represents 95% uncertainty intervals. Total diabetes is the sum of type 1 and type 2 diabetes.

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2021 to 0·3% (0·3–0·4) in 2050 (figure 4; appendix figure S26).

Discussion

The international community has become increasingly aware that diabetes is a monumental global health threat posing increasing challenges to public health and health-care systems worldwide. WHO has identified diabetes as one of three target diseases in its *WHO Global Action Plan for the Prevention and Control of NCDs*,[41] and the WHO Global Diabetes Compact was established in 2021 to improve access to health care for, and work closely with, those living with diabetes. The UN uses diabetes treatment as an indicator of countries' health-care systems when assessing universal health coverage objectives[42] and has established a target of reducing rates of premature death due to diabetes and other non-communicable diseases by a third by 2030, as detailed in Goal 3 of the UN Sustainable Development Goals.[43] To drive improvements in diabetes prevention and care, the *Lancet* Commission on diabetes[7] called for an increased reliance on high-quality data, with a focus on LMICs, to allow policy makers to better understand risks and define needs.

To contribute to this undertaking, as part of GBD we generated estimates of diabetes prevalence and burden, stratified by geographical and demographic factors; examined the contribution of leading risk factors; and forecast location-specific diabetes prevalence in 2050. There were an estimated 529 million people living with diabetes in 2021, a number we project will more than double to about 1·31 billion by 2050. The global age-standardised diabetes prevalence rate in 2021 was 6·1%, with highs of 9·3% in the north Africa and Middle East super-region and 12·3% in the Oceania region. Diabetes was especially prevalent in people aged 65 years and older in every location, but in some locations the prevalence rates were high even in younger adults, exceeding 10% among those aged 30–34 years in ten countries, all in Oceania.

Because diabetes prevalence rates are driven almost entirely by type 2 diabetes, which accounted for more than 96% of diabetes cases worldwide in 2021, much of the following discussion will focus on type 2 diabetes.

Our estimates showed high BMI to be the primary risk factor for type 2 diabetes, contributing more than 50% of global DALYs in 2021. The association between high BMI and type 2 diabetes has intensified in recent decades, with the proportion of global type 2 diabetes DALYs attributable to high BMI growing by nearly 25% between 1990 and 2021. By 2050, we project a global increase in age-standardised type 2 diabetes prevalence of more than 60%, with increases of more than 70% in six regions: north Africa and the Middle East, east Asia, central sub-Saharan Africa, southern sub-Saharan Africa, central Latin America, and Australasia.

Major behavioural shifts and changes in food systems contributing to high BMI include greater availability of shelf-stable and high-calorie products; limited financial and proximal access to healthy food options; increased consumption of ultra-processed foods[44] and fat, sugar, and animal products; and reductions in physical activity related to global work and transportation trends.[45] Particularly in low-income and middle-income populations, the shift away from a traditional diet to an industrialised one has been abrupt and is associated with considerable increases in nutrition-related non-communicable diseases such as type 2 diabetes.[46] In some instances, high type 2 diabetes prevalence rates might also be partly associated with a population-specific genetic disposition to developing diabetes.[47] A high diabetes burden in LMICs is also related to economic and sociopolitical challenges, including limited health spending on diabetes[10] and inadequate or incomplete coverage for pharmacological treatment. Fewer than one in ten people with diabetes in LMICs receive coverage for comprehensive diabetes treatment; Oceania, for example, has the lowest medication coverage in the world despite its very high prevalence rates.[7]

The regional variation in sex differences in age-standardised diabetes prevalence rates revealed by our estimates is probably also related to variation in patterns in and the impact of obesity on type 2 diabetes, socioeconomic factors, and biological and hormonal differences.[48] Evidence suggests that males might develop type 2 diabetes at lower BMI thresholds and might be more insulin resistant than females.[49] Moreover, in a study of diabetes treatment coverage in LMICs, females had better treatment coverage than males.[10] Conversely, obesity tends to be more common in females,[50] and diabetes treatments rarely account for reported differences in the risk of developing diabetes at different ages between males and females, which are likely to be due to the impact of a combination of genetic, hormonal, and psychosocial dimensions.[20]

Although obesity is theoretically reversible[51–53] and addressing it could provide the biggest opportunity to limit the advance of diabetes, current trends suggest that obesity rates are likely to continue to climb.[51] Various interventions and policies to address obesity have been developed and studied,[54–56] but no programme to date has shown long-term, sustained, population-level reductions in obesity.[57] This is probably because no strategy has attempted to deal with the multiple factors that potentially contribute to obesity. Creating change that relies on behavioural and structural shifts in interconnected, complex, and dynamic systems requires a multifaceted, long-term approach with contributions from policy makers, regulators, educators, public health officials, and the medical community.[58] This is clearly not a simple challenge.

In 2022, the WHO Global Diabetes Compact outlined five diabetes targets to reach by 2030,[59] focused on addressing metabolic risks, access to medication, and diagnosis. Although 77 countries, representing every region and socioeconomic level, have created

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recommendations, guidelines, and targets to monitor and control diabetes in their populations.[60–62] preparedness varies considerably between countries. A survey of 160 WHO member states revealed that approximately 60% have conducted national surveys of blood glucose concentrations, 50% have a diabetes registry, and 80% have an action plan in place.[63] Ultimately, effective testing, diagnosis, treatment, and diabetes control are lacking, particularly in LMICs.[17] As our forecasts suggest that nearly 50% of the increases in diabetes prevalence will be due to changing demographic profiles, countries will need to invest in health systems to handle the surge in expected patients.

The outlook for a healthy future is further marred by the lack of sustained progress in strategies designed to remediate diabetes.[64] Interventions that have yielded successful results for more than 2 years in people with type 2 diabetes involve bodyweight loss through aggressive control of calorie intake and physical activity or bariatric surgery.[65–68] Both options involve close oversight and are unlikely to be scalable at a population level globally. Pharmacological agents such as SGLT2 inhibitors and GLP1 agonists have shown some promising results in weight reduction and cardiovascular protection in individuals with type 2 diabetes,[69,70] but the viability of these interventions at the population level remains unclear. Moreover, disparities in medication coverage remain widespread.[71b] Early diagnosis, patient education, and regular visits to health-care providers can offer clinicians, public health professionals, and policy makers opportunities for potentially effective early intervention through pharmacological approaches and other strategies such as lifestyle changes.[71,72] Evidence from studies done in China, Finland, and the USA suggests that these interventions can prevent or at least delay the onset of type 2 diabetes.[73–75] Few countries have health-care systems, however, that are positioned to take a proactive approach or possess the infrastructure to prioritise early interventions. Developing and implementing strategies that will have long-lasting impacts at the population level remains a persistent challenge.

Although this study does not explicitly report the impact of diabetes on diseases such as chronic kidney disease, ischaemic heart disease, and cancer, since these relationships are captured in the GBD risk factor framework via high fasting plasma glucose, the impact of diabetes extends beyond the results presented here. Strategies and policies aimed at mitigating the diabetes burden should also consider the additional nuance that diabetes can lead to irreversible microvascular damage and increase the risk of morbidity and mortality due to other infectious and non-communicable diseases.[8,51,52] Furthermore, efforts that succeed in halting the rise in diabetes could mitigate or delay associated health complications if implemented early.[76–80] These are important considerations given that in many places in the world, increases in disability due to diabetes have outpaced diabetes mortality.[8,81]

In addition to the estimates presented here, previous studies have reported global diabetes estimates from earlier rounds of GBD, and two other organisations, the IDF and NCD-RisC, have also generated global and multi-country estimates of diabetes for specific age groups.[4,10] Differences between estimates produced by GBD and IDF or NCD-RisC are likely to be due to differences in methods, case inclusion criteria, and data sources used. For example, our GBD analysis deliberately excluded sources that rely on self-reported diabetes data because we assumed that reporting bias would change over time and across location (eg, due to variability in diagnostic and screening efficacy), thus making bias adjustment challenging. Although we omitted these data sources, which were included in the IDF and NCD-RisC models, we were still able to incorporate data expressly gathered in 172 countries, exceeding the 144 locations with data in the IDF analysis and 146 locations with data in the NCD-RisC analysis. Moreover, our modelling approach—which used a Bayesian meta-regression tool, MR-BRT,[39,41] to develop coefficients to adjust non-reference case definitions, as well as DisMod-MR, which allows us to estimate prevalence by taking into account diabetes mortality—is unique to GBD. Other differences include our estimates for locations not reported by IDF (Cook Islands, Niue, and Tokelau) or NCD-RisC (Guam, Monaco, Northern Mariana Islands, San Marino, South Sudan, and Virgin Islands; appendix table S19). Our estimates covered the entire age spectrum, whereas IDF only reported total diabetes estimates for people aged 20–79 years and type 1 diabetes estimates for those younger than 19 years, and NCD-RisC reported estimates of total diabetes for individuals aged 18 years and older. Finally, we projected type-specific and total diabetes through 2050 for every age group, while IDF and NCD-RisC did not make projections as far out and did not generate forecasts for the entire population.

Despite methodological and reporting differences, our estimates of particularly high total diabetes prevalence rates in the north Africa and the Middle East super-region are supported by similar estimates reported in the 2021 IDF Atlas.[4] Similar to many other regions, north Africa and the Middle East has experienced a rise in obesity due to rapid urbanisation and a concomitant rise in sedentary lifestyles and unhealthy eating patterns.[82,83]

Our study has several strengths. The robust location-specific, age-specific, and sex-specific diabetes estimates produced by our analysis are largely due to our ability to leverage the rigorous evidentiary and methodological framework provided by the larger GBD enterprise. GBD integrates all available data, critically examines and standardises differences in methods, and draws upon the expertise of a network comprising more than 9000 researchers located in more than 160 countries to generate estimates of mortality and morbidity associated

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The unfinished agenda of communicable diseases among children and adolescents before the COVID-19 pandemic, 1990–2019: a systematic analysis of the Global Burden of Disease Study 2019

GBD 2019 Child and Adolescent Communicable Disease Collaborators*

Summary

Background Communicable disease control has long been a focus of global health policy. There have been substantial reductions in the burden and mortality of communicable diseases among children younger than 5 years, but we know less about this burden in older children and adolescents, and it is unclear whether current programmes and policies remain aligned with targets for intervention. This knowledge is especially important for policy and programmes in the context of the COVID-19 pandemic. We aimed to use the Global Burden of Disease (GBD) Study 2019 to systematically characterise the burden of communicable diseases across childhood and adolescence.

Methods In this systematic analysis of the GBD study from 1990 to 2019, all communicable diseases and their manifestations as modelled within GBD 2019 were included, categorised as 16 subgroups of common diseases or presentations. Data were reported for absolute count, prevalence, and incidence across measures of cause-specific mortality (deaths and years of life lost), disability (years lived with disability [YLDs]), and disease burden (disability-adjusted life-years [DALYs]) for children and adolescents aged 0–24 years. Data were reported across the Socio-demographic Index (SDI) and across time (1990–2019), and for 204 countries and territories. For HIV, we reported the mortality-to-incidence ratio (MIR) as a measure of health system performance.

Findings In 2019, there were 3·0 million deaths and 30·0 million years of healthy life lost to disability (as measured by YLDs), corresponding to 288·4 million DALYs from communicable diseases among children and adolescents globally (57·3% of total communicable disease burden across all ages). Over time, there has been a shift in communicable disease burden from young children to older children and adolescents (largely driven by the considerable reductions in children younger than 5 years and slower progress elsewhere), although children younger than 5 years still accounted for most of the communicable disease burden in 2019. Disease burden and mortality were predominantly in low-SDI settings, with high and high-middle SDI settings also having an appreciable burden of communicable disease morbidity (4·0 million YLDs in 2019 alone). Three cause groups (enteric infections, lower-respiratory-tract infections, and malaria) accounted for 59·8% of the global communicable disease burden in children and adolescents, with tuberculosis and HIV both emerging as important causes during adolescence. HIV was the only cause for which disease burden increased over time, particularly in children and adolescents older than 5 years, and especially in females. Excess MIRs for HIV were observed for males aged 15–19 years in low-SDI settings.

Interpretation Our analysis supports continued policy focus on enteric infections and lower-respiratory-tract infections, with orientation to children younger than 5 years in settings of low socioeconomic development. However, efforts should also be targeted to other conditions, particularly HIV, given its increased burden in older children and adolescents. Older children and adolescents also experience a large burden of communicable disease, further highlighting the need for efforts to extend beyond the first 5 years of life. Our analysis also identified substantial morbidity caused by communicable diseases affecting child and adolescent health across the world.

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Introduction

The substantial reductions in burden and mortality from communicable diseases among children younger than 5 years have been one of the success stories of global health.[1–3] Key initiatives contributing to these gains include the WHO Integrated Management of Childhood Illness, the WHO and UNICEF child survival strategy, and the integrated Global Action Plan for Prevention and Control of Pneumonia and Diarrhoea (GAPPD). The Millennium Development Goals also brought a focus to

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Research in context

Evidence before this study

The 2016 Lancet Commission on Adolescent Health and Wellbeing, a subsequent analysis of 12 headline indicators for adolescent health, and recent analyses of adolescent mortality each identified communicable diseases to be a key contributing cause of mortality and morbidity among adolescents globally. In 2016, 40% of the burden of disease (measured in disability-adjusted life-years [DALYs]) among adolescents was accounted for by communicable, maternal, and nutritional diseases. Yet these analyses report communicable diseases as an aggregate group and do not provide estimates of specific communicable disease burden, essential for targeted policy and programming. We could not find any further analyses of communicable disease burden for adolescents, or indeed for older children. In November, 2021, we searched for reports and publications describing the burden of communicable diseases among children and adolescents aged 0–24 years in the past 10 years (2012–21) using the following search terms: "communicable diseases/epidemiology" AND child* OR adoles* OR youth* OR paed* OR ped*. We also searched for specific causes (including pneumonia, diarrhoea, malaria, HIV, and tuberculosis) supplemented by recent Global Burden of Disease (GBD) publications on pneumonia and diarrhoea in young children. We reviewed peer-reviewed and selected grey literature sources: UN agencies including WHO, UNICEF, and UNAIDS, key policy and monitoring agencies, including the Independent Accountability Panel, The Partnership for Maternal, Newborn and Child Health, and Countdown to 2030; and funding bodies such as The Global Fund to Fight AIDS, Tuberculosis and Malaria and the Bill & Melinda Gates Foundation. We screened more than 6000 titles but found no report or systematic analysis of communicable disease burden across childhood and adolescence. Available evidence either focused on specific age groups (particularly children <5 years of age), specific diseases, or both, or on mortality only. Available summary reports of population health (including the WHO Global Health Observatory and the Institute for Health Metrics and Evaluation GBD capstone papers) often describe communicable disease at an aggregate level, which again is insufficient for targeted policy and actions. Countdown to 2030 and associated country profiles and available data dashboards for child and adolescent health through UNICEF and WHO include indicators of some communicable diseases, but again mostly for young children. Formerly known as the WHO and UNICEF's Child Health Epidemiology Reference Group, the Maternal Child Epidemiology Estimation (MCEE) group published global, national, and regional mortality estimates in 2019 for diarrhoea, malaria, tuberculosis, lower-respiratory-tract infections, and HIV and AIDS for children and adolescents aged 0–19 years in 5-year age groups and disaggregated by sex for those aged 15–19 years. These MCEE estimates make an essential contribution to the literature, but they are focussed on mortality. For tuberculosis, the WHO 2022 Global Tuberculosis Report (and other tuberculosis surveillance data) describes data for children and adolescents aged 0–5 years, 5–14 years, and 15–24 years by WHO region; however, there is no country-level age disaggregated data for children and adolescents. The one exception is a paper by Snow and colleagues that reported tuberculosis notification data by 5-year age groups in people aged 10–24 years. For malaria, the Malaria Atlas Project (which informs the WHO World Malaria Report) reports total all-age estimates of cases and deaths in endemic countries and regions, but does not present detailed specific estimates of incidence or burden by age or sex; data are typically reported in children and adolescents younger than 5 years, aged 5–14 years, and aged 15–49 years. For HIV, UNAIDS provides annual estimates on populations living with HIV but does not typically stratify by age or gender across the developmental window (0–24 years) in their annual global updates, with the WHO Global Health Estimates (informed by UN partners, GBD, and other scientific studies) providing global, regional, and country-level estimates for various age bands, although adolescents older than 15 years are typically aggregated with adults. One exception is a paper by Zhang and colleagues based on GBD 2019 data that reported the burden associated with HIV and sexually transmitted infections for adolescents aged 10–24 years at the global, regional, and national level in 1990–2019. For diarrhoeal disease and pneumonia, available data are focussed on children younger than 5 years.

Added value of this study

This study provides a systematic and comprehensive analysis of communicable disease across the entire developmental window from birth to 24 years of age. Data are reported at a global level, across the gradient of sociodemographic development, and at a country level, disaggregated by age and sex where possible. Although aggregate data enable advocacy, granular data are essential for targeted action and monitoring of progress. We report data on incidence and mortality (typical metrics for communicable diseases), but add value by also reporting morbidity to illustrate the true effect of these largely preventable diseases; this is especially important for children and adolescents living in settings with high sociodemographic development. To further ensure as complete a picture of communicable disease burden as possible, we reviewed all the 369 causes modelled in GBD and included all communicable diseases, their clinical presentations, or direct sequelae in our reported estimates, resulting in 83 million DALYs that are in addition to the 420 million DALYs traditionally reported as communicable diseases in GBD 2019. We report the burden of vaccine-preventable diseases and the mortality-to-incidence ratio for HIV (a non-curable communicable disease) across available age groups as measures of health system performance.

(Panel continues on next page)

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elsewhere.[22,23] Hereafter we present specific methods and assumptions of relevance to this secondary data analysis.

For more on the IHME Global Health Data Exchange see http://ghdx.healthdata.org/gbd-results-tool

Data sources

We used GBD 2019 data accessed from the IHME Global Health Data Exchange. We accessed data between Dec 10, 2021, and Nov 4, 2022 for all causes (at level 4) as absolute numbers and rates per 100 000 population for the following metrics: mortality (deaths and years of life lost [YLLs]); disability (years lived with disability [YLDs]); and disease burden (measured as disability-adjusted life-years [DALYs]). We accessed data for all ages (in 5-year age bands up to 24 years, then for 25 years and older), for males and females, for all years between 1990 and 2019, and for 204 countries and territories. We also accessed the IHME sociodemographic index to group countries at similar levels of sociodemographic development, comprising high, mid-high, middle, mid-low, and low socioeconomic development (appendix pp 1–4). GBD 2019 complies with the Guidelines for Accurate and Transparent Health Estimates Reporting statement and all data input sources and statistical codes are available online.[24,25]

See Online for appendix

Definitions

GBD 2019 includes 369 causes of disease and injury organised within three disease groups: communicable, maternal, neonatal, and nutritional diseases (group A); non-communicable diseases (group B); and injuries (group C; appendix pp 5–6). For this analysis we included all causes of communicable disease in group A (cause groups A1 to A5); these causes include specific infectious diseases (eg, cause group A.1.1, HIV) and clinical presentations of communicable disease (eg, cause group A.2.2, lower-respiratory-tract infection). We then reviewed all other causes included within group B and group C in GBD 2019 and their corresponding International Classification of Disease codes to identify other relevant communicable diseases.[26] Maternal sepsis (A.6.1.2), neonatal sepsis (A.6.2.3), rheumatic heart disease (B.2.1), bacterial skin disease (B.9.3), scabies (B.9.4), fungal skin disease (B.9.5), viral skin disease (B.9.6), liver cancer caused by hepatitis B and C (B.1.7.1 and B.1.7.2), and cirrhosis and chronic liver disease caused by hepatitis B and C (B.4.1.1 and B.4.1.2) were all included within our definition of communicable diseases (appendix pp 5–6). We did not include cervical cancer (B.1.15), given that modelled estimates are not specific to human papillomavirus. Our definition of communicable diseases yielded a total disease burden of 503 296 274 DALYs in 2019 for all age groups compared with 420 392 536 for cause groups A1–A5. All communicable diseases included in our analysis were additionally subcategorised into 16 sub-groupings that represent common communicable diseases or clinical presentations (appendix pp 5–6).

This analysis focused on the developmental window of childhood and adolescence. Consistent with newer understandings of neurodevelopment but also global shifts in the timing of key social role transitions (such as completion of education and parenthood), we define childhood as being younger than 10 years and adolescence as being 10–24 years of age.[27] Data are reported in 5-year age bands within these broad definitions of childhood and adolescence; we did not further disaggregate the under 5-year age band given that these estimates are extensively reported elsewhere. We additionally defined three aggregate groups that represent key target populations within the health sector: young children (younger than 5 years), older children and young adolescents who are still consistently cared for by paediatric services (5–14 years),[28] and older adolescents (15–24 years).[29] We use the Socio-demographic Index, a composite indicator of development based on the geometric mean of total fertility (younger than 25 years), mean education (15 years and older), and lag-distributed income per capita.

Analysis and reporting

Data were reported as absolute count, cases per 100 000 population, and incidence (cases of new disease per 100 000 population per year) across measures of mortality, disability (YLD or morbidity), and disease burden (DALYs). Of note, GBD 2019 does not model deaths caused by scabies, or fungal or viral skin conditions. Estimates were reported for each of 204 locations separately, across sociodemographic development groupings, and for adolescents globally, noting that the global estimate is greater than the sum of 204 individual nations or territories, because it includes people who are stateless and additional nations and territories. We estimated the percentage change between 1990 and 2019 (difference between 2019 and 1990 value divided by the 1990 value and multiplied by 100), reporting this value as an annualised percentage change (dividing by 30 years). Where possible, we also reported the corresponding uncertainty interval (UI) for each estimate. This interval is produced for each estimate by running 1000 draws of the posterior distribution, ordering the draws, and selecting the 25th and 975th draw values.[25] The code that is used to produce the estimates is available online.[25] Given that UIs are obtained during modelling, these intervals are not available for some aggregate estimates that we provide in this analysis (eg, the 0–24-year age group for total communicable disease cause). Uncertainty for each cause that contributes to our aggregate estimates by age, sex, and country is detailed in the appendix (pp 87–167).

As a measure of health system response to communicable disease, we reported the mortality-to-incidence ratio (MIR) for HIV.[30–32] The MIR calculation was only estimated for HIV given that it is a true chronic communicable disease for which a definitive objective diagnosis is typical, and treatment, remission, and several incident infections are not possible. MIR was calculated by dividing the number of cause-specific

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deaths by the number of new cases for a given year. We reported this metric for children younger than 5 years, adolescents aged 15–19 years, and adolescents aged 20–24 years; HIV incidence for children and adolescents aged 5–14 years was modelled to be negligible by GBD and is excluded here.

Data were analysed in Stata 17.0 and visualisations prepared in Stata and Tableau 2021.3.20.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Total burden of communicable disease across childhood and adolescence

In 2019, there were 3·0 million deaths and 30·0 million years of healthy life lost to disability (as measured by YLDs) from communicable diseases globally among people aged 0–24 years, corresponding to a total disease burden of 288·4 million DALYs (table 1). This burden represents 57·3% of the total communicable disease burden across all ages. For children and adolescents specifically, communicable disease accounted for 44·1% of the total 6·9 million deaths in this age group, 16·6% of the total disability, and 37·9% of the total 760·0 million all-cause DALYs (table 1). Globally, the proportion of deaths caused by communicable diseases in 2019 was between 41·2% and 55·9% for those aged 0–14 years, decreasing to between 20·6% and 33·9% among those aged 15–24 years (table 1). This pattern was similar for disability, with the proportion of YLDs attributable to communicable diseases consistently declining with increasing age. Communicable disease burden among children and adolescents was predominantly in countries of low sociodemographic development, with 1·8 million deaths (58·2% of all communicable disease deaths among children and adolescents) and 161·4 million DALYs (56·0% of all communicable disease DALYs among children and adolescents; appendix pp 7–10). More than half of the mortality among children and adolescents in settings of low sociodemographic development was caused by communicable diseases compared with just 5·6% of deaths and 7·1% of DALYs in settings of high sociodemographic development (appendix pp 8–10).

There were important changes in communicable disease epidemiology across childhood and adolescence

	Population	Mortality (deaths)			Disability (YLDs)			Disease burden (DALYs)		
		All-cause	Communicable disease	Percentage due to communicable diseases	All-cause	Communicable disease	Percentage due to communicable diseases	All-cause	Communicable disease	Percentage due to communicable diseases
Under 5 years										
Female	320 443 936	2 311 567	1 154 730	50·0%	12 885 506	3 340 269	25·9%	216 217 639	104 465 025	48·3%
Male	342 398 784	2 732 001	1 245 653	45·6%	14 180 583	3 600 909	25·4%	254 728 403	112 733 037	44·3%
5–9 years										
Female	316 754 496	167 708	93 711	55·9%	13 824 110	3 389 243	24·5%	27 539 772	11 053 467	40·1%
Male	337 949 216	210 032	106 164	50·5%	15 108 732	3 693 489	24·4%	32 722 747	12 370 285	38·3%
10–14 years										
Female	310 852 512	129 193	60 936	47·2%	17 981 681	2 754 176	15·5%	22 855 730	7 451 772	26·8%
Male	331 334 176	170 079	70 032	41·2%	16 907 999	2 974 792	17·6%	29 903 595	8 326 975	27·8%
15–19 years										
Female	301 758 880	196 977	66 846	33·9%	23 120 691	2 660 741	11·5%	32 193 646	7 436 890	20·0%
Male	317 782 112	302 328	77 986	25·8%	18 794 347	2 490 160	13·2%	40 371 375	8 058 373	20·0%
20–24 years										
Female	295 776 256	255 141	81 310	31·9%	27 205 707	2 676 953	9·8%	44 184 802	8 087 317	18·3%
Male	304 368 224	437 271	90 184	20·6%	20 650 899	2 420 924	11·7%	49 742 667	8 421 269	16·9%
≥25 years										
Female	2 310 904 064	32 784 061	2 905 445	12·8%	381 599 235	18 658 273	4·9%	837 038 989	92 819 766	11·1%
Male	2 247 142 144	26 830 603	3 589 282	13·4%	298 712 790	18 158 028	6·1%	940 950 706	122 872 101	13·0%
0–24 years										
Female and male	3 179 418 560	6 912 296	3 047 551	44·1%	180 661 755	30 041 657	16·6%	760 030 375	288 404 407	37·9%
Female	1 545 586 080	3 060 585	1 457 533	47·6%	95 017 694	14 861 382	15·6%	353 011 588	138 494 470	39·2%
Male	1 633 832 512	3 851 711	1 590 018	41·3%	85 644 061	15 180 275	17·7%	407 018 787	149 909 938	36·8%

DALYs=disability-adjusted life-years. YLDs=years of life lost to disability.

Table 1: Age and sex-specific estimates of deaths, YLDs, and DALYs (all-cause and communicable-disease specific), in 2019

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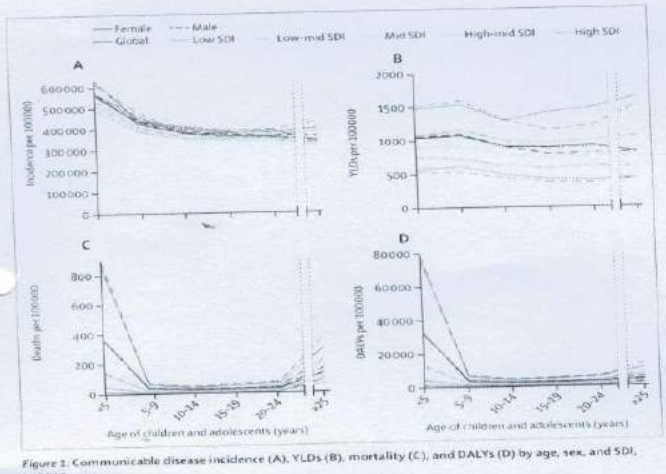

Figure 1: Communicable disease incidence (A), YLDs (B), mortality (C), and DALYs (D) by age, sex, and SDI, in 2019
DALYs=disability-adjusted life years. SDI=Socio-demographic index. YLDs=years of life lost to disability

(figure 1). Communicable disease incidence (figure 1A; appendix pp 11–12) was highest in children younger than 5 years (569 924 communicable diseases per 100 000 population for both sexes globally) but remained high across childhood (431 375 communicable diseases per 100 000 population for children aged 5–9 years) and for adolescents aged 15–19 years (369 246 communicable diseases per 100 000 population). There appeared to be little difference in all-cause incidence by sex or socioeconomic development. Disability caused by communicable disease as measured by YLDs (figure 1B; appendix pp 13–14) was relatively similar across age and sex, but with marked variation across socioeconomic development. Communicable diseases in children and adolescents aged 0–24 years caused 1407 YLDs per 100 000 population in settings of low sociodemographic development and 458 YLDs per 100 000 population in settings of high sociodemographic development. There was marked variation in mortality (figure 1C; table 1; appendix pp 15–16) by age and sociodemographic development, with mortality caused by communicable diseases greatest for children younger than 5 years (mortality rate 362·1 per 100 000 population and a total of 2·4 million deaths in 2019), especially for children younger than 5 years in settings of low sociodemographic development (869 deaths per 100 000 population for both sexes). However, the relatively low mortality throughout later childhood and adolescence still corresponded to a substantial number of deaths: overall, 647 169 deaths from communicable diseases occurred among children and adolescents aged 5–24 years in 2019, corresponding to 6·8% of total deaths from communicable diseases and 34·6% of all deaths in this age group (table 1). In 2019, DALYs (figure 1D; appendix pp 17–18) were largely driven by mortality (288·4 million DALYs for individuals aged 0–24 years, comprising 30·0 million YLDs and 258·4 million YLLs), and as a result, trends in DALYs largely mirrored trends in mortality.

Communicable disease epidemiology changed over time from 1990 to 2019 (figure 2). Incidence of communicable diseases (figure 2A) declined most markedly for children younger than 5 years, and especially for those younger than 5 years in settings of low sociodemographic development, with an annual decline of around 0·5% in settings of low sociodemographic development compared with a 0·03% decline in settings of high sociodemographic development. As a result, in 2019 the incidence of communicable diseases for children younger than 5 years in settings of high sociodemographic development (597 655 communicable diseases per 100 000 population) was similar to that in settings of low sociodemographic development (633 509 communicable diseases per 100 000 population). The change in incidence among older children and adolescents across sociodemographic development settings was small. Disability as measured by YLDs (figure 2B) changed little globally for children and adolescents (0·5% for those aged <5 years, 0·4% for those aged 5–14 years, and 0·5% for those aged 15–24 years); however, there were marked reductions in YLDs in settings of low sociodemographic development (1·0% annual decline for individuals aged 0–24 years overall). Mortality caused by communicable diseases (figure 2D; appendix pp 15–16) declined most markedly for children younger than 5 years (2·2% annual decline globally) and those aged 5–14 years (1·9% annual decline globally), with changes for adolescents aged 15–24 years being more modest (1·3% annual decline globally). Declines in mortality between 1990 and 2019 were most marked in settings of low sociodemographic development, where for children younger than 5 years, deaths decreased from 2752 per 100 000 to 869 deaths per 100 000 between 1990 and 2019 (an average decline of 2·3% per year). Shifts in total disease burden (figure 2C) largely mirrored shifts in mortality. At a global level, these relative transitions in epidemiology by age and sociodemographic development resulted in the communicable disease burden increasingly shifting from children younger than 5 years to older children and adolescents between 1990 and 2019 (DALYs in appendix p 20 and mortality in appendix p 21). In 1990, 85% of the communicable disease burden across the developmental window was among children younger than 5 years, decreasing to 75% in 2019.

Total disease burden caused by communicable diseases (as measured by DALYs) for children and adolescents at a country level changed from 1990 to 2019 (figure 3). For

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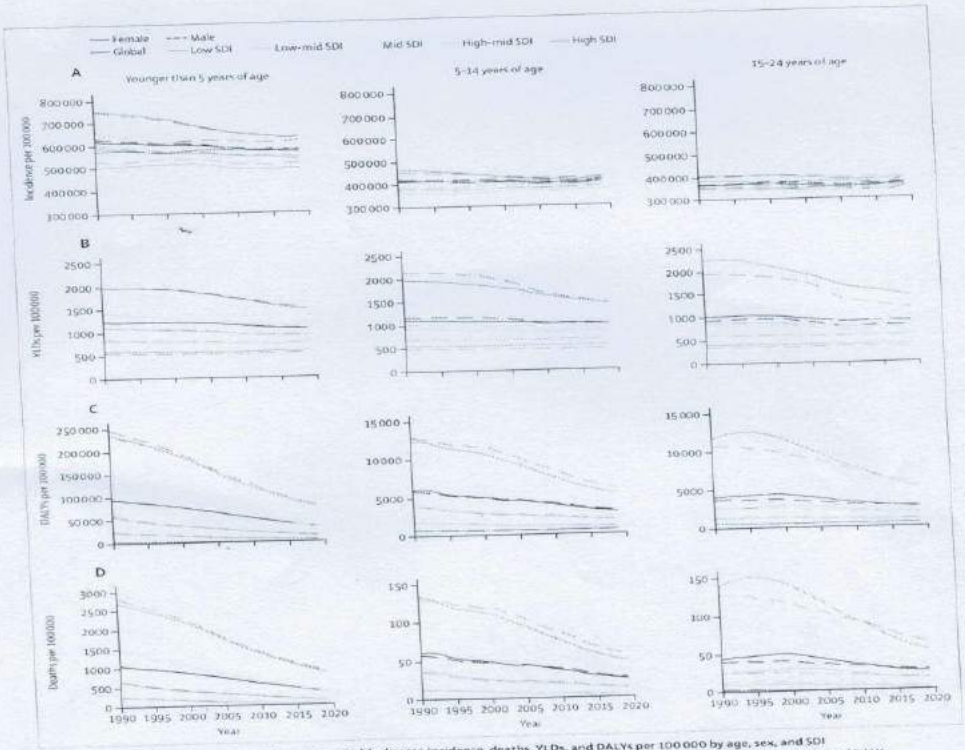

Figure 2: Trends over time (1990–2019) for communicable disease incidence, deaths, YLDs, and DALYs per 100 000 by age, sex, and SDI
The incidence graphs start at 300 000 cases per 100 000 per year and the age groups younger than 5 years are on a different y-scale for deaths and DALYs. DALY=disability-adjusted life-years. SDI=Socio-demographic Index. YLD=years of life lost to disability.

children younger than 5 years, there were uniform and significant reductions in the communicable disease burden (especially noting the magnitude of reduction among these children under 5 years given the unique axis values for DALYs in figure 3), compared with children and adolescents aged 5–14 years and adolescents aged 15–24 years. Although most countries showed a decline in communicable disease burden, large increases (>2% annual increase) in burden were seen in Eswatini, Lesotho, and South Africa, for the 15–24-year age group. In 2019, countries in sub-Saharan Africa and some countries in Asia had the largest burden of communicable diseases for children and adolescents.

Cause-specific estimates of communicable diseases in children and adolescents

60% of communicable disease burden (as measured by DALYs) among children and adolescents was accounted for by three cause groups in 2019 (table 2), comprising enteric infections (69·5 million DALYs, 24·1% total),

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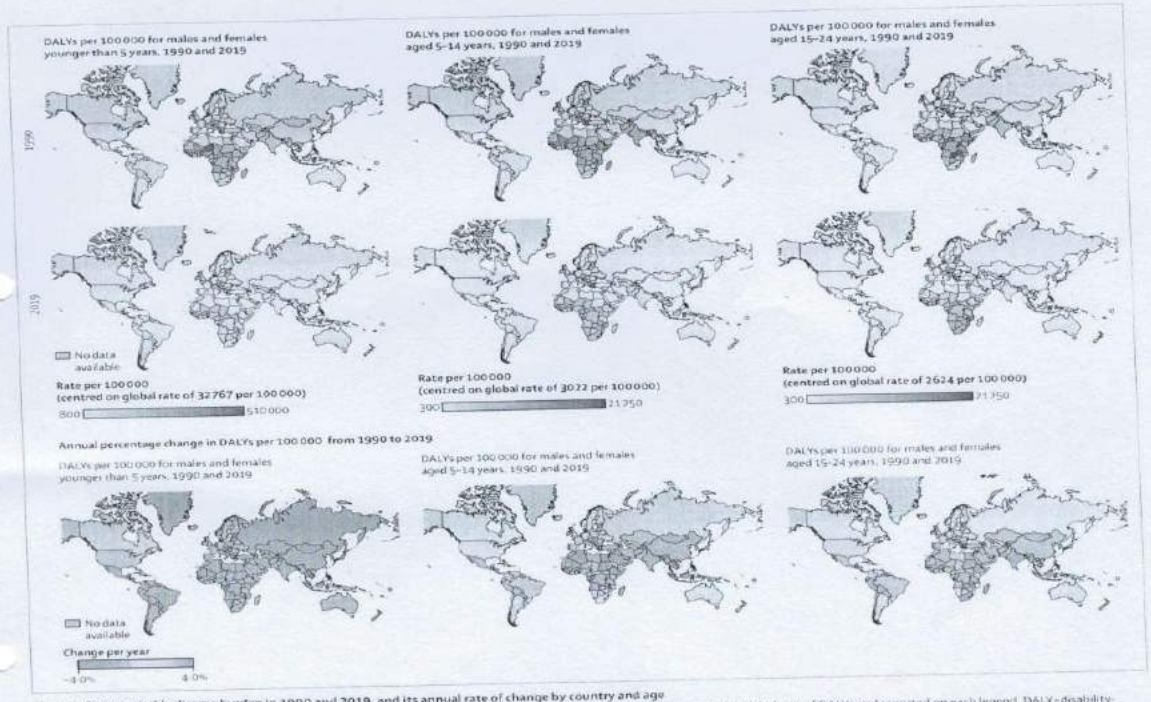

Figure 3: Communicable disease burden in 1990 and 2019, and its annual rate of change by country and age
The colour scheme for the DALY rate in 1990 and 2019 is specific for each age group and the colour band is centred around the global rate of DALYs and reported on each legend. DALY=disability-adjusted life-years.

lower-respiratory-tract infections (LRTIs; 64·7 million DALYs, 22·4% total), and malaria (38·3 million DALYs, 13·3% total). These same cause groups together accounted for almost two-thirds of all deaths caused by communicable diseases among children and adolescents: enteric infections (763 545 deaths, 25·1% of deaths caused by communicable diseases), LRTIs (743 546 deaths, 24·4% of deaths caused by communicable diseases), and malaria (427 469 deaths, 14·0% of deaths caused by communicable diseases).

Contributors to communicable disease burden (as measured by DALYs) by age, sex, and sociodemographic development are presented in the appendix (p 19). LRTIs and enteric infections were the leading causes of communicable disease burden across childhood and early-to-mid adolescence globally. For older adolescents aged 20–24 years, HIV and tuberculosis emerged as leading causes. HIV caused 20·9% of the communicable disease burden in females and 10·3% in males, and 28·3% of age-specific deaths in females and 13·2% in males aged 20–24 years (appendix pp 15, 18). Tuberculosis caused 18·7% of the communicable disease burden in males and 14·0% in females, and 22·6% of age-specific deaths in males and 15·9% in females aged 20–24 years. Rheumatic heart disease, maternal sepsis, and sexually transmitted infections (STIs; excluding HIV) were other communicable diseases that predominantly emerged during adolescence, albeit with a considerably smaller burden.

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Causes of communicable diseases varied substantially by sociodemographic development (appendix pp 11–19). In settings of low sociodemographic development, enteric diseases and LRTIs were the leading causes of DALYs among children and adolescents (with enteric diseases causing 25·9% of the burden and LRTIs causing 21·6% of the burden in settings of low sociodemographic development), whereas infectious skin conditions and upper-respiratory-tract infections (URTIs) were the leading causes in settings of high sociodemographic development (with infectious skin diseases causing 28·7% of the burden and URTIs causing 22·1% of the burden).

	Mortality (deaths)			Disability			Disease Burden		
	Number of deaths	Deaths per 100 000 (percentage change per year)	Percentage of deaths caused by communicable diseases	Number of YLDs	YLDs per 100 000 (percentage change per year)	Percentage of YLDs caused by communicable diseases	Number of DALYs	DALYs per 100 000 (percentage change per year)	Percentage of DALYs caused by communicable diseases
Enteric infections									
Female	355 016	23·0 (−2·3%)	24·4%	2 425 525	157·0 (−0·4%)	16·3%	32 420 734	2 097·6 (−2·2%)	23·4%
Male	408 529	25·0 (−2·2%)	25·7%	2 599 957	159·1 (−0·4%)	17·1%	37 102 149	2 270·9 (−2·2%)	24·7%
Lower-respiratory-tract infections									
Female	363 980	23·5 (−2·4%)	25·0%	102 760	6·6 (−1·4%)	0·7%	31 693 882	2 050·6 (−2·4%)	22·9%
Male	379 566	23·2 (−2·4%)	23·9%	119 028	7·3 (−1·3%)	0·8%	33 012 492	2 020·6 (−2·4%)	22·0%
Malaria									
Female	211 315	13·7 (−1·5%)	14·5%	1 126 500	72·9 (0·0%)	7·6%	19 166 971	1 240·1 (−1·5%)	13·8%
Male	216 154	13·2 (−1·5%)	13·6%	889 966	54·5 (−0·2%)	5·9%	19 172 678	1 173·5 (−1·4%)	12·8%
Neonatal sepsis and other neonatal infections									
Female	100 303	6·5 (−0·8%)	6·9%	1 000 143	64·7 (7·9%)	6·7%	9 908 325	641·1 (−0·6%)	7·2%
Male	126 234	7·7 (−0·8%)	7·9%	1 060 359	64·9 (8·8%)	7·0%	12 270 216	751·0 (−0·7%)	8·2%
Vaccine-preventable disease									
Female	117 278	7·6 (−2·8%)	8·0%	105 275	6·8 (−2·0%)	0·7%	10 215 074	651·0 (−2·8%)	7·4%
Male	111 480	6·8 (−2·8%)	7·0%	94 315	5·8 (−2·1%)	0·6%	9 703 923	593·9 (−2·8%)	6·5%
Meningitis and encephalitis									
Female	82 954	5·4 (−2·2%)	5·7%	257 998	16·7 (−1·1%)	1·7%	7 207 750	466·3 (−2·2%)	5·2%
Male	101 377	6·2 (−2·0%)	6·4%	274 568	16·8 (−1·3%)	1·8%	8 767 715	536·6 (−2·0%)	5·8%
HIV and AIDS									
Female	74 914	4·8 (0·2%)	5·1%	345 448	22·4 (2·4%)	2·3%	6 051 479	391·5 (0·2%)	4·4%
Male	64 035	3·9 (0·8%)	4·0%	219 471	13·4 (6·1%)	1·4%	5 226 905	319·9 (0·6%)	3·5%
Tuberculosis									
Female	54 313	3·5 (−2·5%)	3·7%	708 837	45·9 (−1·3%)	4·8%	4 985 849	322·6 (−2·4%)	3·6%
Male	62 201	3·8 (−2·3%)	3·9%	492 195	30·1 (−1·1%)	3·2%	5 287 041	323·6 (−2·3%)	3·5%
Neglected tropical diseases									
Female	15 834	1·0 (−2·4%)	1·1%	2 330 032	150·8 (−1·4%)	15·7%	3 621 573	234·3 (−1·9%)	2·6%
Male	24 779	1·5 (−2·3%)	1·6%	2 476 188	151·6 (−1·7%)	16·3%	4 465 254	273·3 (−2·1%)	3·0%
Infectious skin conditions									
Female	3278	0·2 (−1·3%)	0·2%	3 344 347	216·4 (−0·1%)	22·5%	3 612 997	234·1 (−0·3%)	2·6%
Male	2421	0·1 (−1·5%)	0·2%	3 678 489	225·1 (−0·1%)	24·2%	3 872 245	237·0 (−0·2%)	2·6%
Sexually transmitted infections excluding HIV									
Female	37 374	2·4 (−0·9%)	2·6%	75 221	4·9 (0·4%)	0·5%	3 378 042	218·6 (−0·9%)	2·4%
Male	45 258	2·8 (−0·9%)	2·8%	58 207	3·6 (0·2%)	0·4%	4 068 741	249·0 (−0·9%)	2·7%
Upper-respiratory-tract infections									
Female	2043	0·1 (−2·8%)	0·1%	1 991 663	128·9 (−0·1%)	13·4%	2 167 122	140·2 (−1·1%)	1·6%
Male	2915	0·2 (−2·7%)	0·2%	2 282 909	139·7 (−0·1%)	15·0%	2 532 781	155·0 (−1·0%)	1·7%
Other unspecified infectious diseases									
Female	10 559	0·7 (−1·2%)	0·7%	562 619	36·4 (−0·2%)	3·8%	1 452 672	94·0 (−0·9%)	1·0%
Male	14 644	0·9 (−1·3%)	0·9%	515 999	31·6 (−0·3%)	3·4%	1 723 549	105·5 (−1·1%)	1·1%
Hepatitis									
Female	14 837	1·0 (−2·3%)	1·0%	91 598	5·9 (−0·2%)	0·6%	1 249 223	80·8 (−2·3%)	0·9%
Male	23 079	1·3 (−2·1%)	1·3%	105 110	6·4 (−0·3%)	0·7%	1 714 000	104·9 (−2·1%)	1·1%

(Table 2 continues on next page)

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	Mortality (deaths)			Disability			Disease Burden		
	Number of deaths	Deaths per 100 000 (percentage change per year)	Percentage of deaths caused by communicable diseases	Number of YLDs	YLDs per 100 000 (percentage change per year)	Percentage of YLDs caused by communicable diseases	Number of DALYs	DALYs per 100 000 (percentage change per year)	Percentage of DALYs caused by communicable diseases
(Continued from previous page)									
Rheumatic heart disease									
Female	8719	0·6 (−1·9%)	0·6%	351 987	22·8 (0·9%)	2·4%	987 240	63·9 (−1·5%)	0·7%
Male	9366	0·6 (−1·6%)	0·6%	313 517	19·2 (0·9%)	2·1%	989 751	60·6 (−1·2%)	0·7%
Maternal sepsis and other maternal infections									
Female	4815	0·3 (−2·3%)	0·3%	40 429	2·6 (−1·2%)	0·3%	369 940	23·9 (−2·2%)	0·3%
Male									
Total communicable diseases									
Female	1 457 533	94·3 (−2·2%)	100·0%	14 861 382	961·5 (−0·4%)	100·0%	138 494 470	8960·4 (−2·2%)	100·0%
Male	1 590 018	97·3 (−2·2%)	100·0%	15 180 275	929·1 (−0·5%)	100·0%	149 909 936	9175·6 (−2·2%)	100·0%

Results are ordered by largest DALYs burden. For skin disease, only bacterial skin disease contributes to the value for deaths, because there were no deaths recorded for fungal and viral skin diseases or scabies.
DALY=disability-adjusted life-years. YLD=years of life lost to disability.

Table 2: Cause-specific estimates of DALYs and deaths for each communicable disease in 2019 and annual rate of change since 1990, for children and adolescents aged 0–24 years by sex

Of note, DALYs caused by skin infections and URTIs were mostly caused by YLDs, with little mortality attributed to these causes (total of 10 647 deaths globally, 0·3% of the total communicable disease deaths). Conditions such as neonatal sepsis, maternal sepsis, and meningitis predominantly affected children and adolescents in settings of low and middle sociodemographic development, with rheumatic heart disease and neglected tropical diseases exclusively so. By contrast, hepatitis and STIs affected children and adolescents across all sociodemographic development settings.

There was an overall reduction in burden (as measured by DALYs) for the key causes of communicable diseases globally between 1990 and 2019 (table 2). Annual declines in DALYs of at least 2% were seen for eight cause groups, comprising enteric infections, LRTIs, vaccine-preventable diseases, meningitis and encephalitis, tuberculosis, neglected tropical diseases, hepatitis, and maternal sepsis and other maternal infections. Malaria burden only declined 1·5% annually and declines in infectious skin conditions, neonatal infections, and STIs were less than 1% annually. These declines were seen largely in settings of low and middle sociodemographic development (appendix p 17). The only disease to increase in burden over time was HIV (0·2% annual increase for males and 0·6% for females). HIV burden increased most for children and adolescents in settings of middle sociodemographic development (14·3% annual increase for females and 12·1% for males; appendix p 17), and across settings, increases were most marked for children aged 5–9 years (13·3% annual increase for females and 13·5% increase for males globally), those aged 10–14 years (128·2% for females and 83·6% for males globally), and male adolescents aged 15–19 years (22·3% annual increase; appendix p 18). With respect to incidence, the causes where there has been an increased incidence over time globally included rheumatic heart disease and neglected tropical diseases (particularly among adolescents), and STIs in some settings of low and low-middle sociodemographic development.

Key findings by leading cause groups for children and adolescents

In this section we focus on five major contributors to overall communicable disease burden in children and adolescents, comprising enteric infections, LRTIs, and malaria, as well as tuberculosis and HIV which both emerged as key causes of burden in older adolescents; these five conditions accounted for 71·9% of communicable disease-related deaths and 67·3% of DALYs in those aged 0–24 years (69·7% of DALYs for children <5 years, 58·8% for those aged 5–14 years, and 61·2% for adolescents aged 15–24 years; appendix p 18). The countries that contribute the largest burden for these five causes are reported in table 3 (the specific burden in each country is reported in figure 4). It is worth additionally noting the burden of vaccine-preventable disease given that it is a marker of health-system performance. In 2019, globally there were 228 758 deaths in children and adolescents from diphtheria, pertussis, tetanus, measles, and varicella, 153 169 (66·9%) of which occurred in settings of low sociodemographic development.

In 2019, there were about 69·5 million DALYs caused by enteric infections among children and adolescents (table 2; appendix pp 18, 22–26), of which about 41·9 million occurred in settings of low sociodemographic development and 19·6 million in settings of low-middle sociodemographic development (88·3% of total combined). Globally, almost three quarters of this burden (74·5%) was in children younger than 5 years, but the burden among those aged 5–24 years was substantial (17·7 million DALYs and 190 487 deaths globally). Three

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countries (India, Nigeria, and Pakistan) together accounted for almost half (47·7%) of the total burden of enteric infection globally for children and adolescents (table 3). The largest burden per capita (figure 4) was in Chad (21 278 DALYs per 100 000 population), Central African Republic (16 202 DALYs per 100 000), Niger (14 883 DALYs per 100 000), and Nigeria (11 323 DALYs per 100 000). Most countries (187 [92%] of 204) saw reductions in enteric disease burden for children and adolescents between 1990 and 2019 (appendix p 22–26), with the greatest reductions seen in Equatorial Guinea (a decrease of 3·3% per year) and Nicaragua (a decrease of 3·2% per year). Among specific groups, boys aged 5–14 years in Zimbabwe (an increase of 2·1% per year, 95% UI −0·13 to 6·0), and boys aged 15–24 years in Puerto Rico (an increase of 1·7% per year) showed the greatest increases.

In 2019, there were a total of 64·7 million DALYs caused by LRTIs among children and adolescents (table 2; appendix pp 18, 22–26). Similar to enteric infections, the

	Enteric infections	Lower-respiratory-tract infections	Malaria	Tuberculosis	HIV and AIDS
<5 years					
1	Nigeria (27·1%)	Nigeria (19·2%)	Nigeria (26·8%)	Nigeria (14·2%)	Mozambique (19·8%)
2	India (11·2%)	India (19·2%)	Democratic Republic of the Congo (12·4%)	India (10·2%)	Nigeria (12·9%)
3	Pakistan (6·3%)	Pakistan (6·9%)	Uganda (4·9%)	Pakistan (10·0%)	Ethiopia (6·8%)
4	Chad (4·4%)	Ethiopia (2·9%)	Niger (4·6%)	Democratic Republic of the Congo (8·6%)	Zambia (6·2%)
5	Ethiopia (4·4%)	Niger (2·6%)	Burkina Faso (4·6%)	Somalia (4·1%)	Kenya (5·2%)
6	Niger (4·4%)	Tanzania (2·5%)	Côte d'Ivoire (3·9%)	Tanzania (3·2%)	South Africa (3·9%)
7	Democratic Republic of the Congo (3·7%)	Burkina Faso (2·4%)	Mali (3·9%)	Ethiopia (3·1%)	India (3·8%)
8	Cameroon (2·5%)	China (2·2%)	Tanzania (3·7%)	Angola (3·1%)	Uganda (3·6%)
9	Burkina Faso (2·2%)	Somalia (2·1%)	Ethiopia (3·2%)	Chad (3·0%)	Zimbabwe (2·9%)
10	Madagascar (1·9%)	Democratic Republic of the Congo (2·1%)	Ghana (2·8%)	Burkina Faso (3·0%)	Tanzania (2·7%)
5–14 years					
1	India (35·6%)	India (21·3%)	Nigeria (24·5%)	India (20·2%)	Mozambique (34·4%)
2	Pakistan (11·7%)	Pakistan (7·5%)	India (15·2%)	Pakistan (17·6%)	South Africa (13·7%)
3	Nigeria (9·9%)	Nigeria (5·7%)	Democratic Republic of the Congo (8·8%)	Democratic Republic of the Congo (7·4%)	Ethiopia (6·0%)
4	Bangladesh (4·0%)	Bangladesh (4·1%)	Mozambique (4·9%)	Nigeria (5·2%)	Kenya (5·8%)
5	Ethiopia (2·7%)	Democratic Republic of the Congo (3·8%)	Pakistan (4·0%)	Indonesia (4·2%)	Uganda (5·4%)
6	Indonesia (2·5%)	Ethiopia (3·3%)	Uganda (3·6%)	Philippines (3·6%)	Nigeria (5·0%)
7	Democratic Republic of the Congo (2·0%)	Philippines (3·2%)	Côte d'Ivoire (3·4%)	Bangladesh (3·4%)	Zimbabwe (4·8%)
8	Tanzania (1·6%)	China (2·6%)	Cameroon (2·7%)	Ethiopia (2·6%)	Tanzania (4·5%)
9	Kenya (1·5%)	Egypt (2·4%)	Niger (2·5%)	Somalia (2·3%)	Malawi (4·3%)
10	Mali (1·3%)	Tanzania (2·4%)	Burkina Faso (2·4%)	South Africa (2·2%)	Zambia (3·5%)
15–24 years					
1	India (39·2%)	India (28·3%)	Nigeria (29·2%)	India (26·8%)	South Africa (14·7%)
2	Pakistan (9·7%)	Nigeria (4·8%)	India (6·2%)	Pakistan (9·3%)	Mozambique (12·4%)
3	Nigeria (6·4%)	Democratic Republic of the Congo (4·0%)	Democratic Republic of the Congo (6·0%)	Indonesia (6·2%)	Kenya (6·9%)
4	Ethiopia (3·4%)	Ethiopia (3·5%)	Côte d'Ivoire (4·7%)	Democratic Republic of the Congo (4·8%)	Nigeria (6·6%)
5	Indonesia (3·3%)	Philippines (3·4%)	Yemen (4·5%)	Ethiopia (4·1%)	India (5·4%)
6	Bangladesh (3·0%)	Pakistan (3·2%)	Cameroon (4·0%)	Nigeria (3·8%)	Ethiopia (5·1%)
7	Kenya (1·9%)	China (2·9%)	Ghana (3·9%)	Somalia (2·9%)	Uganda (4·4%)
8	Democratic Republic of the Congo (1·9%)	Brazil (2·7%)	Mozambique (3·7%)	Mozambique (2·8%)	Tanzania (4·2%)
9	Tanzania (1·6%)	Tanzania (2·0%)	Burkina Faso (3·0%)	Tanzania (2·4%)	Zambia (3·5%)
10	China (1·5%)	Kenya (2·0%)	Uganda (2·5%)	Philippines (2·3%)	Cameroon (2·7%)

(Table 3 continues on next page)

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	Enteric infections	Lower-respiratory-tract infections	Malaria	Tuberculosis	HIV and AIDS
(Continued from previous page)					
0–24 years					
1	Nigeria (22·4%)	India (19·3%)	Nigeria (26·7%)	India (18·4%)	Mozambique (15·6%)
2	India (17·8%)	Nigeria (18·0%)	Democratic Republic of the Congo (11·6%)	Pakistan (10·8%)	South Africa (10·2%)
3	Pakistan (7·5%)	Pakistan (6·9%)	Uganda (4·6%)	Nigeria (8·6%)	Nigeria (8·7%)
4	Ethiopia (4·0%)	Ethiopia (3·0%)	India (4·3%)	Democratic Republic of the Congo (6·9%)	Kenya (5·1%)
5	Niger (3·5%)	Tanzania (2·5%)	Burkina Faso (4·3%)	Indonesia (4·3%)	Ethiopia (6·0%)
6	Chad (3·5%)	Niger (2·5%)	Niger (4·2%)	Ethiopia (3·4%)	Zambia (4·5%)
7	Democratic Republic of the Congo (3·2%)	Burkina Faso (2·3%)	Côte d'Ivoire (3·9%)	Somalia (3·3%)	India (4·3%)
8	Cameroon (2·0%)	China (2·1%)	Mali (3·5%)	Tanzania (2·6%)	Uganda (4·3%)
9	Indonesia (2·0%)	Democratic Republic of the Congo (2·3%)	Tanzania (3·4%)	Mozambique (2·5%)	Tanzania (3·7%)
10	Burkina Faso (1·9%)	Somalia (2·1%)	Mozambique (2·9%)	Philippines (2·2%)	Zimbabwe (3·2%)

The denominator is the total number of DALYs associated with the condition and age grouping. DALY=daily-adjusted life-years.

Table 3: **The ten countries with the highest percentage burden (DALYs) for enteric infections, HIV and AIDS, lower-respiratory-tract infections, malaria, and tuberculosis, for the three age groups**

burden of LRTIs was greatest in settings of low and low-middle sociodemographic development (54·4 million DALYs) and in children younger than 5 years globally (59·2 million DALYs, 95% UI 48·5 to 72·7). Nigeria, India, and Pakistan accounted for the largest cause-specific burden (44·2% of the global burden of LRTIs for 0–24 year olds), although it is noteworthy that the percentage burden in Nigeria and Pakistan is greater for enteric infection compared with LRTI (table 3). Countries with the largest per-capita burden (figure 4) of LRTIs were Chad (11 090 DALYs per 100 000 population), Burkina Faso (10 277 per 100 000), and Somalia (9708 per 100 000). In six countries across Asia and Europe, more than half of the disease burden for children and adolescents was accounted for by LRTIs (Azerbaijan [65%], Cambodia [51%], Romania [51%], Tajikistan [56%], Turkmenistan [68%], and Uzbekistan [73%]). With the exception of Dominica (with an increase of 0·1% per year), all countries showed a decline in LRTI-related DALYs between 1990 and 2019 (appendix p 22–26), with the greatest being in Türkiye and Equatorial Guinea (both with declines of 3·2% per year). However, increases over time were seen within specific groups, with the greatest increases among adolescent males aged 15–24 years in Argentina (an increase of 2·9% per year), Sao Tome and Principe (increase of 3·0% per year), and Ukraine (increase of 2·9% per year). Given that enteric disease and LRTIs are the focus of combined intervention such as the GAPPD, the relationship between these two diseases at a country level is detailed in the appendix (p 57). Overall, there was a strong relationship between these two diseases (R^2 0·7).

There were 38·3 million DALYs in 2019 caused by malaria across 89 countries (115 countries had no malaria burden; table 2, appendix pp 18, 22–26). 299 052 malaria deaths, representing 70·0% of all malaria deaths globally in those aged 0–24 years, occurred in settings of low sociodemographic development (appendix p 15). Although global disease burden from malaria was highest in children younger than 5 years, with 31·6 million DALYs (95% UI 15·1 to 55·3) and 356 363 deaths (95% UI 169 469 to 630 387), there were 6·7 million DALYs and 71 106 deaths among children and adolescents aged 5–24 years in 2019. Nigeria alone accounted for 26·7% of the malaria burden among children and adolescents globally (table 3), with the highest per-capita burden (figure 4) found in Burkina Faso (11 083 DALYs per 100 000 population), Niger (9827 DALYs per 100 000), and Sierra Leone (13 267 DALYs per 100 000). Most countries demonstrated decreasing burden of malaria (appendix pp 22–56). Within countries with low sociodemographic development, the greatest rate of malaria DALY change was observed in Nepal and Bhutan (a decrease in burden of 3·3% per year). However, notable increases in malaria burden (DALYs) were observed in countries with low-to-middle sociodemographic development, such as North Korea (increase of 190·0% per year) and Cabo Verde (increase of 23·1% per year).

In 2019 there were 10·3 million DALYs caused by tuberculosis among children and adolescents (table 2; appendix pp 18, 22–26), most from drug-susceptible tuberculosis (appendix p 79). Tuberculosis-related mortality and DALYs were greatest in children younger than 5 years (globally, 4·6 million DALYs, 95% UI 3·6 to 5·7, and 50 163 deaths, 95% UI 39 248 to 63 385), but incidence and disability from tuberculosis, as measured by YLDs, increased mostly after the age of

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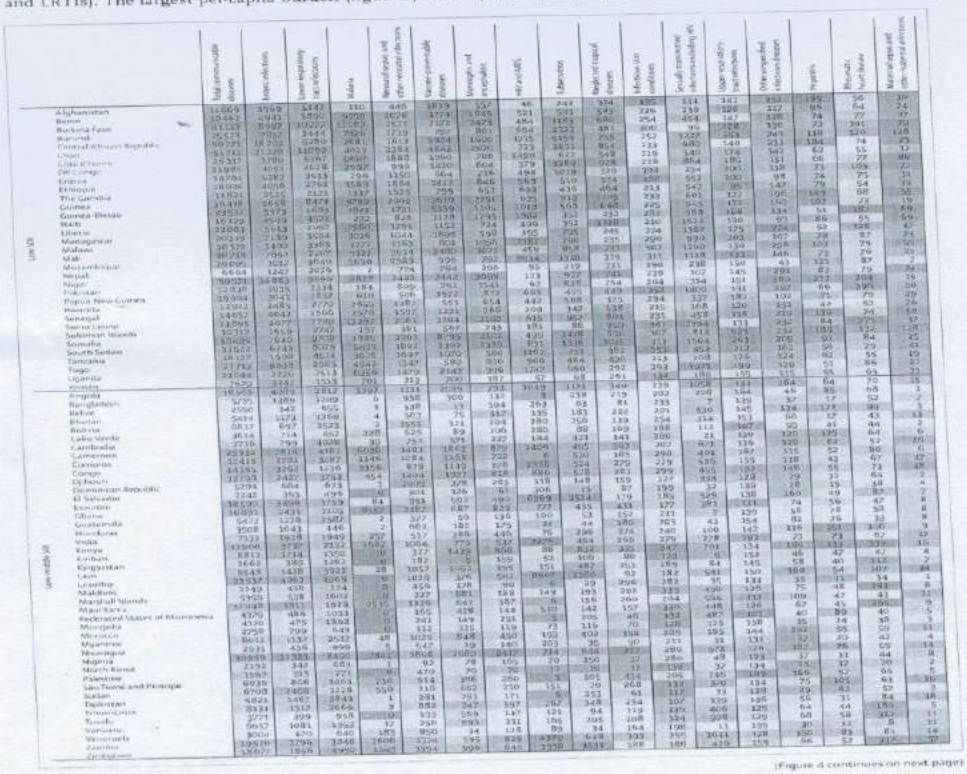
14 years. India, Nigeria, and Pakistan together accounted for 38% of the global tuberculosis burden (table 3) across the developmental window (similar to enteric infections and LRTIs). The largest per-capita burden (figure 4) was in the Central African Republic (5159 DALYs per 100 000 population), Chad (1631 DALYs per 100 000), Lesotho (2380 DALYs per 100 000), and Somalia (2478 DALYs per 100 000). Most countries showed



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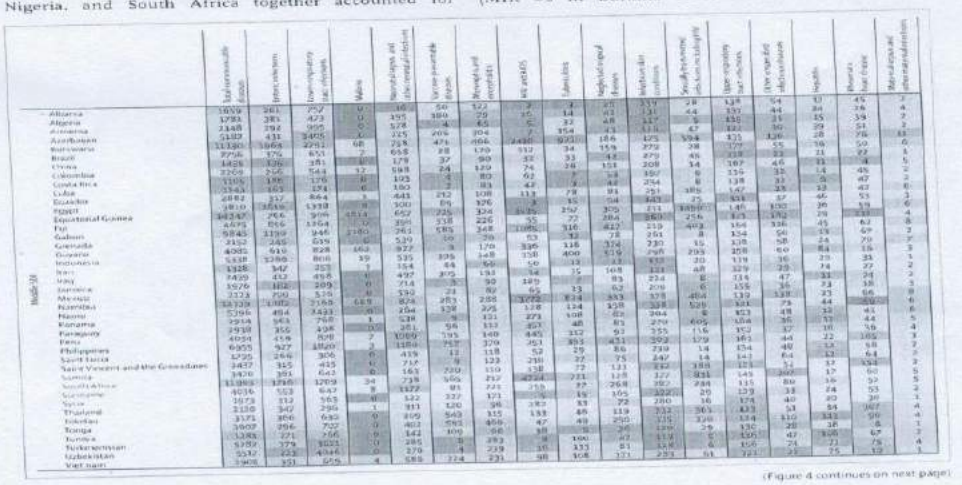
decreasing tuberculosis burden between 1990 and 2019 (appendix pp 22–26), except for Ukraine (increase of 3·4% per year) and Zimbabwe (increase of 0·5% per year). These increases were largely driven by male adolescents aged 15–24 years in Ukraine (increase in males of 7·0% and increase in females of 3·8%) and in Zimbabwe (increase in males of 1·7% and increase in females of 0·8%).

In 2019, there were 138 949 deaths and about 11·3 million DALYs caused by HIV and AIDS among children and adolescents (table 2; appendix pp 16, 18, 27–56), predominantly in settings of low-to-middle sociodemographic development, where 11·0 million DALYs were recorded, accounting for 97% of the global HIV burden. HIV burden varied substantially by age and sex (appendix pp 17, 18); it was high among children younger than 5 years (4·3 million DALYs, 95% UI 3·4 to 5·4, and 48 928 deaths, 95% UI 38 663 to 61 262), lower during childhood 5–9 years (1·1 million DALYs, 95% UI 889 333 to 1 299 952, and 12 379 deaths, 95% UI 10 049 to 15 024), but higher again during adolescence, such that adolescents aged 15–24 years accounted for 4·7 million DALYs and 62 529 deaths. Mozambique, Nigeria, and South Africa together accounted for 34·5% of all HIV burden across the developmental window (table 3), with the largest burden of HIV and AIDS per capita (figure 4) in Eswatini (6369 DALYs per 100 000 population), Lesotho (8907 DALYs per 100 000), and Mozambique (8934 DALYs per 100 000). There were also important sex differences in burden. Countries with the greatest HIV burden for females aged 15–24 years included Lesotho (15 826 DALYs per 100 000 population *vs* 7283 per 100 000 for males), Mozambique (12 503 per 100 000 *vs* 6535 per 100 000 for males), Eswatini (10 992 per 100 000 *vs* 5383 per 100 000 for males), and South Africa (10 253 per 100 000 *vs* 4088 per 100 000 for males). Over time there has been an increased HIV burden in many settings, with HIV burden only declining in 47 (23%) of 204 countries for children and adolescents aged 5–14 years and 65 (32%) of 204 locations for adolescents aged 15–24 years.

The HIV MIR for children younger than 5 years, adolescents aged 15–19 years, and adolescents aged 20–24 years, and the MIR at a country level are shown in the appendix (pp 84–86). The highest MIRs were observed for adolescents aged 15–19 years and mostly for males in settings of low sociodemographic development (MIR >3 in Burkina Faso, Burundi, Côte d'Ivoire,



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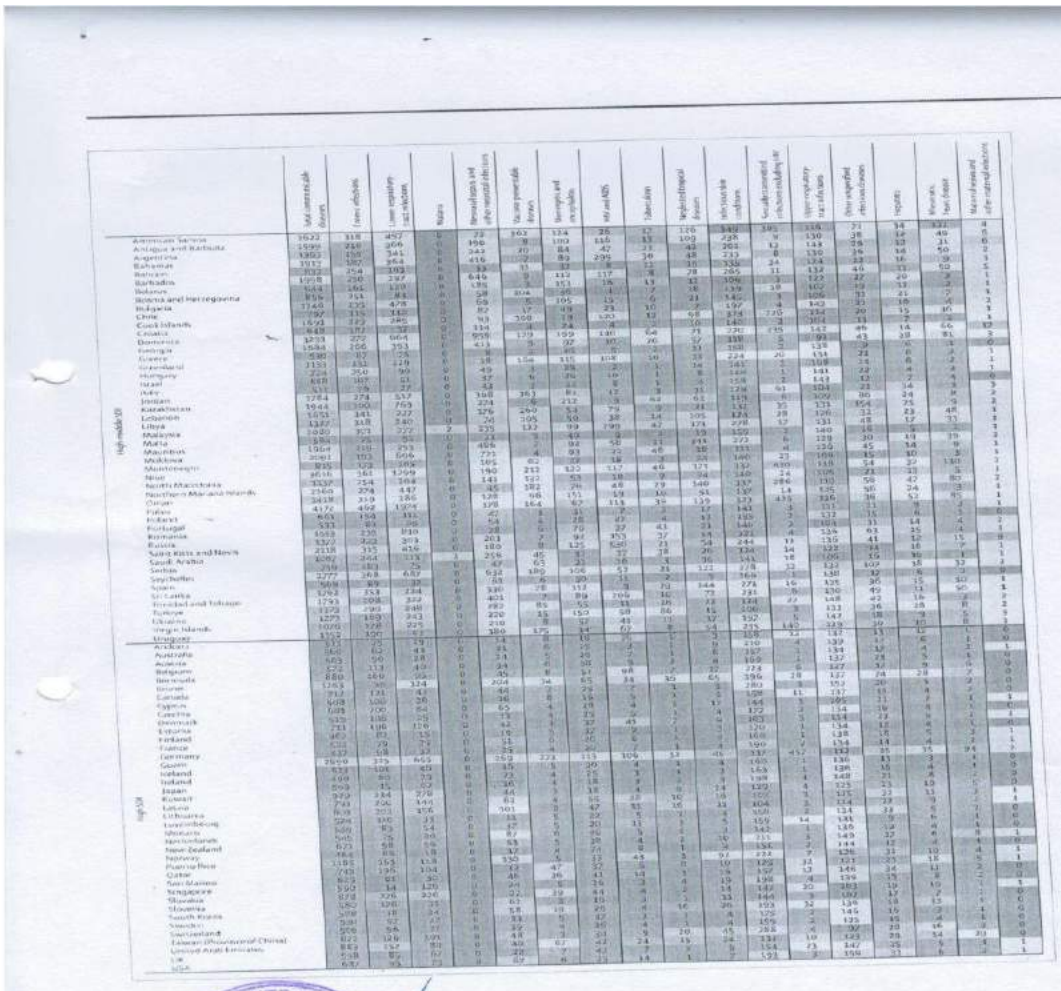

Figure 4: Country-level heatmap of communicable disease DALYs by cause for children and adolescents aged 0–24 years in 2019, grouped by SDI
The shading ranges from green, which indicates a low number of DALYs per 100 000 for that country within the disease, whereas the highest rates are shaded in a dark orange colour, which indicates a country has a large DALY burden. DALY=disability adjusted life-years. SDI=Socio-demographic index.

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Ethiopia, Eritrea, Somalia, and Togo), with females aged 15–19 years in Syria having an MIR of 32.

Discussion

Much remains to be done to reduce the 3 million deaths each year from communicable diseases among children and adolescents globally, approximately one death every 10 sec. Our analysis supports a continued focus on mortality reduction among children under 5 years in settings of low sociodemographic development, with a continued focus on gastroenteritis, pneumonia, and malaria.[1,2] However, policy and programming actions need to be inclusive of older children and adolescents, who accounted for 647 168 deaths from communicable diseases in 2019. Within this action, we also need to shift our focus to other diseases, including HIV and tuberculosis; the marked increases in deaths in older children and adolescents infected with HIV in some settings are at odds with overall reductions in communicable diseases across the developmental window. We also need to look beyond mortality reduction and focus on morbidity reduction; the 30 million years of healthy life lost to disability in 2019 among children and adolescents signifies an opportunity for health gain; this estimated burden does not include effects on education or social engagement, and as such, effects on human capital will be even greater. This reframing also brings into scope the substantial burden of disability related to communicable diseases in countries of high and high-middle sociodemographic development (8·9 million DALYs and 4·0 million YLDs in 2019 alone), often at the margins of communicable disease control.

This analysis documents the substantial unmet needs in communicable disease control before the COVID-19 pandemic. These findings highlight the need for health systems, particularly in settings of low sociodemographic development where disease burden is focussed, to continue to build capacity to respond to communicable diseases across the life course. Excess mortality-to-incidence ratios for HIV, especially for male adolescents in settings of low sociodemographic development, suggests barriers (supply or demand) to quality health care. The findings also suggest the need to strengthen prevention. Required preventive efforts include established interventions, such as immunisation (the large number of vaccine-preventable deaths suggests incomplete coverage), but also investment in broader approaches that address social determinants. For example, the excess burden of HIV among female individuals in some settings suggests harmful gender norms that might drive differential risk exposure (eg, intimate partner violence),[3] or limit access to quality health care; these same gender norms might be driving the excess mortality-to-incidence ratio for male adolescents.[3] The findings also highlight the need for communicable disease-focussed programme policies to be inclusive of older children and adolescents. As such, although the replenishment of The Global Fund is welcomed, these resources need to stretch further, and especially if we are to extend our focus while also maintaining efforts where progress has been made.[4]

To our knowledge, this study is the first systematic analysis of all causes of communicable-disease morbidity and mortality across the developmental window. Available estimates of diarrhoea and pneumonia have been largely limited to children younger than 5 years and focussed on mortality.[1,2,5] Estimates of malaria and tuberculosis have typically not reported disaggregated data for adolescents,[6,7] and global data coverage for HIV in adolescents remains limited,[8,9] but is improving. Our HIV results replicate, yet extend, previously published GBD 2019 incidence and DALY data disaggregated for adolescents aged 10–14 years, 15–19 years, and 20–24 years.[10] We also extend upon currently available HIV data from UNAIDS that are limited to incidence and mortality.[11] In short, existing reporting frameworks do not consistently disaggregate data for children and adolescents,[8] focus on conditions in isolation, or are limited to measures of mortality. This incomplete reporting is reflected in key data dashboards, including Countdown to 2030[12] (dependent on available primary data), and means that there are important areas of data and knowledge scarcity in policy and programming. As an example, the inter-UN agency OneHealth tool, developed to inform national strategical planning and resource allocation, does not model interventions for diarrhoea and pneumonia beyond the age of 5 years.[13]

Our analysis, which explored all causes of communicable diseases for children and adolescents across the globe, identified some clear targets for action. Five cause groups (enteric infection, LRTIs, malaria, tuberculosis, and HIV) account for more than two-thirds of the burden from communicable diseases across the developmental window. There are also some countries that contribute the greatest burden of these conditions, allowing for targeted actions. India, Nigeria, and Pakistan together account for 47·7% of disease burden related to enteric infections among children and adolescents, 44·2% of lower-respiratory-tract infections, and 37·8% of tuberculosis cases. For tuberculosis, these three countries are identified as priority countries in the WHO Global Tuberculosis Report,[7] but countries such as Chad and Somalia that we identified as having an excess tuberculosis burden for children and adolescents were not included. For malaria, we found that the Democratic Republic of the Congo, Nigeria, and Uganda together account for 42·9% of the malaria burden among children and adolescents, consistent with priority countries in the WHO World Malaria Report.[6] For HIV, just six sub-Saharan countries (Ethiopia, Kenya, Mozambique, Nigeria, South Africa, and Zambia) contribute to more than half of the global HIV burden for children and adolescents. These findings can help inform where efforts can be focussed, but not at the expense of children and adolescents in other settings, and not at the expense

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of opportunities to tackle morbidity. In this regard, it is important to also keep in scope the diseases for which the overall burden might be small, but for which the incidence has increased over time (including STIs, rheumatic heart disease, and neglected tropical diseases), because they pose future threats.

We identify that HIV needs to be a particular priority for global health action. Our trend analysis (annualised change over the past 30-year period and less sensitive to recent improvements as reported elsewhere)[1,2] showed that although incidence has declined, mortality and burden have increased over time for older children and adolescents. These findings probably reflect the success of Prevention of Parent to Child Transmission interventions and early antiretroviral therapy on improved survival in young children, but unmet health-care needs in older children and adolescents living with HIV. For example, we found that male adolescents in Burkina Faso, Burundi, Côte d'Ivoire, Ethiopia, Eritrea, Somalia, and Togo and female adolescents in Syria have an MIR higher than 3, substantially greater than other age groups and greater than the global all-age average of 1·6 as reported by UNAIDS. As such, accessible and responsive health care for adolescents living with HIV must be prioritised along with efforts to prevent HIV transmission and acquisition. High-quality subnational data are central to this endeavour, including data on the mode and timing of HIV acquisition.

Our analysis provides estimates up to 2019, and there is no doubt that the COVID-19 pandemic has radically shifted the landscape for communicable disease control. COVID-19 vaccine hesitancy and disruptions to education and primary care services pose real risks to preventive and promotive interventions for communicable disease.[46,47] However, the COVID-19 pandemic has also highlighted the need to address social inequity and has highlighted interventions (decreasing social contact when unwell, hand sanitation, and interventions to improve air quality)[48] that might favourably affect broader communicable disease control.[49–51] There are additional threats that will probably affect communicable disease control. The first is climate change, which increases the incidence and burden associated with numerous communicable diseases, particularly malaria and enteric infections.[52] Global warming impacts the built environment and natural habitats, causing expansion in the range and movement of wildlife vectors present in populated areas. In response, proven and effective tools to fight malaria will need to be introduced to new areas. The second is population growth, with the global population forecast to peak in 2064.[53] In 2100 it is forecasted that the majority of the world's population (including children and adolescents) will live in countries of low and low-middle sociodemographic development (eg, Democratic Republic of the Congo, India, and Nigeria),[53] settings that have an excess burden of communicable diseases. The third is an increasing demand on the shrinking global health budget. Mental health and non-communicable diseases, long neglected, are increasingly included within global health policy, and rightly so; however, these investments must not displace the required efforts to address communicable diseases.

To maximise data coverage and ensure comparability across locations and over time we used modelled data from the GBD 2019 Study. The disease models employed within the GBD 2019 Study are robust for communicable diseases, and a particular strength is that they harmonise what are often disparate (and sometimes conflicting) epidemiological surveillance data.[19] Indeed, burden of disease data are increasingly being used in global health, including in the UNICEF adolescent health dashboard.

In these analyses we extended the definition of communicable diseases to include all communicable diseases and their direct sequalae, as modelled in the GBD 2019 Study, resulting in 83 million DALYs in addition to the 420 million DALYs traditionally reported as communicable diseases in GBD 2019. There are also some important limitations associated with using GBD data. Notably, the quality of primary data for communicable diseases is dependent on diagnostic accuracy and population-based surveillance; the burden of diseases such as tuberculosis, STIs, rheumatic heart diseases, and neglected tropical diseases might be underestimated.[54] Data are also limited in settings of low sociodemographic development (in which burden is greatest) and for older children and adolescents; however, we detailed UIs for each cause, and these give some indication of where the data need to be strengthened (appendix p 87–167). Historical data are also limited in quality, and these limitations might have affected our trend analysis. Cause of death data might also underestimate the contribution of some communicable diseases; for example, deaths among people with HIV might be caused by other causes such as tuberculosis. Within GBD, estimates of morbidity are dependent on disease weights, which are not age or gender specific, and do not include educational and social burdens, which are especially relevant for children and adolescents. GBD also does not include the lifelong or intergenerational effects of disease, and so the true burden might be underestimated. However, these modelled estimates do provide guidance on where the burden of disease is and can inform current efforts to strengthen measurement and reporting of child and adolescent health globally.[55,56]

Following the COVID-19 pandemic, communicable disease control among children and adolescents must be central to efforts ensuring sustainable development.[6] Our findings support the continued focus of policy and action on diarrhoea, pneumonia, and malaria, and on young children. However, widening the scope to include older children and adolescents, extending the disease focus to include tuberculosis and HIV, and investing in actions to reduce morbidity and mortality are needed to

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REVIEW ARTICLE

Fused Deposition Modelling (FDM) in Personalized Medicine- An Overview on the Rise of Fused Deposition Model

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ABSTRACT

Three-dimensional 3D printing is a trending technology that makes complex 3D bodies by settling materials layer by layer. 3D printing uses different materials. And to make this technique sustainable, research has been going on. FDM is one of the special 3d printing techniques that are more affordable than other techniques due to its ease of availability and affordability. FDM 3D printing is based on the melt extrusion of thermoplastic polymers for the creation of objects. It is an emerging technique for creating customized and complex dosage forms tailored to the needs of the patient. This customizability makes FDM a powerful method for fabricating personalized and patient-tailored dosage forms. Therefore, in the last few years research has increased in demonstrating the utilization of FDM to produce solid dosage forms. There are many research articles published in the last 7 years. This review gives a basic overview of FDM and several stages involved in FDM 3D printing, filament preparation from hot-melt extrusion, and various new applications.

Keywords

Additive manufacturing, Clinical trials, Computer-aided drug design, Drug delivery, Fused deposition modeling, personalized medicine, Stereolithography, Selective laser sintering, 3D Printing technology.

Introduction

3D printing technology was established by Charles Hull in the early 1980s. This is an additive manufacturing process in which thin layers of metal, liquid, or powdered plastic are laid down and fused to create a complex 3-dimensional solid device or object[1]. The construction of a 3D object is done using computer-aided drug design (CAD). Whereas the technology was established in the 1980s, 3D printers were not commonly accepted until 2015 when the USFDA has given green light to the first 3D printed product, Spritam, manufactured by Aprecia Pharmaceuticals[2]. This technology has led to immense attention within the pharmaceutical industry, mostly due to its strength to resolve the challenge the industry is struggling with regarding personalized medicine[3]. It offers many advantages over the conventional rapid prototyping process, as 3D printers produce objects with

Abbreviations: 3DP: Three-dimensional printing; FDM: Fused deposition model; FDA: Food and drug administration; API: Active pharmaceutical ingredients; PVA: Polyvinyl alcohol; PVP: Polyvinyl pyrrolidone; PLA: Polylactic acid; ABS: Acrylonitrile butadiene styrene; SLS: Selective laser sintering; SLA: Stereolithography; HME: Hot melt extrusion; HPMC: Hydroxyl propyl methylcellulose; TID: Tablet in device; ODF: Oro-dispersible films.

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high complexity, less expensive material, and are less time-consuming, and it can produce dosage forms that consist of multiple APIs in different layers of the same unit dosage form as needed to a particular patient[4].

Various 3D printing techniques have been developed for different purposes including stereolithography, selective laser sintering, inkjet printing, zip dose technology, and fused deposition model (FDM)[5]. FDM is the exceptional technology that is found in open source, which makes this 3D printing technology the most affordable one, as compared to others such as powder-based 3D printing, selective laser sintering (SLS), and stereolithography (SLA). The focus of this article is on the application of fused deposition modeling (FDM). The goal for choosing FDM from all the 3D printing processes is because of its broad applications in many industries. The FDM process is the simplest, cheap, and more feasible technique for polymer-based materials, and it has been highly used in various industries[6].

Marketed product of 3D printing

Spritam- the first drug fabricated using 3D printing technology (3DP) has been given the green light by the US food and drug administration. Levetiracetam is a generic name for spritam as shown in figure 1 which is used to treat epilepsy[7]. Levetiracetam has been used in seizures for 15 years but by coupling 3D printing technology with epilepsy treatment, spritam is fabricated to fulfill the requirements of patients who battle with conventional medicine.

A future to 3D printing- Graphical records of the 3D printed medicine

3D printing create a base for personalized medicine in the 1990s. Several advancements have taken place in this field. In 1995, the first paper was published that narrates the production of 3d printing devices by binder jet[8]. First 3D printed medicine was fabricated using FDM in 2014. 3D printed polypill with various geometries was first fabricated in 2015[9]. In 2016, Spritam was the first 3D printed medicine that is FDA approved[10]. World-first clinical study to treat children for maple syrup urine disease in 2019[9]. For personalized medicine, the world's first pharmaceutical 3D printer was launched in 2020. Figure 2 shows the map of 3D printing advancements from 1995 to 2020.

Papers and patents related to 3D printing technology

3D printing can boost treatment efficacy while decreasing the adverse effect risks resulting in inaccurate treatment. 3D printing can create a poly-printlet containing more than one drug. It can also alter design, shape, and size with the help of auto CAD.

Scoutaris et al. fabricated candy-like formulations of indomethacin in 2018 using FDM. They studied that 3D printing can formulate palatable drugs for children[11].

Januskaite et al. prepared a placebo with a similar size and shape using a fused deposition model, selective laser sintering, semi-solid extrusion, and digital light photopolymer. They explored children's perception of printlets. Around 79% of children choose 3D printed chewable dosage form[12].

A world-first clinical study was executed utilizing a 3D printer to formulate personalized drugs in the pharmacy. In 2019, this technique was combined in the clinic hospital at the University de Santiago de compostela, Spain to fabricate personalized medicine for maple syrup urine disease for 3-16 years aged children[13].

Robles-Martinez et al. fabricated 3D printed polyprintlets containing six different drugs (aspirin, paracetamol, caffeine, naproxen, prednisolone, chloramphenicol) parted into 6 compartments which reduces dosing frequency[8].

Antihypertensive polypills consisting of ramipril, aspirin, atenolol, hydrochlorothiazide, and pravastatin were fabricated by a fused deposition model[14].

Oncology hospital in Paris announced a partnership with FabRx in 2021 June focusing to convert 3d printed formulations into the clinic. In this, multidrug or personalized drugs will be produced for the therapy of breast cancer patients.

A study includes patents relating to cancer applications using three-dimensional printing technology. A total of 51 patents are there in cancer applications[15]. Figure 3 has shown different patents relating to 3D printing.

Fused Deposition Model (FDM)

FDM is the most commonly used 3D printing technology (Additive manufacturing). It was first invented by S. Scott Crump in the late 1980s and was marketed in 1990 by Stratasys. The history of the fused deposition model is shown in figure 4. The FDM process comes under an extrusion-based additive method, where the material is forced out with the help of a nozzle and spread in successive layers on the build plate. Thermoplastic material is used and heated to a semi-molten state and then forced out to build a 3D object layer-by-layer[4]. It has been observed that the attraction toward FDM topics has increased over the years, as the amount of articles increases. Figure 5, has shown the rising interest in the fused deposition model technique[16].

Working of fused deposition model-

- A reel of thermoplastic filament is delivered to the FDM printer then these filaments are melted and softened and then forced out from the print head that has potential, to move in x, y, and z directions.
- Each layers of melted thermoplastic are bonded to another layer that gives rise to a solid object.
- As one layer cools down and solidifies, another layer of melted filament will be deposited on top of it until the printing of the object is finished[17]. Figure 6 has shown a diagrammatic view of the fused deposition model.

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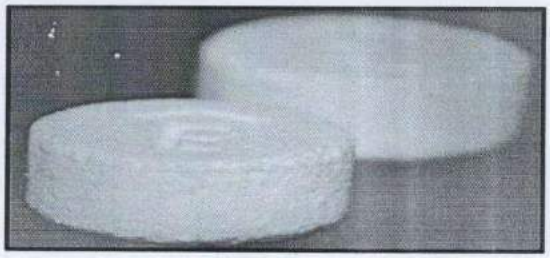

Fig. 1. Spritam- first 3D printed tablet approved by USFDA to treat epilepsy.

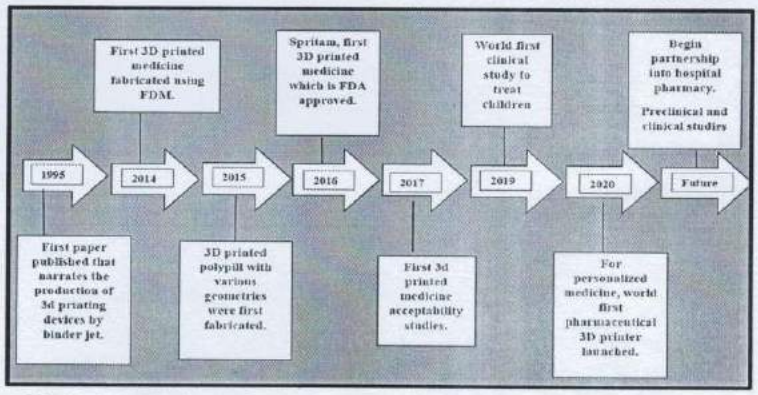

Fig. 2. Graphical record of the 3D printing advancements from 1995 to 2020

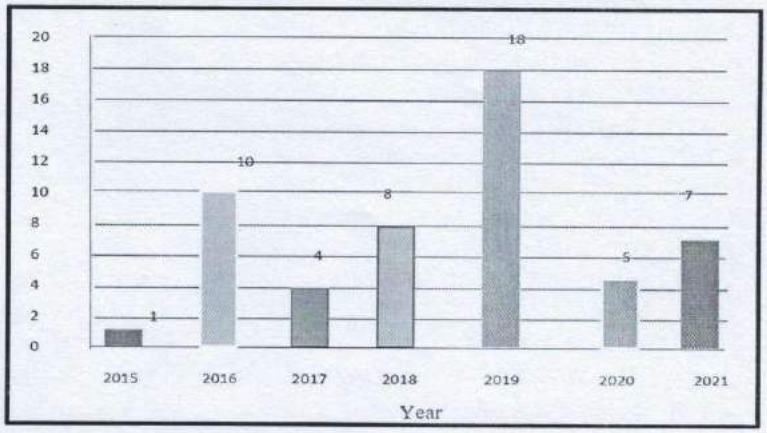

Fig. 3. Patents in 3D printing technology relating to cancer therapies

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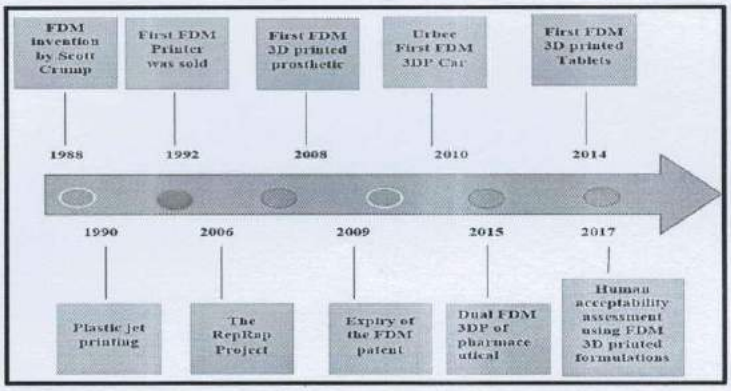

Fig. 4. History of fused deposition model (FDM)

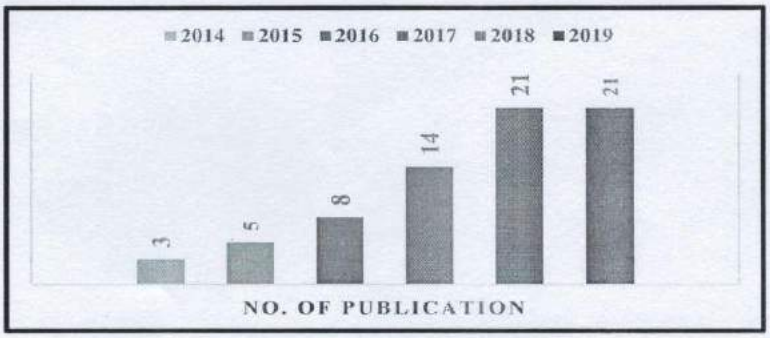

Fig. 5. Evolution of the FDM articles

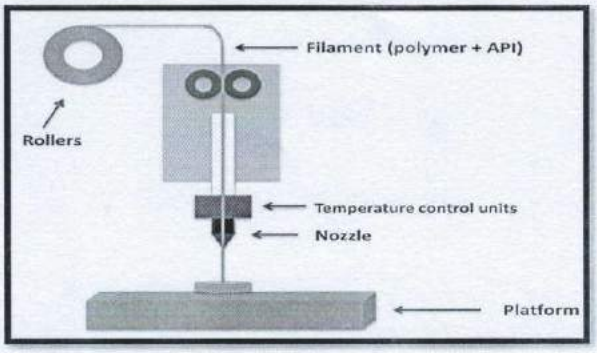

Fig. 6. Fused deposition model machine with temperature control units

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Materials used in FDM –

Mainly thermoplastic materials are used in a filament form and these filaments are melted and softened and then forced out from the print head. These thermoplastic filaments are used for deposition purposes. Polyvinyl alcohol (PVA), Acrylonitrile butadiene styrene (ABS) and polylactide (PLA) are some thermoplastics used in this process[18]. Several filaments are discussed in **table 1.**

Fused deposition model parameters-

The fused deposition model is affected by numerous parameters as compiled in **figure 7**. There are two broad classes of FDM parameters, namely material and machine parameters. The quality of prints and printed parts depends on these two parameters. Material parameters such as mechanical and thermal affect both performances of print and extrusion. On the other hand, machine parameters include printing speed, layer thickness, infill density, nozzle, air gap, temperature, etc[17].

Fabrication of 3D solid oral dosage form using FDM-

There are three different strategies to load Active Ingredients into the 3D printed material.

Impregnation-FDM

In this strategy, the filament is immersed in the solution of active ingredients for several days. The filament is dried in an oven after impregnation. Then this active ingredient-loaded filament is used as raw material for the printer as shown in **figure 8**[19]. An example of impregnation- FDM is discussed in **table 2**

Print and Fill process

In this process printing an empty shell and then filling it with the active ingredient in the form of liquid or powder as shown in **figure 9.** The steps of printing and filling can be either sequential or simultaneous. Some example of the print and fill process is discussed in **table 3.**

HME-FDM

In this process, FDM is combined with hot-melt extrusion. An API and excipients are combined in a single or twin-screw extruder to form an API-loaded filament[19]. Some example of HME- FDM is discussed in **table 4.**

HME-FDM 3D Printing (pairing of HME with FDM)

Hot-melt extrusion technology has gained much attention from the pharmaceutical industry for the manufacturing of oral dosage forms and implants. The amount of HME patents has increased since the 1980s. A clear view of an increased amount of patents has shown in **figure 10.** This is because the hot-melt extrusion process has the potential to reach the regulatory requirement for the greater demand for personalized medicine.

FDM 3D printers used thermoplastic materials that needed to be in the form of a filament. But several filaments are not acceptable for pharmaceutical applications. This problem has been a great hurdle for FDM 3D printing. Besides, most conventional polymer excipients that have been utilized in pharmaceutical industries do not have the proper mechanical and thermal properties. Some thermoplastic materials suitable to be made into filaments are polyvinyl alcohol (PVA), polyvinyl pyrollidone (PVP), and polylactic acid (PLA) filaments because of their excellent mechanical and thermal properties. They require to be of good quality so that we can get a high-quality 3D printed product. HME method can produce good quality thermoplastic filament.

There has been some research on combining HME technology and FDM to obtain a more effective process. HME-FDM opens up an opportunity for creating any dosage form for immediate use. Coupling these two technologies makes the manufacturing of oral dosage forms more economical, cost-effective, and efficient[20]. In **figure 11,** the combined HME-FDM technique has shown.

Table 1. Thermoplastic material used in FDM

Materials	Bio-degradability	Physical Property	Melting Point
Acrylonitrile butadiene styrene (ABS)	Non-biodegradable	It offers shiny and smooth surfaces. It is soluble in ketones, acetone, esters, etc.	105°C
Polylactic acid (PLA)	Biodegradable	It is soluble in hot benzene, dioxane, and chlorinated solvents. Heat-resistant PLA can withstand temperatures of 110°C.	105°C-160°C
Polyvinyl alcohol (PVA)	Biodegradable	It shows high tensile strength and flexibility. It is soluble in water. It is insoluble in organic solvents.	180°C-228°C
Polycaprolactone (PCL)	Biodegradable	It is partially crystalline and soluble in chloroform, tetrahydrofuran.	60°C
Ethylene-vinyl acetate (EVA)	Non-biodegradable	Insoluble Pill or powder form.	59°C-185°C

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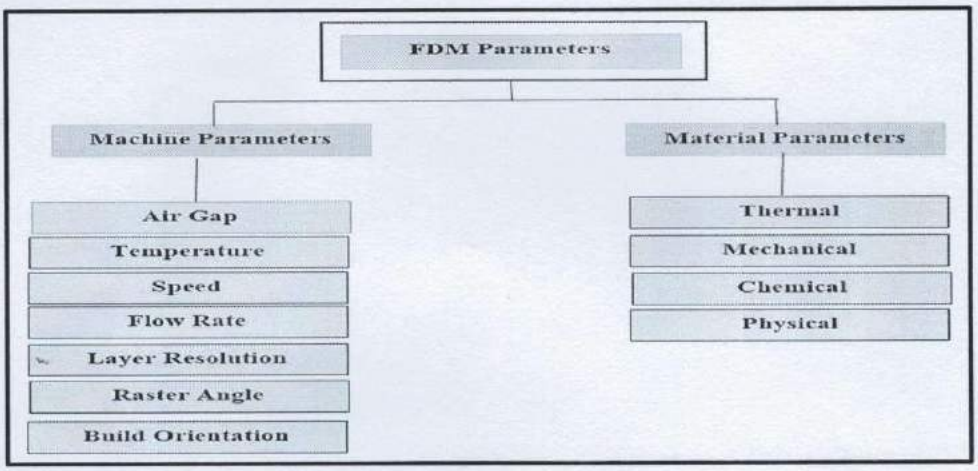

Fig. 7. Process Parameters of FDM

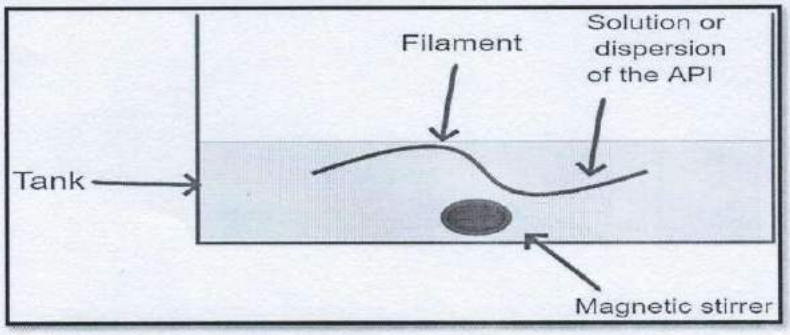

Fig. 8. Impregnation –FDM process

Table 2. Some examples of Impregnation FDM

API	Polymer Used	Solvent Used	Incubation Time	Resulted kinetics	References
Curcumin	PVA	Ethanol	24 h	Sustained release	21
Fluorescein	PVA	Ethanol	24 h	Sustained release	22
Metformin	PVA	Ethanol	72 h	Sustained release	23
Prednisolone	PVA	Methanol	24 h	Sustained release	24
4-aminosalicylic acid	PVA	Ethanol	24 h	Sustained release	25
Deflazacort	Eudragit	Acetone	24 h	Sustained release	26

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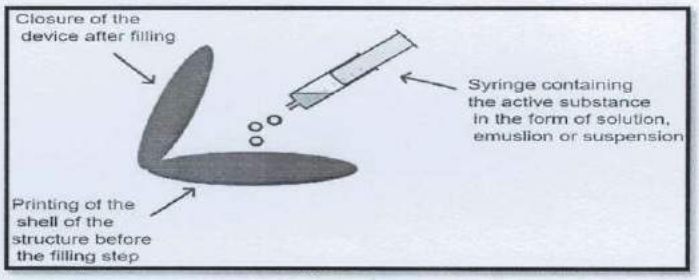

Fig. 9. Print and Fill process

Table 3. Some examples of the Print and Fill process

API	API blend method	Shell material	Shape	Release kinetics	References
Paracetamol	Manually filled	HPMC	Two half-shells of a capsule	Delayed-release	27
Paracetamol	Manually filled	Eudragit	A hollow capsule	Delayed-release	28
Paracetamol	Manually filled	HPC	A hollow capsule	Delayed-release	29
Acyclovir	Manually filled	PLA	Windowed floating capsules	Sustained-release	30
Lamivudine	Filled by printer syringe manually	PVA	Cylindrical hollow capsule with internal scaffold	Delayed-release	31
Metformin	Filled by printer syringe manually	PVA	Cylindrical hollow capsule with internal scaffold	-	31
Riboflavin	Manually filled	PLA	Capsules with modified head and body parts	Sustained-release	32
Theophylline	Filled by printer syringe manually	PLA Eudragit	A hollow capsule	Sustained release	33

Table 4. Some examples of HME- FDM

API	Polymers	Type of extruders	Shape	Release kinetics	References
Paracetamol	HPC HPMC	Twin extruder	Cylindrical tablet	sustained release	34
Baclofen	PVA	Twin extruder	Square films	sustained release	35
Budesonide	PVA	Single extruder	Caplet	sustained release	36
Caffeine	Kollindon	Twin extruder	Cylindrical table	sustained release	37
Haloperidol	Kollindon	Twin extruder	Cylindrical tablet	Immediate release	38
Ibuprofen	Ethylcellulose	Twin extruder	Cylindrical tablet	sustained release	39
Furosemide	PVA Kollindon HPMC	Twin-screw	Thin disks	Immediate and sustained release	40

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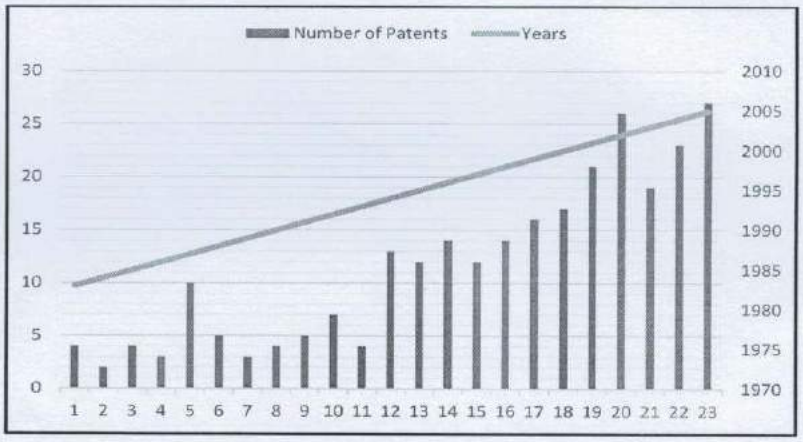

Fig. 10. Number of HME patents issued over the year

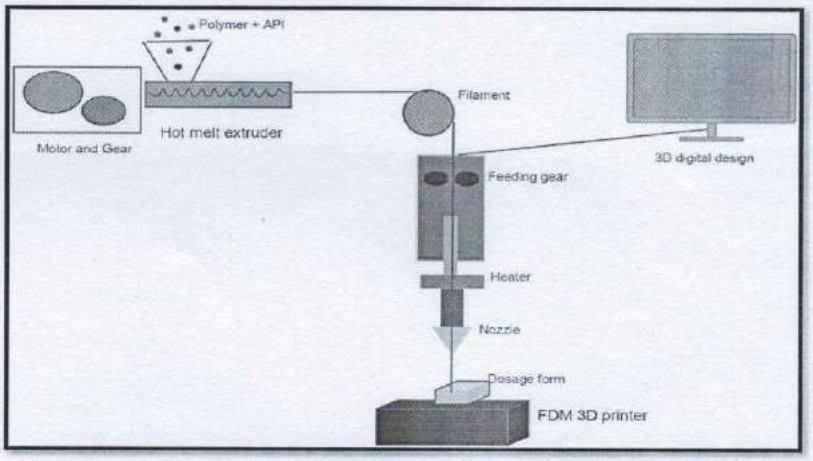

Fig. 11. Hot melt extrusion and Fused Deposition Model combined in one integrated continuous process.

FDM 3D Printing for Various Drug Delivery Devices

FDM 3D Printers can manufacture a broad range of various drug delivery devices. Many drug delivery devices were fabricated by FDM, as published between the years 2014 and 2018 in research papers. Tablets consist of the largest share of the graph (63%), as the greater studies explored the manufacturing of tablet dosage form, followed by capsules (11%) as shown in figure 12.

Domperidone disks were printed with low infill, which let the drug stay in the stomach for a long time through floating, thus improving patient compliance by decreasing the frequency of tablet dose. Polypills are also fabricated and consist of paracetamol and caffeine, resulting in the release of both drugs at the same time. Tablet's shapes and sizes can also control, which helps regulate the drug release rate[6].

Pharmaceutical application of FDM in drug delivery-

Controlled release dosage form

To achieve a good therapeutic effect, the release of drugs from dosage forms plays an important role. The controlled release allows the slow release of active content, reducing

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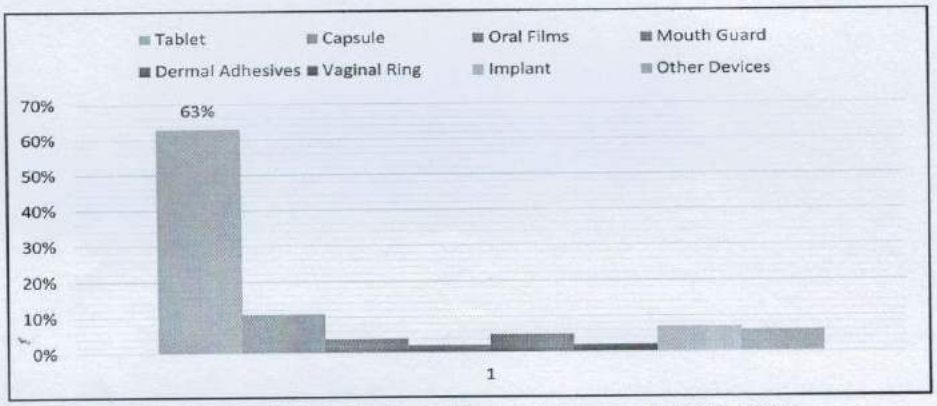

Fig. 12. Graph of various drug delivery systems manufactured by FDM

dose fluctuation related to IR dosage forms at regular intervals. 3D printing can get control some problems of traditional sustained-release tablets, like the release of drug is non-constant from dosage form because of low total surface area. Some controlled-release tablets that were invented from FDM.

Zhao et al. fabricate a 3D printed shell with an FDM printer using polyvinyl alcohol (PVA). They gave a spherical shape to the shell with a blank internal tetrahedral cavity. The blend of PVA and a drug was filled in that shell. When a tablet makes contact with water, it breaks down, resulting in increased drug dissolution over time. Patients who need rapid drug release can be administered this tablet at night and the maximum drug concentration in the blood will spike in the morning[41].

Kadry et al. fabricated tablets with several infill patterns using an FDM printer. Diltiazem and HPMC powder were mixed by extrusion. The results revealed that the tablets with hexagonal infill patterns dissolved quickly[42].

Yang et al. observed that the drug release rate was affected based on tablet patterns. The tablets were fabricated by using an FDM printer, using filaments containing ibuprofen and ethylcellulose[43].

Skowyra et al. printed extended-release personalized prednisolone tablets with a FDM 3D printer. The drug prednisolone was loaded into a PVA polymeric matrix. PVA filament was soaked in a prednisolone methanolic solution. This study revealed that FDM-based 3D printing is a favorable method for fabricating extended-release tablets, and future products can be tailored to controlled-release tablets[44].

Multiple API tablets

3D printing can play an important role in multiple API dosage forms, where the multilayer printed tablets are formulated with sustained-release properties. This minimizes the burden of more than one pill a day and many tablets taken by the patient on a daily routine. The polypill concept leads to the therapy of consuming more than one drug to one or more active content in single dosage form.

Wald and law in 2003 suggest a polypill, to reduce cardiovascular diseases. This polypill contains 6 active pharmaceutical ingredients in one dosage form[45].

Melocchi et al. printed capsular devices with two-compartment by FDM. This device has the potential to carry chemical incompatible drugs or a different drug. The thickness of the capsule is important for the dissolution profile[29].

Gioumouxouzis et al. also applied the same concept in which a dosage form containing two anti-diabetic drugs, glimepiride and metformin was fabricated. The tablets were fabricated by FDM from two nozzles[48].

This study revealed that the FDM-based 3d printer has a capacity of producing a single oral caplet with multiple APIs and has the potential of attaining delayed release profiles.

Gastro-floating devices

Floating drug delivery systems have developed to remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period. Effervescent and non- effervescent systems are used to increase gastric retention time.

Shin et al. developed a gastro retentive delivery system for acyclovir by FDM and evaluate *invivo* pharmacokinetics in beagle dogs. The system contains a gastro-floating device that can float in the gastric fluid, printed by an FDM 3D printer and a traditional SR acyclovir tablet. The tablet was placed in the floating devices to bring the sustained release of the drug in the stomach[47].

Chai et al. fabricated a hollow tablet using FDM by using hydroxypropyl cellulose (HPC). Domperidone drug

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was loaded into HPC filaments through hot-melt extrusion. Prolonged floating and released were noted because of low density and rigid shells[48].

Huanbutta and sangnim developed zero-order drug release gastro retentive floating tablets printed by FDM. The floating housing was fabricated from acrylonitrile butadiene styrene (ABS) or polyvinyl alcohol (PVA) which are insoluble and water-soluble synthetic polymers, respectively. It can provide drug release orifice for constant drug release rate[49].

Fabricated riboflavin floating tablet-in-device (TID) systems by 3D printing techniques. The device consists of two parts-a body and a cap. Two types of TID systems are applied-single and double net TID. Riboflavin tablet inserted in the device, that's why it is named as tablet-in-device[50].

Transdermal Microneedle Patches-

A transdermal drug delivery system is applied onto a healthy skin for delivering drugs systemically. It is a painless method. In this, the drug penetrates subcutaneous without accumulation of the drug in the dermal layer. Microneedles deliver the drugs by piercing through the skin. It is an array of needles of a hundred micron in size. Recently, Wu et al. used a new microfabrication technique (FDM 3D printer) with an improved resolution to fabricate biodegradable microneedles. Polylactic acid is used as a polymer that showed the degradability in the skin[51].

Oro-dispersible films-

Oro-dispersible films have been used to improve patient compliance and ease of swallowing in patients who have got problems swallowing food or medicine. Advantage of ODF is that it doesn't need water and also avoids first-pass metabolism.

Fabricated aripiprazole oro-dispersible films using FDM 3D printing. The drug is mixed with PVA and filament was produced using HME. Dimensions of films 20, 30, and 0.15 mm were designed and printed. As compared to cast films, 3d printed films have more flexibility and lower durability. In dissolution profiles, standard deviations were reported to be smaller than cast films, suggesting the ability of FDM 3D printing for manufacturing ODFs[52].

Examined the application of FDM in fabricating multi-layered fast-dissolving films. PVA was prepared as feedstock filament and paracetamol as a model drug. To increase the disintegration of films, sodium starch glycolate and cross-carmellose sodium were added to the formulation, and to increase the dissolution rate, sodium lauryl sulfate was added. Films had appropriate flexibility and faster drug release. This work showed the potential of FDM 3D printing to prepare fast dissolving films[53]. **Figure 13** displays various examples of drug delivery devices fabricated by FDM 3D printing.

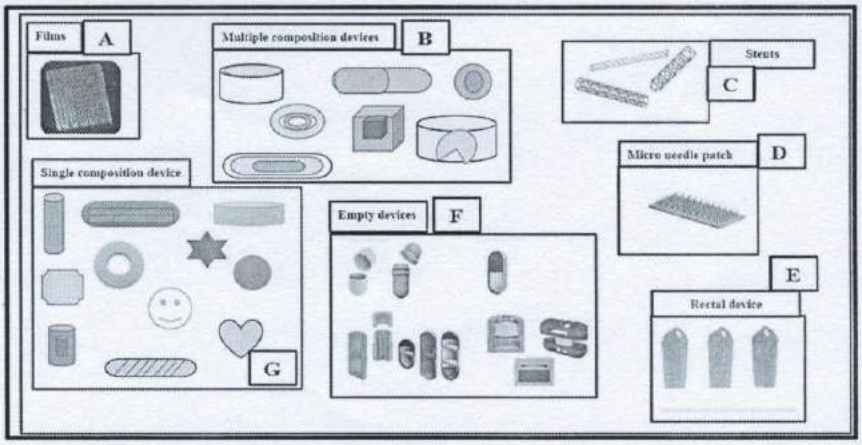

Fig. 13. Examples of different drug delivery device fabricated by 3D printing. **(A)** Orodispersible films fabricated by 3D printing (Jamróz et al., 2017). (B) Mulitple composition devices which contains two or more drug composition in single devices. (C) Drug eluting stents in treatment of coronary artery diseases (Wald, 2003). (D) Biodegradable 3D printed microneedle patch using FDM (Luzuriaga et al., 2018). (E) 3D printed rectal suppositories (Rodríguez-García et al., 2021). (F) Empty devices fabricated from 3D printing technology and then assembled with conventional tablet also called Tablet-in-device (TID) (Huanbutta & Sangnim, 2019; Melocchi, Uboldi, Maroni, et al., 2020; Shin et al., 2019). (G) Single composition device printed by FDM.

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Future Perspectives

Fused deposition modeling for drug production express the future of pharmaceuticals. FDM is an additive manufacturing technology that has a high ability for personalized medicine. Also, the concept 'On Demand production' can be useful when manufactured using FDM technologies. Thus, such technologies have the potential for manufacturing low-cost patient-specific formulations. FDM can also manufacture complex geometries and can also tailor the drug release kinetics as needed. Additionally, FDM is relatively cheap compared to conventional technologies.

Research has been increasing in the 3D printing field for manufacturing personalized medicine, and we believe this will attain advanced possibility, and by this particular application of 3D printing, pharmacies will be revolutionized.

Conclusion

FDM 3D printing is a flexible technology broadly studied for the manufacturing of multiple drug delivery devices. The ability of FDM 3D printing is indisputable in the area of personalized medicines. The FDA approval of the first 3D printed drug has given interest to many researchers in the industrial feasibility of this technique. This review found that every parameter in the FDM 3D printing process affected the quality and mechanical properties. This study has discussed increased research in FDM, the history of FDM 3D printers, the material used in FDM and their working, and different methods of filament and drug loading in addition to applications of FDM. Although there are still several challenges that are required to be in control like high thermal exposure, poor resolution, low production capabilities, safety, and regulatory concerns. Putting regulations and resolving these limitations can revolutionize the process in addition to safety, adherence, and efficacy.

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References

1. Park BJ, Choi HJ, Moon SJ, Kim SJ, Bajracharya R, Min JY, et al. Pharmaceutical applications of 3D printing technology: current understanding and future perspectives. J Pharm Investig [Internet]. 2018 Oct 29 [cited 2022 Mar 14]; Available from: http://link.springer.com/10.1007/s40005-018-00414-y
2. Samiei N. Recent trends on applications of 3D printing technology on the design and manufacture of pharmaceutical oral formulation: a mini review. Beni-Suef Univ J Basic Appl Sci. 2020 Dec;9(1):12.
3. Melnyk LA, Oyewumi MO. Integration of 3D printing technology in pharmaceutical compounding: Progress, prospects, and challenges. Ann 3D Print Med. 2021 Dec;4:100035.
4. Dumpa N, Butreddy A, Wang H, Komanduri N, Bandari S, Repka MA. 3D printing in personalized drug delivery: An overview of hot-melt extrusion-based fused deposition modeling. Int J Pharm. 2021 May;600:120501.
5. Vaz VM, Kumar L. 3D Printing as a Promising Tool in Personalized Medicine. AAPS PharmSciTech. 2021 Jan;22(1):49.
6. Araújo M, Sa-Barreto L, Gratieri T, Gelfuso G, Cunha-Filho M. The Digital Pharmacies Era: How 3D Printing Technology Using Fused Deposition Modeling Can Become a Reality. Pharmaceutics. 2019 Mar 19;11(3):128.
7. Hsiao WK, Lorber B, Reitsamer H, Khinast J. 3D printing of oral drugs: a new reality or hype? Expert Opin Drug Deliv. 2018 Jan 2;15(1):1–4.
8. Mostafaei A, Elliott AM, Barnes JE, Li F, Tan W, Cramer CL, et al. Binder jet 3D printing—Process parameters, materials, properties, modeling, and challenges. Prog Mater Sci. 2021 Jun;119:100707.
9. Robles-Martinez P, Xu X, Trenfield SJ, Awad A, Goyanes A, Telford R, et al. 3D Printing of a Multi-Layered Polypill Containing Six Drugs Using a Novel Stereolithographic Method. Pharmaceutics. 2019 Jun 11;11(6):274.
10. Wang Z, Han X, Chen R, Li J, Gao J, Zhang H, et al. Innovative color jet 3D printing of levetiracetam personalized paediatric preparations. Asian J Pharm Sci. 2021 May;16(3):374–86.
11. Scoutaris N, Ross SA, Douroumis D. 3D Printed "Starmix" Drug Loaded Dosage Forms for Paediatric Applications. Pharm Res. 2018 Feb;35(2):34.
12. Januskaite P, Xu X, Ranmal SR, Gaisford S, Basit AW, Tuleu C, et al. I Spy with My Little Eye: A Paediatric Visual Preferences Survey of 3D Printed Tablets. Pharmaceutics. 2020 Nov 17;12(11):1100.
13. Goyanes A, Madla CM, Umerji A, Duran Piñeiro G, Giraldez Montero JM, Lamas Diaz MJ, et al. Automated therapy preparation of isoleucine formulations using 3D printing for the treatment of MSUD: First single-centre, prospective, crossover study in patients. Int J Pharm. 2019 Aug;567:118497.

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14. Tagami T, Fukushige K, Ogawa E, Hayashi N, Ozeki T. 3D Printing Factors Important for the Fabrication of Polyvinylalcohol Filament-Based Tablets. Biol Pharm Bull. 2017;40(3):357–64.
15. Li R, Ting YH, Youssef S, Song Y, Garg S. Three-Dimensional Printing for Cancer Applications: Research Landscape and Technologies. Pharmaceuticals. 2021 Aug 10;14(8):787.
16. Shaqour B, Samaro A, Verleije B, Beyers K, Vervaet C, Cos P. Production of Drug Delivery Systems Using Fused Filament Fabrication: A Systematic Review. Pharmaceutics. 2020 Jun 5;12(6):517
17. Mwema FM, Akinlabi ET. Basics of Fused Deposition Modelling (FDM). In: Fused Deposition Modeling [Internet]. Cham: Springer International Publishing; 2020 [cited 2022 Mar 14]. p. 1–15. (SpringerBriefs in Applied Sciences and Technology). Available from: http://link.springer.com/10.1007/978-3-030-48259-6_1
18. Nasereddin J. An investigation into the feasibility of Fused Deposition Modelling for 3D printing oral pharmaceuticals. 2020 [cited 2022 Mar 14]; Available from: http://rgdoi.net/10.13140/RG.2.2.18012.41603
19. Cailleaux S, Sanchez-Ballester NM, Gueche YA, Bataille B, Soulairol I. Fused Deposition Modeling (FDM), the new asset for the production of tailored medicines. J Controlled Release. 2021 Feb;330:821–41.
20. Tan D, Maniruzzaman M, Nokhodchi A. Advanced Pharmaceutical Applications of Hot-Melt Extrusion Coupled with Fused Deposition Modelling (FDM) 3D Printing for Personalised Drug Delivery. Pharmaceutics. 2018 Oct 24;10(4):203
21. Tagami T, Fukushige K, Ogawa E, Hayashi N, Ozeki T. 3D Printing Factors Important for the Fabrication of Polyvinylalcohol Filament-Based Tablets. Biol Pharm Bull. 2017;40(3):357–64.
22. Goyanes A, Buanz ABM, Basit AW, Gaisford S. Fused-filament 3D printing (3DP) for fabrication of tablets. Int J Pharm. 2014 Dec;476(1–2):88–92.
23. Ibrahim M, Barnes M, McMillin R, Cook DW, Smith S, Halquist M, et al. 3D Printing of Metformin HCl PVA Tablets by Fused Deposition Modeling: Drug Loading, Tablet Design, and Dissolution Studies. AAPS PharmSciTech. 2019 Jul;20(5):195.
24. Skowyra J, Pietrzak K, Alhnan MA. Fabrication of extended-release patient-tailored prednisolone tablets via fused deposition modelling (FDM) 3D printing. Eur J Pharm Sci. 2015 Feb;68:11–7.
25. Goyanes A, Buanz ABM, Hatton GB, Gaisford S, Basit AW. 3D printing of modified-release aminosalicylate (4-ASA and 5-ASA) tablets. Eur J Pharm Biopharm. 2015 Jan;89:157–62.
26. Beck RCR, Chaves PS, Goyanes A, Vukosavljevic B, Buanz A, Windbergs M, et al. 3D printed tablets loaded with polymeric nanocapsules: An innovative approach to produce customized drug delivery systems. Int J Pharm. 2017 Aug;528(1–2):268–79.
27. Maroni A, Melocchi A, Parietti F, Foppoli A, Zema L, Gazzaniga A. 3D printed multi-compartment capsular devices for two-pulse oral drug delivery. J Controlled Release. 2017 Dec;268:10–8.
28. Krause J, Bogdahn M, Schneider F, Koziolek M, Weitschies W. Design and characterization of a novel 3D printed pressure-controlled drug delivery system. Eur J Pharm Sci. 2019 Dec;140:105060.
29. Melocchi A, Uboldi M, Cerea M, Foppoli A, Maroni A, Moutaharrik S, et al. A Graphical Review on the Escalation of Fused Deposition Modeling (FDM) 3D Printing in the Pharmaceutical Field. J Pharm Sci. 2020 Oct;109(10):2943–57.
30. Shin S, Kim TH, Jeong SW, Chung SE, Lee DY, Kim DH, et al. Development of a gastroretentive delivery system for acyclovir by 3D printing technology and its in vivo pharmacokinetic evaluation in Beagle dogs. Uzun E, editor. PLOS ONE. 2019 May 15;14(5):e0216875.
31. Smith DM, Kapoor Y, Klinzing GR, Procopio AT. Pharmaceutical 3D printing: Design and qualification of a single step print and fill capsule. Int J Pharm. 2018 Jun;544(1):21–30.
32. Fu J, Yin H, Yu X, Xie C, Jiang H, Jin Y, et al. Combination of 3D printing technologies and compressed tablets for preparation of riboflavin floating tablet-in-device (TiD) systems. Int J Pharm. 2018 Oct;549(1–2):370–9.
33. Linares V, Casas M, Caraballo I. Printfills: 3D printed systems combining fused deposition modeling and injection volume filling. Application to colon-specific drug delivery. Eur J Pharm Biopharm. 2019 Jan;134:138–43.
34. Zhang J, Feng X, Patil H, Tiwari RV, Repka MA. Coupling 3D printing with hot-melt extrusion to produce controlled-release tablets. Int J Pharm. 2017 Mar;519(1–2):186–97.
35. Palekar S, Nukala PK, Mishra SM, Kipping T, Patel K. Application of 3D printing technology and quality by design approach for development of age-appropriate pediatric formulation of baclofen. Int J Pharm. 2019 Feb;556:106–16.
36. Goyanes A, Chang H, Sedough D, Hatton GB, Wang J, Buanz A, et al. Fabrication of controlled-release budesonide tablets via desktop (FDM) 3D printing. Int J Pharm. 2015 Dec;496(2):414–20.
37. Fuenmayor E, Forde M, Healy A, Devine D, Lyons J, McConville C, et al. Material Considerations for

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Fused-Filament Fabrication of Solid Dosage Forms. Pharmaceutics. 2018 Apr 2;10(2):44.

38. Solanki NG, Tahsin M, Shah AV, Serajuddin ATM. Formulation of 3D Printed Tablet for Rapid Drug Release by Fused Deposition Modeling: Screening Polymers for Drug Release, Drug-Polymer Miscibility and Printability. J Pharm Sci. 2018 Jan;107(1):390–401.
39. Yang Y, Wang H, Li H, Ou Z, Yang G. 3D printed tablets with internal scaffold structure using ethyl cellulose to achieve sustained ibuprofen release. Eur J Pharm Sci. 2018 Mar;115:11–8.
40. Melocchi A, Parietti F, Maroni A, Foppoli A, Gazzaniga A, Zema L. Hot-melt extruded filaments based on pharmaceutical grade polymers for 3D printing by fused deposition modeling. Int J Pharm. 2016 Jul;509(1–2):255–63.
41. Zhao J, Xu X, Wang M, Wang L. A New Model of a 3D-Printed Shell with Convex Drug Release Profile. Dissolution Technol. 2018;25(1):24–8.
42. Kadry H, Al-Hilal TA, Keshavarz A, Alam F, Xu C, Joy A, et al. Multi-purposable filaments of HPMC for 3D printing of medications with tailored drug release and timed-absorption. Int J Pharm. 2018 Jun;544(1):285–96.
43. Yang Y, Wang H, Li H, Ou Z, Yang G. 3D printed tablets with internal scaffold structure using ethyl cellulose to achieve sustained ibuprofen release. Eur J Pharm Sci. 2018 Mar;115:11–8.
44. Skowyra J, Pietrzak K, Alhnan MA. Fabrication of extended-release patient-tailored prednisolone tablets via fused deposition modelling (FDM) 3D printing. Eur J Pharm Sci. 2015 Feb;68:11–7.
45. Wald NJ. A strategy to reduce cardiovascular disease by more than 80%. BMJ. 2003 Jun 26;326(7404):1419–0.
46. Gioumouxouzis CI, Baklavaridis A, Katsamenis OL, Markopoulou CK, Bouropoulos N, Tzetzis D, et al. A 3D printed bilayer oral solid dosage form combining metformin for prolonged and glimepiride for immediate drug delivery. Eur J Pharm Sci. 2018 Jul;120:40–52.
47. Shin S, Kim TH, Jeong SW, Chung SE, Lee DY, Kim DH, et al. Development of a gastroretentive delivery system for acyclovir by 3D printing technology and its in vivo pharmacokinetic evaluation in Beagle dogs. Uzun E, editor. PLOS ONE. 2019 May 15;14(5):e0216875.
48. Chai X, Chai H, Wang X, Yang J, Li J, Zhao Y, et al. Fused Deposition Modeling (FDM) 3D Printed Tablets for Intragastric Floating Delivery of Domperidone. Sci Rep. 2017 Dec;7(1):2829.
49. Huanbutta K, Sangnim T. Design and development of zero-order drug release gastroretentive floating tablets fabricated by 3D printing technology. J Drug Deliv Sci Technol. 2019 Aug;52:831–7.
50. Fu J, Yin H, Yu X, Xie C, Jiang H, Jin Y, et al. Combination of 3D printing technologies and compressed tablets for preparation of riboflavin floating tablet-in-device (TiD) systems. Int J Pharm. 2018 Oct;549(1–2):370–9.
51. Wu L, Park J, Kamaki Y, Kim B. Optimization of the fused deposition modeling-based fabrication process for polylactic acid microneedles. Microsyst Nanoeng. 2021 Dec;7(1):58.
52. Jamróz W, Szafraniec J, Kurek M, Jachowicz R. 3D Printing in Pharmaceutical and Medical Applications – Recent Achievements and Challenges. Pharm Res. 2018 Sep;35(9):176.
53. Ehtezazi T, Algellay M, Islam Y, Roberts M, Dempster NM, Sarker SD. The Application of 3D Printing in the Formulation of Multilayered Fast Dissolving Oral Films. J Pharm Sci. 2018 Apr;107(4):1076–85.

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REVIEW ARTICLE

BENTHAM SCIENCE

Current Pharmaceutical Biotechnology

Promising Repurposed Antiviral Molecules to Combat SARS-CoV-2: A Review

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Abstract: COVID-19, an extremely transmissible and pathogenic viral disease, triggered a global pandemic that claimed lives worldwide. To date, there is no clear and fully effective treatment for COVID-19 disease. Nevertheless, the urgency to discover treatments that can turn the tide has led to the development of a variety of preclinical drugs that are potential candidates for probative results. Although most of these supplementary drugs are constantly being tested in clinical trials against COVID-19, recognized organizations have aimed to outline the prospects in which their use could be considered. A narrative assessment of current articles on COVID-19 disease and its therapeutic regulation was performed. This review outlines the use of various potential treatments against SARS-CoV-2, categorized as fusion inhibitors, protease inhibitors, and RNA-dependent RNA polymerase inhibitors, which include antiviral drugs such as Umifenovir, Baricitinib, Camostatmesylate, Nafamostatmesylate, Kaletra, Paxlovide, Darunavir, Atazanavir, Remdesivir, Molnupiravir, Favipiravir, and Ribavirin. To understand the virology of SARS-CoV-2, potential therapeutic approaches for the treatment of COVID-19 disease, synthetic methods of potent drug candidates, and their mechanisms of action have been addressed in this review. It intends to help readers approach the accessible statistics on the helpful treatment strategies for COVID-19 disease and to serve as a valuable resource for future research in this area.

Keywords: COVID-19, SARS-CoV-2, COVID-19 treatment, coronavirus, corona treatment, antiviral molecules.

1. INTRODUCTION

The year 2019 ended with a frightening catastrophic outbreak of the 2019 coronavirus disease (COVID-19), which is highly catastrophic and has a high transmission rate. On December 8, 2019, a considerable number of cases of pneumonia of unclear etiology were recorded for the first time in the Chinese city of Wuhan, which had a higher clinical symptomatic analogy with viral pneumonia and rapidly resulted in severe illness with fatal outcomes [1, 2]. It was designated a pandemic and declared a community health emergency of international concern by WHO on March 11, 2020. The first cases of pneumonia-like symptoms were reported at Huanan Seafood Wholesale in Wuhan, Hubei Province, China, where various wildlife species were sold, including birds, rabbits, frogs, bats, and snakes, leading to the closure of the seafood market on January 1, 2020 [3, 4].On January 9, 2020, the Chinese CDC confirmed a novel coronavirus from the throat swab of infected individuals as the cause of the disease [1, 5]. Subsequently, the disease began to spread dramatically from Wuhan to other parts of China and other countries. By December 2020, COVID-19 had spread to and affected 218 countries [5].

The history of SARS-CoV infection includes the emergence of the beta coronavirus, SARS-CoV, which emerged in Guangdong, southern China, in November 2002 and spread rapidly to 5 continents, infecting more than 8000 people and causing 774 (95% of cases) deaths in 37 countries in 2002-2003 [6, 7]. In 2012, MERS-CoV broke out in Middle Eastern countries and was first detected in Saudi Arabia, resulting in the infection of 2494 people and 858 deaths (34.4% of cases) in 27 countries [6].

The genetic sequence of COVID-19 was identified and made available to the public on January 11-12, 2020 [3]. Further phylogenetic analysis depicted that the current human coronavirus responsible for COVID-19 has a complete genomic nucleotide identity of 79.5% with SARS-CoV and 96% with bat SARSr-CoV-RaTG13 [6, 7]. On February 11, 2020, WHO announced that COVID-19 would be the authorized name for this disease, meaning Coronairus Disease-2019, and the International Committee on Taxonomy of Viruses identified the coronavirus for COVID-19 as a related

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species to SARS-CoV. It officially designated it as SARS-CoV-2 [8]. Adding the currently spreading COVID-19, a total of seven human CoVs (HCoV) have been reported to date: alpha-CoV: HCoV-229E & NL63, and beta-CoV: HCoV-OC43 & HKU1, SARS-CoV, MERS-CoV, SARS-CoV-2 [1, 5, 6]. In contrast to SARS-CoV (9.5%) and MERS-CoV (34.4%), COVID-19 had a lower mortality rate of 2.3% [9, 10] Figs. (**1** and **2**) show the prevalent COVID-19 disease infecting the world.

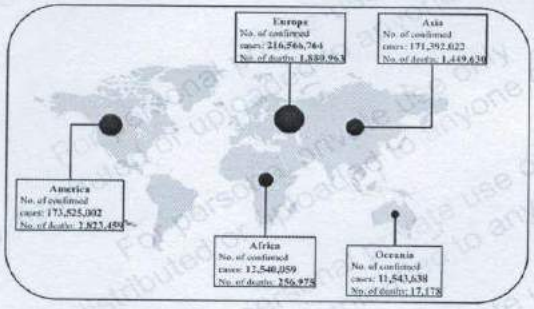

Fig. (1). Worldwide case report of SARS-CoV-2 (as of August 04, 2022, 07:14 GMT). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

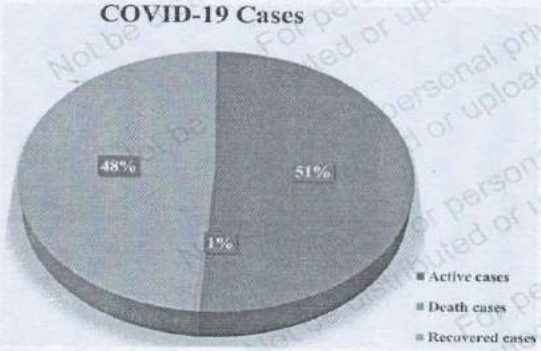

Fig. (2). Worldwide COVID-19 cases as of August 04, 2022, 07:14 GMT. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

2. SARS-CoV-2 STRUCTURE AND VIROLOGY

2.1. SARS-CoV-2 Structure

The SARS-CoV-2 genome and nucleocapsids are enclosed in a viral lipid bilayer membrane surrounded by a substantial number of spike proteins, along with envelope and membrane proteins, as illustrated in Fig. (**3**).

2.2. SARS-CoV-2 Virology

Understanding the SARS-CoV-2 virology (Fig. **4**) is a prerequisite for establishing potential drug targets to suppress viral replication [11]. Currently, antiviral drugs are being developed that target basic biochemical steps and components in the coronavirus replication process. These include spike proteins, RNA-dependent RNA polymerase, and proteases [12]. SARS-CoV-2 is similar to other coronaviruses whose genome consists of a single-stranded RNA with a spike protein coat that facilitates virus entry into the host unit [13].

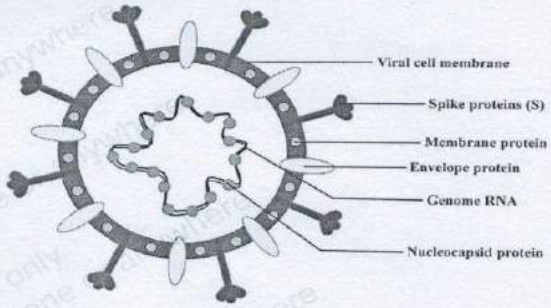

Fig. (3). Structure of SARS-CoV-2. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

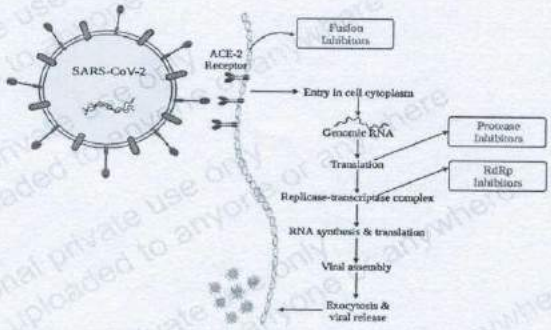

Fig. (4). SARS-CoV-2 invasion in a host cell. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Once the viral spike (S) protein binds to the ACE2 receptor [14, 15], the S protein is cleaved to form two non-covalently linked subunits, wherein S1 figures the virus-host & cellular tropism while S2 promotes viral membrane fusion [16-18]. Fusion inhibitors could be a way to prevent viral cell membrane fusion [19]. After fusion, the SARS-CoV-2 genome invades the host's cytoplasm and generates two polyproteins, pp1, and pp2, which help to capture the host ribosome for viral translation. These are required for the replication and transcription process of the virus. Then, the SARS-CoV-2 virus begins its replication phase. The mechanism of SARS-CoV-2 is not fully known and is still under investigation, so its replication could only be elucidated using SARS-CoV and MERS-CoV models. In SARS-CoV, nsp12 gener-

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ates a complementary RNA strand from the original template. The viral replicate then uses the negative-strand RNA to make new positive RNA molecules, which are employed in other translation and replication steps to build the new viral genome. This is conducted by the topoisomerase III - beta in SARS-CoV [11].

3. ANTIVIRALS FOR SARS-CoV-2 INFECTION

It is clear from the virology of SARS-CoV-2 that three types of inhibitors that work against this deadly virus can be used.

1. Fusion inhibitors can prevent the fusion of the viral membrane with the host.
2. Protease inhibitors: target structural and non-structural proteins and could be competent anti-COVID-19 agents.
3. RNA-dependent RNA polymerase inhibitors: can be used to prevent the viral replication step.

3.1. Fusion Inhibitors

3.1.1. Umifenovir (Arbidol)

About 55 years ago, the development of Umifenovir began as part of a joint venture between the Chemical-Pharmaceutical Scientific Research Institute of Russia, the Scientific Research Institute of Medical Radiology in Obninsk, and the Scientific Research Institute of Epidemiology and Microbiology in Leningrad-Pasteur [20]. It is an indole-derived compound that has been used in Russia for more than 20 years and in China since 2006 for the prophylactic treatment of pulmonary diseases in humans due to influenza A and B viruses and other human respiratory viruses [21, 22]. It has been reported to be effective against influenza A (H5N1), influenza B, and H275Y oseltamivir-resistant viruses [23]. Umifenovir is currently being evaluated for its antiviral activity against SARS-CoV-2, but minimal clinical data are available [24]. *In-vitro* studies of Arbidol and Arbidol-mesylate molecules showed inhibition against SARS viral replication and are currently under investigation in COVID-19 patients to assess therapeutic efficacy against pneumonia caused by SARS-CoV-2 [25].

3.1.1.1. Synthesis of Umifenovir

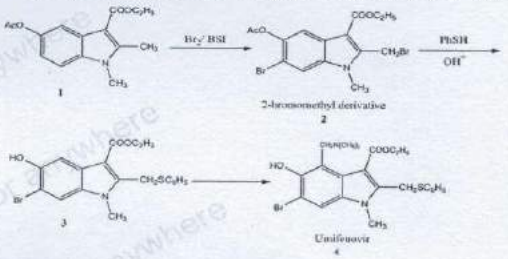
It is an indole-type derivative of Ferkaptolin, with bromine in the sixth position. The ethyl ester of 5-acetocy-1,2-dimethylindole-3-carboxylic acid **1** is incorporated as the starting material in Umifenovir synthesis. First, the benzene ring and the methyl group of **1** undergo bromination in the presence of bromine or bromosuccinimide. Then the condensation of the 2-bromomethyl derivative **2** with potassium thiophenolate in methanol was carried out. After removal of the acetyl-protecting group by acid/alkaline hydrolysis, compound **3** was produced, in which amino methylation was accomplished in the presence of bis(dimethylamino)methane or a combination of dimethylamine and formaldehyde to obtain Arbidol **4** [26] (Scheme **1**).

3.1.1.2. Mechanism of Action

Umifenovir is a fusion inhibitor that interferes with viral cell membrane fusion by S-protein and ACE-2 interaction, halting the hydrogen-bonded framework of phospholipids [27, 28].

3.1.2. Baricitinib

Baricitinib is manufactured by Lilly & Co. under the brand name Olumiant. Baricitinib was approved by the FDA on May 31, 2018, for treating mild to severe rheumatoid arthritis [29, 30]. Studies have shown that the endocytosis of



Scheme 1. Synthesis of umifenovir.

SARS-CoV-2 for host cell invasion is regulated by AP2-associated protein kinase 1 (AAK1). Therefore, disruption of AAK1 will inhibit the virus invasion into the host cell [7]. The Janus kinase (JAK) 1 & 2 inhibitor baricitinib inhibits AAK1 and may be an effective drug candidate for the treatment of COVID-19 [31]. A clinical study (number NCT04401579) depicted that the combination of baricitinib and remdesivir may be more effective than remdesivir alone in reducing recovery time in COVID-19 patients [32]. On November 19, 2020, the FDA granted emergency approval for baricitinib for the treatment of COVID-19 hospitalized patients [33]. However, clinical trial data on baricitinib for COVID-19 are limited and still under investigation [34, 35].

3.1.2.1. Synthesis of Baricitinib

In 2007, Rodgers *et al.* first reported the synthetic pathway of baricitinib [36]. It was an eight-step synthesis process with the limitations of overall low yield, high cost, and adherence to stringent operating conditions. In 2016, Jiaojiao Xu *et al.* reported a baricitinib synthesis method with basic accessible operating necessities, impressive overall yield, and minimal cost suitable for industrial-scale production [37]. The starting material in this synthesis method was tert-Butyl 3-oxoazetidine-1-carboxylate **5**. This was converted to compound **6** using the Horner-Emmons reaction, which was then deprotected under acidic conditions. Sulfonamidation of compound **7** with ethane sulfonyl chloride gave intermediate **8**. Further, the reaction of compound **9** with [2-(chloromethoxy)ethyl]trimethylsilane (SEM -Cl) yielded intermediate **10**, which upon reaction with **11** produced compound **13** followed by intermediate **12**. Baricitinib **15** was obtained after the nucleophilic addition reaction of compound **13** and deprotection of the SEM group in compound **14** [37] (Scheme **2**).

3.1.2.2. Mechanism of Action

A selective Janus kinase (JAK) 1 and 2 inhibitors, baricitinib, inhibits AP2-associated protein kinase 1 (AAK1), which results in the prevention of host cell invasion [31].

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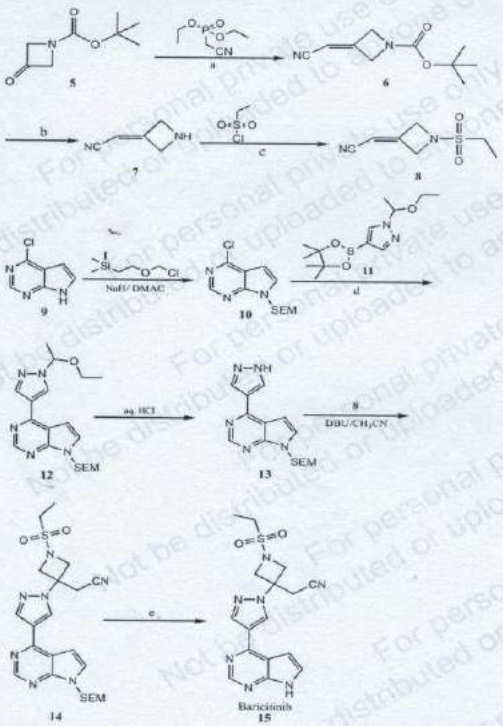
3.1.3. Camostat Mesylate

In the 1980s, Camostat mesylate began to be developed as a protease inhibitor and has been used to treat postoperative reflux esophagitis since 1984 [38]. It is manufactured by Ono pharmaceutical Co. Ltd. in Japan under the brand name Foipan [39]. Camostat mesylate is approved in Japan to treat chronic pancreatitis and postoperative reflux esophagitis [40]. Post-marketing studies in Japan exhibited that camostat mesylate is a drug candidate with minimal side effects and almost no serious adverse effects. TMPRSS2 protease has been reported to facilitate viral entry into cells in SARS, MERS, and influenza [40]. Therefore, Camostat mesylate may be a promising drug candidate against COVID-19 [41].



[Reagents and conditions: (a)- t-BuOK/ THF; (b)- HCl; (c)- DIPEA, CH_3CN; (d)- K_2CO_3/ Pd $(PPh_3)_4$, 1-butanol/ H_2O; (e)- (1) $LiBF_4$/ CH_3CN (2) aq. NaOH.]

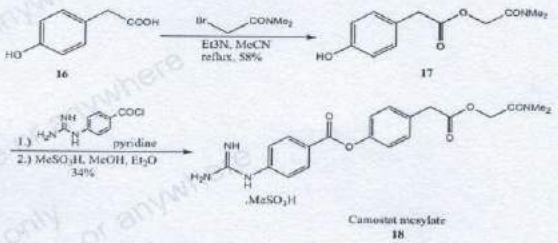
Scheme 2. Synthesis of baricitinib.

3.1.3.1. Synthesis of Camostat Mesylate

Synthetic methodology of camostat mesylate was first developed in 1977 [42]. It was a relatively simple synthetic method consisting of two successive esterification reactions. First, the alkylation of compound **16** was carried out with N,N-dimethyl-2-bromo-acetamide to give the intermediate **17**. Next, camostat was synthesized from p-Guanidinobenzoic acid using crude acyl chloride, followed by precipitation of Camostat mesylate **18** from a methanol/diethyl ether solution (Scheme **3**).

3.1.3.2. Mechanism of Action

It is a protease inhibitor that inhibits the transmembrane protease serine-2 (TMPRSS2), thus invading the fusion step and interfering with the viral cellular entry [43].



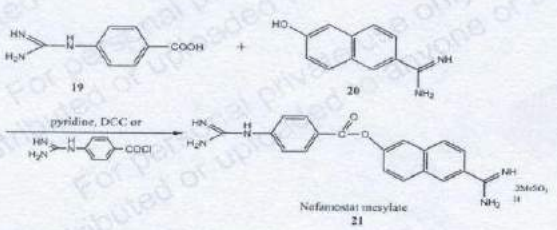
Scheme 3. Synthesis of camostat mesylate.

3.1.4. Nafamostat Mesylate

Setsuro Fujii first synthesized this broad-spectrum protease inhibitor in 1981. It was later commercialized by Japan Tobacco [44, 45]. It was found that nafamostat mesylate, a serine proteinase inhibitor, was 15 times more effective in preventing the entry of the SARS-CoV-2 virus into host cells [46]. Because of its higher efficacy and safe therapeutic profile, nafamostat mesylate is considered a preferable alternative to camostat mesylate. Nafamostat mesylate actively inhibits proteolytic enzymes, plasmin, thrombin, and trypsin and has potent antifibrinolytic activity [47]. In Japan, it has been used for treating pancreatitis, DIC, and dialysis for three decades [48]. Moreover, it was concluded from clinical studies that it might be even more helpful in treating DIC in COVID-19 patients with increased fibrinolysis [49].

3.1.4.1. Synthesis of Nafamostat Mesylate

Nafamostat mesylate **21** was synthesized by condensing guanidinobenzoic acid **19** and 6-amino-2-napthol **20** in the presence of pyridine and DCC or acid chloride of 4-guanidinobenzoic acid at room temperature [50] (Scheme **4**).



Scheme 4. Synthesis of nafamostat mesylate.

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3.1.4.2. Mechanism of Action

It functions by blocking several enzymatic pathways, including pancreatic proteases, the plasma kallikrein-kinin system (KKS), fibrinolysis or coagulation, as well as acting as an antioxidant in Tumour Necrosis Factor α-induced ROS generation [51, 52].

3.2. Protease Inhibitors

3.2.1. Lopinavir/Ritonavir (KALETRA)

Abbott Laboratories developed lopinavir to improve the company's previous protease inhibitor, ritonavir, particularly in terms of serum protein binding properties (reducing serum interference with protease enzyme inhibition) and HIV resistance profile [53]. USA-FDA approved Kaletra on September 15, 2000, and in Europe on March 19, 2001 [54], a coformulation of lopinavir and ritonavir, a viral protease inhibitor [55]. Furthermore, lopinavir-ritonavir is an FDA-approved HIV-1 proteinase inhibitor with the brand name Kaletra [56, 57]. Kaletra showed *in-vitro* activity against both coronaviruses, SARS and MERS. Based on these studies and findings, it was suggested that the drug is being investigated for the treatment of COVID-19 [24]. For SARS-CoV-2, it was reported and found that treatment with lopinavir/ritonavir provided no additional benefit over standard treatment [11, 24, 58].

3.2.1.1. Synthesis of Lopinavir (ABT378)

Large-scale synthesis of ABT378 involves using an intermediate similar to the synthesis of ritonavir. The methodology involves subsequent acylation of the intermediate, which is continued through a diastereomeric mixture until the final step [59]. The general synthetic strategy for lopinavir **30** is similar to that of ritonavir, which involves repeated acylation of the protected "core" diamino alcohol **23** with side-chain acids **22** and **24** (Fig. 5). In the production of ritonavir, the protected diamino alcohol **23** is available in sufficient quantity [60, 61].

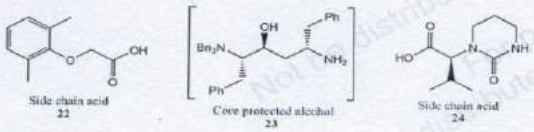
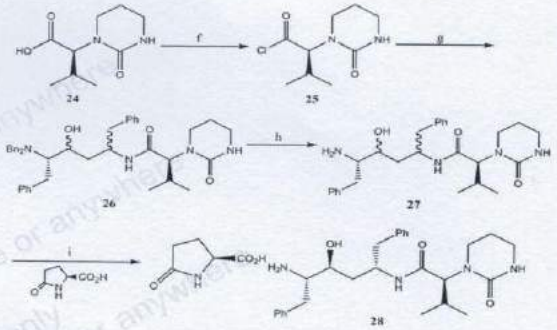


Fig. (5). Core-protected diamino alcohol.

Exposure of acid **24** to thionyl chloride or phosphorus oxychloride produced acyl chloride **25**, which was coupled to compound **23** in the presence of imidazole as a base. This step generated intermediate **26** as a diastereomeric mixture, which on debenzylation, yielded compound **27**. Further reaction of compound **27** in the presence of dioxane with L-pyroglutamic acid at 50°C led to salt **28** (Scheme 5). Finally, acyl chloride **29** was formed from acid **22** in the presence of $SOCl_2$, EtOAc, and DMF at 50°C. Subsequently, salt **28** was reacted with acyl chloride **29** in a heterogeneous reaction (Schotten-Baumann) in the presence of sodium bicarbonate to give lopinavir **30** with high purity (Scheme **6**).

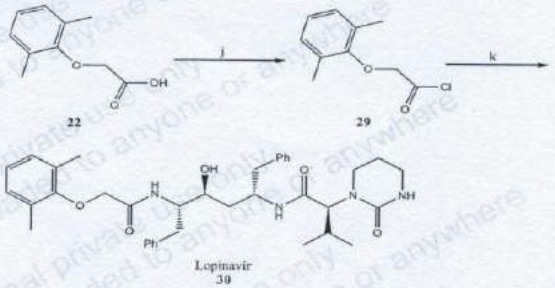
3.2.1.2. Mechanism of Action

In the case of Kaletra, the main active ingredient, lopinavir inhibits viral protease, while ritonavir inhibits the CYP3A4-mediated metabolism of lopinavir and increases its plasma concentrations [24].



[Reagents and conditions: (f)- $SOCl_2$, THF, 20 °C, 100%; (g)- 23, 3.0Eq. imidazole, EtOAc, DMF, 100%; (h)- Pd/C, HCO_2NH_4, MeOH; (i)- dioxane, 50 °C, 80%.]

Scheme 5. Formation of salt **28**.



[Reagents and conditions: (j)- $SOCl_2$, EtOAc, DMF, 50 °C; (k)- **28**, $NaHCO_3$, EtOAc, H_2O.]

Scheme 6. Synthesis of lopinavir.

3.2.2. Paxlovid (PF-07321332)

Pfizer Inc. developed nirmatrelvir, an antiviral that inhibits its C3-like proteases, and is currently used in combination with ritonavir under the brand name Paxlovid against SARS-CoV-2 [62, 63]. On December 22, 2021, the FDA granted a EUA for paxlovid to treat mild to moderate COVID-19 [64].

3.2.2.1. Synthesis of Nirmatrelvir

Pfizer first synthesised it using ethylimino diamine as a coupling agent to couple a homochiral amino acid **31** with a homochiral amino amide **32**, after which the **33**-amide group in the compound was dehydrated to nitrile using the Burgess reagent, resulting in Nirmatrelvir **34** [63] (Scheme **7**).

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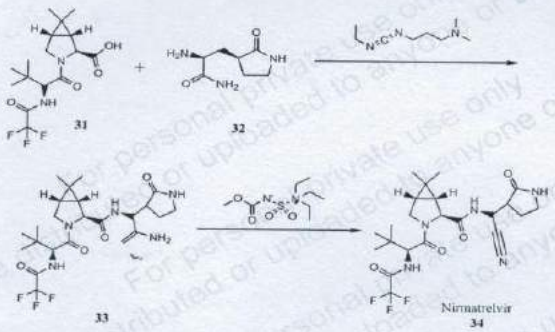
3.2.2.2. Mechanism of Action

Paxlovid includes nirmatrelvir, which prevents viral replication by inhibiting SARS-CoV-2 C3-proteins, and ritonavir, which blocks nirmatrelvir's CYP3A-mediated metabolism and maintains increased plasma concentrations [65].



Scheme 7. Synthesis of nirmatrelvir.

3.2.3. Darunavir, Atazanavir

Professor Arun K. Ghosh discovered darunavir at the University of Illinois at Chicago, manufactured by Tibotec Pharmaceuticals under the brand name Prezista [66]. The FDA first approved Darunaviror to treat HIV infection in June 2006 [67]. The HIV protease inhibitor darunavir showed inhibition of SARS-CoV-2 replication in combination with ritonavir. Clinical trials with darunavir have shown that it may not be an effective drug candidate against COVID-19 [68]. Fintelman-Rodrigues *et al.* reported that atazanavir could bind to the active site of SARS-CoV-2 with higher intensity than lopinavir, inhibiting the replication process [69]. Studies encouraged atazanavir as a potential alternative to lopinavir when used with ritonavir as a therapeutic agent against COVID-19; however, further clinical studies are needed.

3.2.3.1. Mechanism of Action

Darunavir acts by integrating with the protease enzyme and disrupting cleavage of the viral-encoded Gag-Pol proteins, effectively halting viral particle maturation. Atazanavir selectively inhibits the Gag and Gag-Pol proteins encoded by the virus, effectively stopping viral particle maturation [70].

3.3. RNA Dependent RNA Polymerase Inhibitors

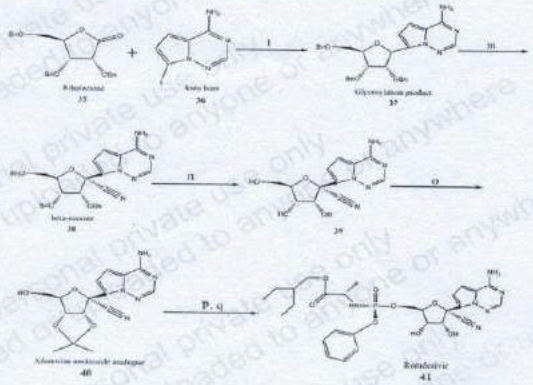
3.3.1. Remdesivir (GS5734)

Gilead Sciences has developed Remdesivir, which was developed in collaboration with USCDC and USAMRIID [71]. The Hepatitis C and Respiratory Syncytial Virus research program in 2009 was the beginning of the research that led to the discovery of remdesivir. Research into various therapeutic applications of remdesivir continued, including antiviral investigations in 2013 and early 2014. These studies demonstrated that remdesivir has a broad antiviral activity window. In 2014, Gilead collaborated with US-CDC and USAMRIID to investigate libraries of Gilead antiviral agents and confirmed that remdesivir is effective against the Ebola virus (EBOV) [72]. Investigational studies on remdesivir depicted potential antagonism against SARS, MERS, HCoV-OC43, and 229E [73].

Remdesivir accelerated the recovery of people who had COVID-19 (if no cure was available). It is the only drug approved by the FDA to date. It is an RNA-dependent RNA polymerase inhibitor that prevents the replication of SARS-CoV-2 [55]. It has an impressively wide radius of antiviral activity against various viruses such as Co-V, Ebola-Marburg, Nipah, Junin, Lassa fever, and Hendra [74]. Its pharmacokinetic attributes have already been studied and reported against SARS and MERS coronaviruses [75].

3.3.1.1. Synthesis of Remdesivir

It involves the formation of a phosphoramidate prodrug and adenosine nucleoside analogs, uniting the two fragments *via* a phosphodiester bond [76, 77]. In 2017, Siegel *et al.* synthesized remdesivir by taking ribolactone **35** and iodobase **36**, which followed a metal-halogen exchange process to form a glycosylated compound **37**. Cyanation of compound **37** yielded beta-anomer **38**, which undergoes benzyl deprotection in the presence of boron trichloride to produce compound **39**. Subsequently, compound **39** reacted with 2,2-dimethoxypropane and was protected by acetonide to produce compound **40**. Further coupling reaction of the prodrug precursor with 40 in the presence of Hunig base produced Remdesivir **41** in excellent yield [78, 79] (Scheme **8**).



[Reagents and conditions: (l)- i-PrMgCl.LiCl, (TMS)Cl, PhMgCl, THF, -20 °C; (m)- TMSOTf, TfOH, TMSCN, CH_2Cl_2, -78 °C; (n)- CH_2Cl_2, BCl_3, -20 °C; (o)- H_2SO_4, acetone, 2,2-dimethoxypropane, rt; (p)- prodrug precursor, $(i\text{-}Pr)_2NEt$, $MgCl_2$, MeCN, 50 °C; (q)- THF37%, HCl, rt.]

Scheme 8. Synthesis of remdesivir.

3.3.1.2. Mechanism of Action

Nucleosides have poor cell permeability, so modified nucleosides are preferred because they have high cell permeability and are metabolized to a permeable nucleoside in the cell [71]. Remdesivir is a nucleotide analog with a monophosphoramidate prodrug metabolized *via* esterase-mediated

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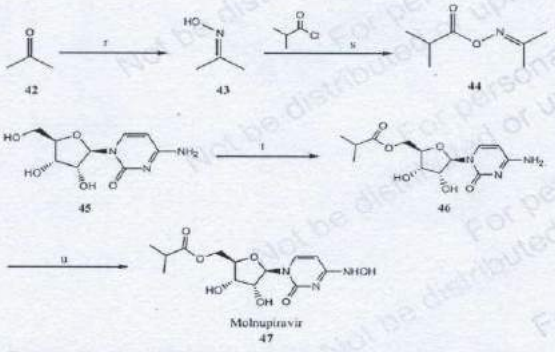
hydrolysis of the nucleoside monophosphate. Phosphorylation of this nucleoside produces a triphosphate analog used by the RNA-dependent RNA polymerase of the virus. This triphosphate analog leads to delayed chain termination due to steric hindrance. It disrupts the functions of viral exonucleases, and viral genomic RNA replication and production decrease as a result of impaired proofreading [11, 55, 80]. Since Remdesivir can inhibit replication steps, it can be suggested for patients with COVID-19.

3.3.2. Molnupiravir

The N-hydroxycytidine prodrug molnupiravir is active against many RNA viruses and has been reported to show activity against SARS-CoV-2 by integrating into viral RNA and eventually terminating the replication step [81]. On December 23, 2021, the FDA approved molnupiravir under EUA for treating patients with COVID-19 [82].

3.3.2.1. Synthesis of Molnupiravir

In 2019, Emory University revealed the first synthetic route of molnupiravir, which had limitations like 4/5 steps that were not optimized and an overall yield of 17%. Ahlqvist *et al.* demonstrated a modified approach to synthesize molnupiravir that was cost-effective, safe and yielded 41% of the maximum yield [83]. Acetone **42** was first reacted with hydroxylamine hydrochloride to produce acetone oxime **43**, which was then used to produce acylating compound **44**. Then, in the presence of novozym 435, cytidine **45** was reacted with compound **44** to produce 5'-O-isobutyryl cytidine **46**. Compound **46** reacted with hydroxylamine sulphate in butanol to produce molnupiravir **47** (Scheme **9**).



[Reagents and conditions: (r)- NH_2OH HCl, H_2O, RT, 16h; (s) Et_3N, DCM, 0 °C to RT, 20h; (t)- 44, Novozym 435, 1,4-dioxane, 60 °C, 43h; (u)- $(NH_2OH)_2$ H_2SO_4, 70% aq. Butenol, 78 °C, 40h.]

Scheme 9. Synthesis of molnupiravir.

3.3.2.2. Mechanism of Action

Molnupiravir hydrolyses and phosphorylates to get integrated into the new viral genome, resulting in aggregation of inactivating mutations [84].

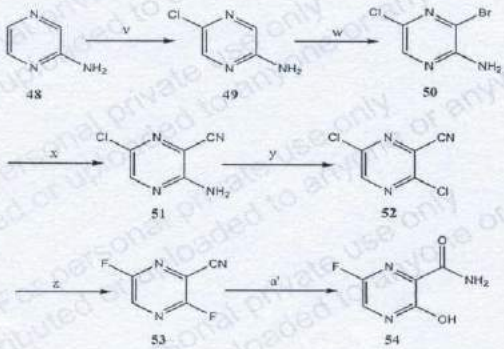
3.3.3. Favipiravir (T705)

The prodrug favipiravir mimics pyrazine carboxamide. Favipiravir, a purine nucleotide analogue, inhibits RNA-dependent RNA polymerase [11, 55]. The antiviral drug favipiravir was developed and manufactured by Toyama Chemical Co. Ltd. [85]. It is not an FDA-approved drug but was approved in Japan against influenza in 2014 under the brand name Avigan [86]. Throughout the world, multiple trials in various stages look at favipiravir for COVID-19 treatment, either alone or in combination with other medicines [87]. Under emergency circumstances, it has been approved for the treatment of COVID-19 in several countries, including Russia, India, and Japan [88-90].

3.3.3.1. Synthesis of Favipiravir

Furuta and scientists at Toyama developed the first approach to favipiravir, in which they chose a cheaper starting material, 3-aminopyrazine-2-carboxylic acid. However, this method could only produce favipiravir in minimal quantities. This method had several disadvantages: the process was lengthy and comprised of many purification steps, the synthesis of a few compounds, the use of an expensive catalyst, and the highly corrosive Olah reagent. Based on these limitations, this synthetic approach was not suitable for the scalable production of favipiravir [91].

In 2017, Liu Feng *et al.* developed an improvised economic route for favipiravir synthesis [92]. The overall yield by this route was 22.3%. However, using large amounts of $POCl_3$ raised severe concerns about scale-up and waste disposal. To overcome these issues associated with favipiravir synthesis, Guo *et al.* 2019 developed a unique production method for favipiravir synthesis from 2-aminopyrazine **48**, which did not require the use of $POCl_3$ and was economical [76, 93]. In this method, the cheaper and readily available starting material **48** was chlorinated in acetonitrile solvent using NCS/TSA. This gave the chloropyrazine compound



[Reagents and conditions: (v)-NCS/ TSA, MeCN, 55-80%; (w)- NBS, DCM, 87%; (x)- NaCN/ CuI, Pd $(PPh_3)_4$/ DMF, 85%; (y)- TiCl, tert-butyl nitrile, 81%; (z)- KF, Bu_4NBr, 60%; (a')- (1) conc. HCl (75%), (2) $NaHCO_3$ (82%).]

Scheme 10. Synthesis of favipiravir.

49, which was further brominated at C-3 position to obtain compound **50**. Further, to form the intermediate **51**, bromine

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atom from compound **50** was replaced by a nitrile group with sodium cyanide in the presence of a palladium coupling agent. The Sand-Meyer reaction with t-butyl nitrite and titanium tetrachloride completed the transition of the functional group of compound **51** to di-chloropyrazine **52**, which was fluorinated with KF in DMSO. It produced compound **53**, which incorporated nitrile hydration and hydroxyl substitution to yield favipiravir **54** [76, 93] (Scheme **10**).

3.3.3.2. Mechanism of Action

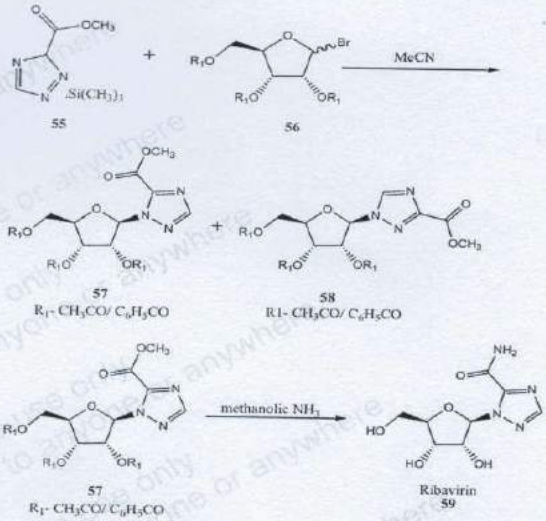
After administration, the prodrug favipiravir converts to the active ingredient favipiravir-ibufuranosyl-5'-triphosphate (T705RTP). This active compound integrates with viral RNA during viral replication and competes with guanine nucleosides, resulting in selective blockade of RNA-dependent RNA polymerase, thereby halting viral RNA production [94].

3.3.4. Ribavirin

Dr. J. T. Witkowski and R. K. Robins first synthesized ribavirin in 1970, and its diverse range of antiviral properties was promulgated in 1972 [95, 96]. The FDA first approved ribavirin to treat hepatitis C infection on April 6, 2004 [97]. Ribavirin is a purine nucleoside connate active against RNA and DNA viruses. *In vitro* studies have shown that ribavirin-interferon-α therapy is effective against SARS-CoV and MERS-CoV [98-100]. This suggests that ribavirin interferon-α therapy may be a suitable treatment for SARS-CoV-2. However, studies by H. Li, N. Xiong, C. Li *et al.* found no clinical benefit of ribavirin/interferon-α therapy in SARS-CoV-2, and another such analysis by S. Tong *et al.* also found that ribavirin did not provide a survival benefit compared with standard treatment [101, 102]. Therefore, further studies are needed to determine ribavirin's benefit in treating COVID-19.

3.3.4.1. Synthesis of Ribavirin

Witkowski *et al.* synthesized ribavirin, as shown in Scheme **11** [103]. In this synthetic approach, the trimethyl silyl compound **55** was reacted in MeCN with the acyl-blocked compound **56** at room temperature to give compounds **57** and **58**. Further treatment of compound **57** with methanolic ammonia gave ribavirin **59**.



Scheme 11. Synthesis of ribavirin.

Table 1. Mode of action, adverse effects & clinical trial phase of antivirals against SARS-CoV-2.

Name of Drug	Mode of Action	FDA Approval for Treatment of	Clinical Trials Phase	Adverse Effects
Fusion Inhibitors				
Umifenovir (Arbidol)	Interrupts S protein & ACE2 interaction.	Influenza	NCT04350684 Phase4	Nausea, vomiting, and diarrhoea [107].
Baricitinib	AP2-associated protein kinase 1 inhibitor.	Rheumatoid arthritis	NCT04358614 Phase 3	Hyperglycaemia, severe venous thrombosis, pulmonary embolism, and serious infection [108].
Camostat Mesylate	Transmembrane protease serine-2 inhibitor.	-	NCT04608266 Phase 3	Nausea, increased liver enzyme, rash, and pruritus [40].
Nafamostat Mesylate	Fusion inhibitor.	-	NCT04418128 Phase 2 Phase 3	Hyponatraemia, abdominal upset, catheter site phlebitis [109].

(Table 1) Contd...

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Name of Drug	Mode of Action	FDA Approval for Treatment of	Clinical Trials Phase	Adverse Effects
Protease Inhibitors				
KALETRA (Lopinavir/ Ritonavir)	Lopinavir- 3CL protease inhibitor Ritonavir- acts by preventing lopinavir's CYP3A4-mediated metabolism.	Lopinavir- HIV	NCT04455958 Phase 2	Nausea, vomiting, diarrhoea, and QT prolongation [110].

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Name of Drug	Mode of Action	FDA Approval for Treatment of	Clinical Trials Phase	Adverse Effects
Protease Inhibitors				
KALETRA (Lopinavir/ Ritonavir)	Lopinavir- 3CL protease inhibitor Ritonavir- acts by preventing lopinavir's CYP3A4-mediated metabolism.	Lopinavir- HIV	NCT04455958 Phase 2	Nausea, vomiting, diarrhoea, and QT prolongation [110].
PAXLOVID (Nirmatrelvir/Ritonavir)	Nirmatrelvir- C3 protein inhibitor.	-	NCT04960202 Phase 3	Dysgeusia, diarrhoea, hypertension, and myalgia [111].
Darunavir/Atazanavir	Virally encoded Gag and Gag-Pol proteins inhibitor.	HIV-1	Darunavir-NCT04252274 Phase3 Atazanavir- NCT04459286 Phase 2	Atazanavir- toxic hepatitis, when used with ritonavir: lymphoma, nephritic syndrome, and peliosis [112].
RNA Dependent RNA Polymerase Inhibitors				
Remdesivir	Prodrug that is metabolised into nucleoside monophosphate, RNA-dependent RNA polymerase inhibitor.	COVID-19, Ebola virus	NCT04292730 Phase 3	Increased level of liver enzymes, injection site reaction, and gastrointestinal upset [113].
Molnupiravir	Inhibits mutation.	-	NCT04575597 Phase 2 Phase 3	Gastrointestinal upset, dizziness, and infections [114].
Favipiravir	Selective RdRp inhibitor.	Influenza	JapicCTI-205238 Phase3	Nausea, increased liver enzymes, diarrhoea, and QT prolongation [115].
Ribavirin	Works by RNA polymerase invasion.	HCV	NCT04828564 Phase 2	Anaemia, nausea, headache, and liver dysfunction [116].

3.3.4.2. Mechanism of Action

Ribavirin is thought to have several mechanisms of action that inhibit viral replication and translation. Ribavirin acts by an invasion of RNA polymerase [104] or by possible insertion into the HCV genome [105], or by blockade of inosine monophosphate dehydrogenase [106].

Table **1** illustrates the FDA approval, drug category, mechanism of action, adverse effects, and clinical trials of the potential antiviral drug candidates used to treat COVID-19 [40, 107-116].

CONCLUSION

COVID-19, this generation's tremendous global public health emergency, is still not entirely in control with the increasing number of infected and death cases. Although no curable treatment for COVID-19 disease has yet been found, the development and screening of potential evidence-based treatment strategies and management of COVID-19 disease are constantly evolving. In this publication, we review and discuss several potential spectrums of treatment approaches against SARS-CoV-2 that reflect the currently accessible literature for ongoing research on COVID-19 disease. This paper reviews various drugs other than antivirals and combination therapies for COVID-19 disease and their historical development and synthetic methods. Remdesivir is the only drug approved by the FDA for the treatment of COVID-19 disease; the other drugs reviewed in this paper mostly have emergency FDA approval. Since research and clinical trials of investigational drug candidates for COVID-19 treatment are ongoing, there is a possibility that a cure will be found in the near future.

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CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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REFERENCES

[1] Ahmed, S.S. The coronavirus disease 2019 (COVID-19): A review. *J. Adv. Med. Med. Res.*, **2020**, *32*(4), 1-9.

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Indore Institute of Pharmacy,
INDORE (M.P.)

http://dx.doi.org/10.9734/jammr/2020/v32i430393
[2] Lou, J.; Tian, S.J.; Niu, S.M.; Kang, X.Q.; Lian, H.X.; Zhang, L.X.; Zhang, J.J. Coronavirus disease 2019: A bibliometric analysis and review. *Eur. Rev. Med. Pharmacol. Sci.*, **2020**, *24*(6), 3411-3421. http://dx.doi.org/10.26355/eurrev_202003_20712 PMID: 32271460
[3] Coronavirus disease - 2019 (COVID-19). *WHO situation report-94*, **2020**. Available from: https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(NCoV)-
[4] Wang, H.; Li, X.; Li, T.; Zhang, S.; Wang, L.; Wu, X.; Liu, J. The genetic sequence, origin, and diagnosis of SARS-CoV-2. *Eur. J. Clin. Microbiol. Infect. Dis.*, **2020**, *39*(9), 1629-1635. http://dx.doi.org/10.1007/s10096-020-03899-4 PMID: 32333222
[5] European Centre for Disease Prevention and Control. Cluster of Pneumonia Cases Caused by a Novel Coronavirus Wuhan, China. **2020**. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Risk assessment - pneumonia Wuhan China January 17 2020.pdf
[6] Bchetnia, M.; Girard, C.; Duchaine, C.; Laprise, C. The outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): A review of the current global status. *J. Infect. Public Health*, **2020**, *13*(11), 1601-1610. http://dx.doi.org/10.1016/j.jiph.2020.07.011 PMID: 32778421
[7] Lu, R.; Zhao, X.; Li, J.; Niu, P.; Yang, B.; Wu, H.; Wang, W.; Song, H.; Huang, B.; Zhu, N.; Bi, Y.; Ma, X.; Zhan, F.; Wang, L.; Hu, T.; Zhou, H.; Hu, Z.; Zhou, W.; Zhao, L.; Chen, J.; Meng, Y.; Wang, J.; Lin, Y.; Yuan, J.; Xie, Z.; Ma, J.; Liu, W.J.; Wang, D.; Xu, W.; Holmes, E.C.; Gao, G.F.; Wu, G.; Chen, W.; Shi, W.; Tan, W. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. *Lancet*, **2020**, *395*(10224), 565-574. http://dx.doi.org/10.1016/S0140-6736(20)30251-8 PMID: 32007145
[8] Gorbalenya, A.E.; Baker, S.C.; Baric, R.S.; De Groot, R.J.; Drosten, C.; Gulyaeva, A.A.; Haagmans, B.L.; Lauber, C.; Leontovich, A.M. Severe acute respiratory syndrome-related coronavirus: the species and its viruses – a statement of the coronavirus study group. *Biorxiv*, **2020**, *5*(4), 536-544. http://dx.doi.org/10.1101/2020.02.07.937862
[9] Petrosillo, N.; Viceconte, G.; Ergonul, O.; Ippolito, G.; Petersen, E. COVID-19, SARS and MERS: Are they closely related? *Clin. Microbiol. Infect.*, **2020**, *26*(6), 729-734. http://dx.doi.org/10.1016/j.cmi.2020.03.026 PMID: 32234451
[10] China CDC Weekly, C. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. *China CDC Weekly*, **2020**, *2*(8), 113-122. http://dx.doi.org/10.46234/ccdcw2020.032 PMID: 34594836
[11] Malinis, M.; McManus, D.; Davis, M.; Topal, J. An overview on the use of antivirals for the treatment of patients with COVID-19 disease. *Expert Opin. Investig. Drugs*, **2021**, *30*(1), 45-59. http://dx.doi.org/10.1080/13543784.2021.1847270 PMID: 33151781
[12] Ghosh, A.K.; Brindisi, M.; Shahabi, D.; Chapman, M.E.; Mesecar, A.D. Drug development and medicinal chemistry efforts toward SARS-coronavirus and covid-19 therapeutics. *ChemMedChem*, **2020**, *15*(11), 907-932. http://dx.doi.org/10.1002/cmdc.202000223 PMID: 32324951
[13] Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S.; Schiergens, T.S.; Herrler, G.; Wu, N.H.; Nitsche, A.; Müller, M.A.; Drosten, C.; Pöhlmann, S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*, **2020**, *181*(2), 271-280. http://dx.doi.org/10.1016/j.cell.2020.02.052 PMID: 32142651
[14] Liu, Z.; Xiao, X.; Wei, X.; Li, J.; Yang, J.; Tan, H.; Zhu, J.; Zhang, Q.; Wu, J.; Liu, L. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. *J. Med. Virol.*, **2020**, *92*(6), 595-601. http://dx.doi.org/10.1002/jmv.25726 PMID: 32100877
[15] Walls, A.C.; Park, Y.J.; Tortorici, M.A.; Wall, A.; McGuire, A.T.; Veesler, D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, **2020**, *181*(2), 281-292.e6. http://dx.doi.org/10.1016/j.cell.2020.02.058 PMID: 32155444
[16] Guo, Y.R.; Cao, Q.D.; Hong, Z.S.; Tan, Y.Y.; Chen, D.; Jin, H.J.; Tan, K.S.; Wang, D.Y.; Yan, Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Eur. Heart J.*, **2020**, *41*(22), 2124-2125. http://dx.doi.org/10.1093/eurheartj/ehaa396 PMID: 32363406
[17] Zhang, N.; Jiang, S.; Du, L. Current advancements and potential strategies in the development of MERS-CoV vaccines. *Expert Rev. Vaccines*, **2014**, *13*(6), 761-774. http://dx.doi.org/10.1586/14760584.2014.912134 PMID: 24766432
[18] Yu, F.; Du, L.; Ojcius, D.M.; Pan, C.; Jiang, S. Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China. *Microbes Infect.*, **2020**, *22*(2), 74-79. http://dx.doi.org/10.1016/j.micinf.2020.01.003 PMID: 32017984
[19] Xia, S.; Zhu, Y.; Liu, M.; Lan, Q.; Xu, W.; Wu, Y.; Ying, T.; Liu, S.; Shi, Z.; Jiang, S.; Lu, L. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell. Mol. Immunol.*, **2020**, *17*(7), 765-767. http://dx.doi.org/10.1038/s41423-020-0374-2 PMID: 32047258
[20] Blaising, J.; Polyak, S.J.; Pécheur, E.I. Arbidol as a broad-spectrum antiviral: An update. *Antiviral Res.*, **2014**, *107*(1), 84-94. http://dx.doi.org/10.1016/j.antiviral.2014.04.006 PMID: 24769245
[21] Boriskin, Y.; Leneva, I.; Pécheur, E.I.; Polyak, S. Arbidol: A broad-spectrum antiviral compound that blocks viral fusion. *Curr. Med. Chem.*, **2008**, *15*(10), 997-1005. http://dx.doi.org/10.2174/092986708784049658 PMID: 18393857
[22] Brooks, M.J.; Sasadeusz, J.J.; Tannock, G.A. Antiviral chemotherapeutic agents against respiratory viruses. *Curr. Opin. Pulm. Med.*, **2004**, *10*(3), 197-203. http://dx.doi.org/10.1097/00063198-200405000-00009 PMID: 15071371
[23] Beigel, J.H.; Nam, H.H.; Adams, P.L.; Krafft, A.; Ince, W.L.; El-Kamary, S.S.; Sims, A.C. Advances in respiratory virus therapeutics – A meeting report from the 6th isirv Antiviral Group conference. *Antiviral Res.*, **2019**, *167*, 45-67. http://dx.doi.org/10.1016/j.antiviral.2019.04.006 PMID: 30974127
[24] Srinivas, P.; Sacha, G.L.; Koval, C. Antivirals for COVID-19. *Cleve. Clin. J. Med.*, **2020**, *1–5* http://dx.doi.org/10.3949/ccjm.87a.ccc030 PMID: 32409433
[25] Zhang, L.; Liu, Y. Potential interventions for novel coronavirus in China: A systematic review. *J. Med. Virol.*, **2020**, *92*(5), 479-490. http://dx.doi.org/10.1002/jmv.25707 PMID: 32052466
[26] Trofimov, F.A.; Tsyshkova, N.G.; Zotova, S.A.; Grinev, A.N. Synthesis of a new antiviral agent, arbidole. *Pharm. Chem. J.*, **1993**, *27*(1), 75-76. http://dx.doi.org/10.1007/BF00772858
[27] Sanders, J.M.; Monogue, M.L.; Jodlowski, T.Z.; Cutrell, J.B. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): A review. *JAMA*, **2020**, *323*(18), 1824-1836. http://dx.doi.org/10.1001/jama.2020.6019 PMID: 32282022
[28] Villalaín, J. Membranotropic effects of arbidol, a broad anti-viral molecule, on phospholipid model membranes. *J. Phys. Chem. B*, **2010**, *114*(25), 8544-8554. http://dx.doi.org/10.1021/jp102619w PMID: 20527735
[29] Mayence, A.; Vanden, E.J. Baricitinib: A 2018 novel FDA-approved small molecule inhibiting Janus kinases. *Pharmaceuticals*, **2019**, *12*(1), 37. http://dx.doi.org/10.3390/ph12010037 PMID: 30871014
[30] Eli Lilly and Company. FDA approves OLUMIANT® (Baricitinib) 2-mg tablets for the treatment of adults with moderately-to-severely active rheumatoid arthritis. *PRNewswire*, **2018**. Available from: https://investor.lilly.com/node/38996/pdf
[31] Richardson, P.; Griffin, I.; Tucker, C.; Smith, D.; Oechsle, O.; Phelan, A.; Rawling, M.; Savory, E.; Stebbing, J. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet*, **2020**, *395*(10223), e30-e31. http://dx.doi.org/10.1016/S0140-6736(20)30304-4 PMID: 32032529
[32] Kalil, A.C.; Patterson, T.F.; Mehta, A.K.; Tomashek, K.M.; Wolfe, C.R.; Ghazaryan, V.; Marconi, V.C.; Ruiz-Palacios, G.M.; Hsieh, L.; Kline, S.; Tapson, V.; Iovine, N.M.; Jain, M.K.; Sweeney, D.A.; El Sahly, H.M.; Branche, A.R.; Regalado Pineda, J.; Lye, D.C.; Sandkovsky, U.; Luetkemeyer, A.F.; Cohen, S.H.; Finberg, R.W.; Jackson, P.E.H.; Taiwo, B.; Paules, C.I.; Arguinchona, H.; Erdmann, N.; Ahuja, N.; Frank, M.; Oh, M.; Kim, E.S.; Tan, S.Y.; Mularski, R.A.; Nielsen, H.; Ponce, P.O.; Taylor, B.S.; Larson, L.;

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Rouphael, N.G.; Saklawi, Y.; Cantos, V.D.; Ko, E.R.; Engemann, J.J.; Amin, A.N.; Watanabe, M.; Billings, J.; Elie, M.C.; Davey, R.T.; Burgess, T.H.; Ferreira, J.; Green, M.; Makowski, M.; Cardoso, A.; de Bono, S.; Bonnett, T.; Proschan, M.; Deye, G.A.; Dempsey, W.; Nayak, S.U.; Dodd, L.E.; Beigel, J.H. Baricitinib plus remdesivir for hospitalized adults with COVID-19. *N. Engl. J. Med.*, **2021**, *384*(9), 795-807.
http://dx.doi.org/10.1056/NEJMoa2031994 PMID: 33306283

[33] Eli Lilly and Company. Baricitinib receives emergency use authorization from the FDA for the treatment of hospitalized patients with COVID-19. *PRNewswire.*, **2020**. Available from: https://investor.lilly.com/node/44041/pdf

[34] Cantini, F.; Niccoli, L.; Nannini, C.; Matarrese, D.; Di Natale, M.E.; Lotti, P.; Aquilini, D.; Landini, G.; Cimolato, B.; Di Pietro, M.A.; Trezzi, M.; Stobbione, P.; Frausini, G.; Navarra, A.; Nicastri, E.; Sotgiu, G.; Goletti, D. Retrospective, multicentre study on the impact of baricitinib in COVID-19 moderate pneumonia. *J. Infect.*, **2020**, *81*(4), 647-679.
http://dx.doi.org/10.1016/j.jinf.2020.06.052 PMID: 32592703

[35] Cantini, F.; Niccoli, L.; Matarrese, D.; Nicastri, E.; Stobbione, P.; Goletti, D. Baricitinib therapy in COVID-19: A pilot study on safety and clinical impact. *J. Infect.*, **2020**, *81*(2), 318-356.
http://dx.doi.org/10.1016/j.jinf.2020.04.017 PMID: 32333918

[36] Rodgers, J.; Shepard, S.; Maduskuie, T.; Wang, H.; Falahatpisheh, N.; Rafalski, M.; Arvanitis, A.; Storace, L.; Jalluri, R.; Fridman, J. Heteroarylsubstituted pyrrolo[2,3-b] pyridines and pyrrolo[2,3-b] pyrimidines as Janus kinase inhibitors. U.S. Patent 2007/0135461, **2007**.

[37] Xu, J.; Cai, J.; Chen, J.; Zong, X.; Wu, X.; Ji, M.; Wang, P. An efficient synthesis of baricitinib. *J. Chem. Res.*, **2016**, *40*(4), 205-208.
http://dx.doi.org/10.3184/174751916X14569294811333

[38] Uno, Y. Camostat mesilate therapy for COVID-19. *Intern. Emerg. Med.*, **2020**, *15*(8), 1577-1578.
http://dx.doi.org/10.1007/s11739-020-02345-9 PMID: 32347443

[39] FOIPAN® Tablets 100 mg. Available from: http://www.shijiebiaopin.net/upload/product/201272318373223.PDF

[40] Breining, P.; Frølund, A.L.; Højen, J.F.; Gunst, J.D.; Staerke, N.B.; Saedder, E.; Cases-Thomas, M.; Little, P.; Nielsen, L.P.; Søgaard, O.S.; Kjolby, M. Camostat mesylate against SARS-CoV-2 and COVID-19—Rationale, dosing and safety. *Basic Clin. Pharmacol. Toxicol.*, **2021**, *128*(2), 204-212.
http://dx.doi.org/10.1111/bcpt.13533 PMID: 33176395

[41] The impact of camostat mesilate on COVID-19 infection. *Clinicaltrials.gov*. Patent NCT04321096, **2020**. Available from: https://clinicaltrials.gov/ct2/show/NCT04321096

[42] De Savi, C.; Hughes, D.L.; Kvaerno, L. Quest for a COVID-19 cure by repurposing small-molecule drugs: mechanism of action, clinical development, synthesis at scale, and outlook for supply. *Org. Process Res. Dev.*, **2020**, *24*(6), 940-976.
http://dx.doi.org/10.1021/acs.oprd.0c00233

[43] Hoffmann, M.; Hofmann-Winkler, H.; Smith, J.C.; Krüger, N.; Arora, P.; Sørensen, L.K.; Søgaard, O.S.; Hasselstrøm, J.B.; Winkler, M.; Hempel, T.; Raich, L.; Olsson, S.; Danov, O.; Jonigk, D.; Yamazoe, T.; Yamatsuta, K.; Mizuno, H.; Ludwig, S.; Noé, F.; Kjolby, M.; Braun, A.; Sheltzer, J.M.; Pöhlmann, S. Camostat mesylate inhibits SARS-CoV-2 activation by TMPRSS2-related proteases and its metabolite GBPA exerts antiviral activity. *EBioMedicine*, **2021**, *65*, 103255.
http://dx.doi.org/10.1016/j.ebiom.2021.103255 PMID: 33676899

[44] Chen, X.; Xu, Z.; Zeng, S.; Wang, X.; Liu, W.; Qian, L.; Wei, J.; Yang, X.; Shen, Q.; Gong, Z.; Yan, Y. The molecular aspect of antitumor effects of protease inhibitor nafamostat mesylate and its role in potential clinical applications. *Front. Oncol.*, **2019**, *9*(9), 852.
http://dx.doi.org/10.3389/fonc.2019.00852 PMID: 31552177

[45] Fujii, S.; Hitomi, Y.; New synthetic inhibitors of C1r, C1 esterase thrombin, plasmin, kallikrein and trypsin. *Biochimica et Biophysica Acta (BBA) - Enzymology*, **1981**, *661*(2), 342-345.
https://doi.org/https://doi.org/10.1016/0005-2744(81)90023-1
http://dx.doi.org/10.1016/0005-2744(81)90023-1 PMID: 6271224

[46] Hoffmann, M.; Schroeder, S.; Kleine-Weber, H.; Müller, M.A.; Drosten, C.; Pöhlmann, S. Nafamostatmesylate blocks activation of SARS-CoV-2: new treatment option for COVID-19. *Antimicrob. Agents Chemother.*, **2020**, *64*(6), e00754-20.
http://dx.doi.org/10.1128/AAC.00754-20 PMID: 32312781

[47] Aoyama, T.; Ino, Y.; Ozeki, M.; Oda, M.; Sato, T.; Koshiyama, Y.; Suzuki, S.; Fujita, M. Pharmacological studies of FUT-175, nafamstat mesilate. I. Inhibition of protease activity in *in vitro* and *in vivo* experiments. *Jpn. J. Pharmacol.*, **1984**, *35*(3), 203-227.
http://dx.doi.org/10.1254/jjp.35.203 PMID: 6482087

[48] Asakura, H.; Ogawa, H. Potential of heparin and nafamostat combination therapy for COVID-19. *J. Thromb. Haemost.*, **2020**, *18*(6), 1521-1522.
http://dx.doi.org/10.1111/jth.14858 PMID: 32302456

[49] Clinical Efficacy of Nafamostat Mesylate for COVID-19 Pneumonia. Gyeongsang National University Hospital. Patent NCT04418128, **2020**. Available from: https://clinicaltrials.gov/ct2/show/NCT04418128

[50] Okajima, K.; Uchiba, M.; Murakami, K. Nafamostat mesilate. *Cardiovasc. Drug Rev.*, **1995**, *13*(1), 51-65.
http://dx.doi.org/10.1111/j.1527-3466.1995.tb00213.x

[51] Kim, H.S.; Lee, K.E.; Oh, J.H.; Jung, C.S.; Choi, D.; Kim, Y.; Jeon, J.S.; Han, D.C.; Noh, H. Cardiac arrest caused by nafamostat mesilate. *Kidney Res. Clin. Pract.*, **2016**, *35*(3), 187-189.
http://dx.doi.org/10.1016/j.krcp.2015.10.003 PMID: 27668164

[52] Kang, M.W.; Song, H.J.; Kang, S.K.; Kim, Y.; Jung, S.; Jee, S.; Moon, J.Y.; Suh, K.; Lee, S.D.; Jeon, B.H.; Kim, C.S. Nafamostat mesylate inhibits TNF-α-induced vascular endothelial cell dysfunction by inhibiting reactive oxygen species production. *Korean J. Physiol. Pharmacol.*, **2015**, *19*(3), 229-234.
http://dx.doi.org/10.4196/kjpp.2015.19.3.229 PMID: 25954127

[53] Sham, H.L.; Kempf, D.J.; Molla, A.; Marsh, K.C.; Kumar, G.N.; Chen, C.M.; Kati, W.; Stewart, K.; Lal, R.; Hsu, A.; Betebenner, D.; Korneyeva, M.; Vasavanonda, S.; McDonald, E.; Saldivar, A.; Wideburg, N.; Chen, X.; Niu, P.; Park, C.; Jayanti, V.; Grabowski, B.; Granneman, G.R.; Sun, E.; Japour, A.J.; Leonard, J.M.; Plattner, J.J.; Norbeck, D.W. ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease. *Antimicrob. Agents Chemother.*, **1998**, *42*(12), 3218-3224.
http://dx.doi.org/10.1128/AAC.42.12.3218 PMID: 9835517

[54] Abbott Laboratoriues. NDA21-226 NDA21-251. **2000**. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/ 2000/21-226_Kaletra.cfm

[55] Frediansyah, A.; Tiwari, R.; Sharun, K.; Dhama, K.; Harapan, H. Antivirals for COVID-19: A critical review. *Clin. Epidemiol. Glob. Health*, **2021**, *9*, 90-98.
http://dx.doi.org/10.1016/j.cegh.2020.07.006 PMID: 33521390

[56] Chandwani, A.; Shuter, J. Lopinavir/ritonavir in the treatment of HIV-1 infection: a review. *Ther. Clin. Risk Manag.*, **2008**, *4*(5), 1023-1033.
PMID: 19209283

[57] Mangum, E.M.; Graham, K.K. Lopinavir-ritonavir: A new protease inhibitor. *Pharmacotherapy*, **2001**, *21*(11), 1352-1363.
http://dx.doi.org/10.1592/phco.21.17.1352.34419 PMID: 11714208

[58] Hussain, N.; Yoganathan, A.; Hewage, S.; Alom, S.; Harky, A. The effect of antivirals on COVID-19: A systematic review. *Expert Rev. Anti Infect. Ther.*, **2021**, *19*(4), 473-486.
http://dx.doi.org/10.1080/14787210.2021.1823832 PMID: 32924650

[59] Ghosh, A.K.; Bilcer, G.; Schiltz, G. Syntheses of FDA approved HIV protease inhibitors. *Synthesis*, **2001**, *2001*(15), 2203-2229.
http://dx.doi.org/10.1055/s-2001-18434 PMID: 30393404

[60] Stoner, E.J.; Cooper, A.J.; Dickman, D.A.; Kolaczkowski, L.; Lallaman, J.E.; Liu, J.H.; Oliver-Shaffer, P.A.; Patel, K.M.; Paterson, J.B.; Plata, D.J.; Riley, D.A.; Sham, H.L.; Stengel, P.J.; Tien, J.H.J. Synthesis of HIV protease inhibitor ABT-378 (Lopinavir). *Org. Process Res. Dev.*, **2000**, *4*(4), 264-269.
http://dx.doi.org/10.1021/op990202j

[61] Stoner, E.J.; Stengel, P.J.; Cooper, A.J. Synthesis of ABT-378, an HIV protease inhibitor candidate: avoiding the use of carbodiimides in a difficult peptide coupling. *Org. Process Res. Dev.*, **1999**, *3*(2), 145-148.
http://dx.doi.org/10.1021/op980214p

[62] Ahmad, B.; Batool, M.; Ain, Q.; Kim, M.S.; Choi, S. Exploring the binding mechanism of PF-07321332 SARS-CoV-2 protease inhibi-

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Principal
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INDORE (M.P.)

tor through molecular dynamics and binding free energy simulations. *Int. J. Mol. Sci.*, **2021**, *22*(17), 9124. http://dx.doi.org/10.3390/ijms22179124 PMID: 34502033

[63] Owen, D.R.; Allerton, C.M.N.; Anderson, A.S.; Aschenbrenner, L.; Avery, M.; Berritt, S.; Boras, B.; Cardin, R.D.; Carlo, A.; Coffman, K.J.; Dantonio, A.; Di, L.; Eng, H.; Ferre, R.; Gajiwala, K.S.; Gibson, S.A.; Greasley, S.E.; Hurst, B.L.; Kadar, E.P.; Kalgutkar, A.S.; Lee, J.C.; Lee, J.; Liu, W.; Mason, S.W.; Noell, S.; Novak, J.J.; Obach, R.S.; Ogilvie, K.; Patel, N.C.; Pettersson, M.; Rai, D.K.; Reese, M.R.; Sammons, M.F.; Sathish, J.G.; Singh, R.S.P.; Steppan, C.M.; Stewart, A.E.; Tuttle, J.B.; Updyke, L.; Verhoest, P.R.; Wei, L.; Yang, Q.; Zhu, Y. An oral SARS-CoV-2 M^{pro} inhibitor clinical candidate for the treatment of COVID-19. *Science*, **2021**, *374*(6575), 1586-1593. http://dx.doi.org/10.1126/science.abl4784 PMID: 34726479

[64] Emergency Use Authorization 105. *Pfizer Inc.*, **2021**. Available from: https://www.fda.gov/media/155049/download

[65] FDA news release. Coronavirus (COVID-19) update: FDA authorizes first oral antiviral for treatment of COVID-19. **2021**. Available from: https://doi.org/https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-oral-antiviral-treatment-covid-19

[66] Ghosh, A.K.; Dawson, Z.L.; Mitsuya, H. Darunavir, a conceptually new HIV-1 protease inhibitor for the treatment of drug-resistant HIV. *Bioorg. Med. Chem.*, **2007**, *15*(24), 7576-7580. http://dx.doi.org/10.1016/j.bmc.2007.09.010 PMID: 17900913

[67] Prezista FDA Approval History. *Drugs.com.*, **2006**. Available from: https://www.drugs.com/history/prezista.html

[68] Riva, A.; Conti, F.; Bernacchia, D.; Pezzati, L.; Sollima, S.; Merli, S.; Siano, M.; Lupo, A.; Rusconi, S.; Cattaneo, D.; Gervasoni, C. Darunavir does not prevent SARS-CoV-2 infection in HIV patients. *Pharmacol. Res.*, **2020**, *157*, 104826. http://dx.doi.org/10.1016/j.phrs.2020.104826 PMID: 32325127

[69] Fintelman-Rodrigues, N.; Sacramento, C.Q.; Ribeiro Lima, C.; Souza da Silva, F.; Ferreira, A.C.; Mattos, M.; de Freitas, C.S.; Cardoso, S.V.; da Silva, G.D.S.; Temerozo, J.R.; Miranda, M.D.; Matos, A.R.; Bozza, F.A.; Carels, N.; Alves, C.R.; Siqueira, M.M.; Bozza, P.T.; Souza, T.M.L. Atazanavir, alone or in combination with ritonavir, inhibits SARS-CoV-2 replication and proinflammatory cytokine production. *Antimicrob. Agents Chemother.*, **2020**, *64*(10), e00825-20. http://dx.doi.org/10.1128/AAC.00825-20 PMID: 32759267

[70] Davis, D.A.; Soule, E.E.; Davidoff, K.S.; Daniels, S.I.; Naiman, N.E.; Yarchoan, R. Activity of human immunodeficiency virus type 1 protease inhibitors against the initial autocleavage in Gag-Pol polyprotein processing. *Antimicrob. Agents Chemother.*, **2012**, *56*(7), 3620-3628. http://dx.doi.org/10.1128/AAC.00055-12 PMID: 22508308

[71] Eastman, R.T.; Roth, J.S.; Brimacombe, K.R.; Simeonov, A.; Shen, M.; Patnaik, S.; Hall, M.D. Remdesivir: A review of its discovery and development leading to emergency use authorization for treatment of COVID-19. *ACS Cent. Sci.*, **2020**, *6*(5), 672-683. http://dx.doi.org/10.1021/acscentsci.0c00489 PMID: 32483554

[72] Development of Remdesivir. *Gilead Sciences*, **2020**. Available from: https://www.gilead.com/-/media/gilead-corporate/files/pdfs/covid-19/gilead_rdv-development-fact-sheet-2020.pdf

[73] Brown, A.J.; Won, J.J.; Graham, R.L.; Dinnon, K.H., III; Sims, A.C.; Feng, J.Y.; Cihlar, T.; Denison, M.R.; Baric, R.S.; Sheahan, T.P. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antiviral Res.*, **2019**, *169*(6), 104541. http://dx.doi.org/10.1016/j.antiviral.2019.104541 PMID: 31233808

[74] Al-Tawfiq, J.A.; Al-Homoud, A.H.; Memish, Z.A. Remdesivir as a possible therapeutic option for the COVID-19. *Travel Med. Infect. Dis.*, **2020**, *34*, 101615. http://dx.doi.org/10.1016/j.tmaid.2020.101615 PMID: 32145386

[75] Agostini, M.L.; Andres, E.L.; Sims, A.C.; Graham, R.L.; Sheahan, T.P.; Lu, X.; Smith, E.C.; Case, J.B.; Feng, J.Y.; Jordan, R.; Ray, A.S.; Cihlar, T.; Siegel, D.; Mackman, R.L.; Clarke, M.O.; Baric, R.S.; Denison, M.R. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. *MBio*, **2018**, *9*(2), e00221-18. http://dx.doi.org/10.1128/mBio.00221-18 PMID: 29511076

[76] Al Bujuq, N. Methods of synthesis of remdesivir, favipiravir, hydroxychloroquine, and chloroquine: four small molecules repurposed for clinical trials during the covid-19 pandemic. *Synthesis*, **2020**, *52*(24), 3735-3750. http://dx.doi.org/10.1055/s-0040-1707386

[77] Zarenezhad, E.; Behrouz, S.; Farjam, M.; Rad, M.N.S. A mini review on discovery and synthesis of remdesivir as an effective and promising drug against COVID-19. *Russ. J. Bioorganic Chem.*, **2021**, *47*(3), 609-621. http://dx.doi.org/10.1134/S1068162021030183 PMID: 34149273

[78] Siegel, D.; Hui, H.C.; Doerffler, E.; Clarke, M.O.; Chun, K.; Zhang, L.; Neville, S.; Carra, E.; Lew, W.; Ross, B.; Wang, Q.; Wolfe, L.; Jordan, R.; Soloveva, V.; Knox, J.; Perry, J.; Perron, M.; Stray, K.M.; Barauskas, O.; Feng, J.Y.; Xu, Y.; Lee, G.; Rheingold, A.L.; Ray, A.S.; Bannister, R.; Strickley, R.; Swaminathan, S.; Lee, W.A.; Bavari, S.; Cihlar, T.; Lo, M.K.; Warren, T.K.; Mackman, R.L. Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo[2,1-f][triazin-4-amino] adenine c-nucleoside (GS-5734) for the treatment of ebola and emerging viruses. *J. Med. Chem.*, **2017**, *60*(5), 1648-1661. http://dx.doi.org/10.1021/acs.jmedchem.6b01594 PMID: 28124907

[79] Khanal, P. Remdesivir for COVID-19 treatment: Mechanism of action, synthesis, and clinical trials. *World J. Pharm. Pharm. Sci.*, **2020**, *9*, 1062-1068. http://dx.doi.org/10.20959/wjpps20208-16808

[80] Ko, W.C.; Rolain, J.M.; Lee, N.Y.; Chen, P.L.; Huang, C.T.; Lee, P.I.; Hsueh, P.R. Arguments in favour of remdesivir for treating SARS-CoV-2 infections. *Int. J. Antimicrob. Agents*, **2020**, *55*(4), 105933. http://dx.doi.org/10.1016/j.ijantimicag.2020.105933 PMID: 32147516

[81] Jayk Bernal, A.; Gomes da Silva, M.M.; Musungaie, D.B.; Kovalchuk, E.; Gonzalez, A.; Delos Reyes, V.; Martín-Quirós, A.; Caraco, Y.; Williams-Diaz, A.; Brown, M.L.; Du, J.; Pedley, A.; Assaid, C.; Strizki, J.; Grobler, J.A.; Shamsuddin, H.H.; Tipping, R.; Wan, H.; Paschke, A.; Butterton, J.R.; Johnson, M.G.; De Anda, C. Molnupiravirfor oral treatment of COVID-19 in non-hospitalized patients. *N. Engl. J. Med.*, **2022**, *386*(6), 509-520. http://dx.doi.org/10.1056/NEJMoa2116044 PMID: 34914868

[82] FDA news release. Coronavirus (COVID-19) Update: FDA Authorizes Additional Monoclonal Antibody for Treatment of COVID-19. **2021**. Available from: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-additional-oral-antiviral-treatment-covid-19-certain

[83] Ahlqvist, G.P.; McGeough, C.P.; Senanayake, C.; Armstrong, J.D.; Yadaw, A.; Roy, S.; Ahmad, S.; Snead, D.R.; Jamison, T.F. Progress toward a large-scale synthesis of molnupiravir(MK-4482, EIDD-2801) from cytidine. *ACS Omega*, **2021**, *6*(15), 10396-10402. http://dx.doi.org/10.1021/acsomega.1c00772 PMID: 34056192

[84] Hampton, T. New flu antiviral candidate may thwart drug resistance. *JAMA-. JAMA*, **2020**, *323*(1), 17. http://dx.doi.org/10.1001/jama.2019.20225 PMID: 31910262

[85] Shiraki, K.; Daikoku, T. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. *Pharmacol. Ther.*, **2020**, *209*, 107512. http://dx.doi.org/10.1016/j.pharmthera.2020.107512 PMID: 32097670

[86] Taisho Toyama Pharmaceutical Co. L. Avigan® (Favipiravir) Tablets Prescribing Information. **2017**. Available from: https://www.cdc.gov.tw/File/Get/ht8jUiB_MI-

[87] Rizzardini, G. Covid-19 clinical study to evaluate the performance and safety of favipiravir in covid-19. *Clinicaltrials.gov.*, **2020**. Available from: https://clinicaltrials.gov/ct2/show/NCT04336904?cond=COVID-19&draw=3&rank=14

[88] Russia approves first covid-19 prescription drug for sale in pharmacies. *Reuters Staf.*, **2020**. Available from: https://www.reuters.com/article/us-health-coronavirus-russia-rpharm-idUSKBN26917Z

[89] Ueda, M.; Tanimoto, T.; Murayama, A.; Ozaki, A.; Kami, M. Japan's drug regulation during the COVID-19 pandemic: lessons from a case study of favipiravir. *Clin. Pharmacol. Ther.*, **2022**, *111*(3), 545-547. http://dx.doi.org/10.1002/cpt.2251 PMID: 33882157

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[90] Pulla, P. Is favipiravir good for COVID-19? clinical trial says no, press release says yes. *Science.thewire.in.*, **2020**. Available from: https://science.thewire.in/the-sciences/favipiravir-glenmark-open-label-trial-primary-endpoints-efficacy-cure-times-misleading-press-release/

[91] Furuta, Y.; Egawa, H. Nitrogenous heterocyclic carboxamide derivatives or salts thereof and antiviral agents containing both. Patent WO2000010569, **2000**. Available from: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2000010569

[92] Liu, F.L.; Li, C.Q.; Xiang, H.Y.; Feng, S. A practical and step-economic route to Favipiravir. *Chem. Pap.*, **2017**, *71*(11), 2153-2158. http://dx.doi.org/10.1007/s11696-017-0208-6

[93] Guo, Q.; Xu, M.; Guo, S.; Zhu, F.; Xie, Y.; Shen, J. The complete synthesis of favipiravir from 2-aminopyrazine. *Chem. Pap.*, **2019**, *73*(5), 1043-1051. http://dx.doi.org/10.1007/s11696-018-0654-9

[94] Furuta, Y.; Komeno, T.; Nakamura, T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. *Proc. Jpn. Acad., Ser. B. Phys. Biol. Sci.*, **2017**, *93*(7), 449-463. http://dx.doi.org/10.2183/pjab.93.027 PMID: 28769016

[95] Lau, J. The story of ribavirin. *Hepcassoc*, **2002**, *35*(5). Available from: http://www.hepcassoc.org/news/ article39.html

[96] Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. Broad-spectrum antiviral activity of virazole: 1-beta-d-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science*, **1972**, *177*, 705-706. http://dx.doi.org/10.1126/science.177.4050.705

[97] Ribasphere FDA Approval History. *Drugs.com.*, **2004**. Available from: https://www.drugs.com/history/ribasphere.html

[98] Chen, F.; Chan, K.H.; Jiang, Y.; Kao, R.Y.T.; Lu, H.T.; Fan, K.W.; Cheng, V.C.C.; Tsui, W.H.W.; Hung, I.F.N.; Lee, T.S.W.; Guan, Y.; Peiris, J.S.M.; Yuen, K.Y. *In vitro* susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. *J. Clin. Virol.*, **2004**, *31*(1), 69-75. http://dx.doi.org/10.1016/j.jcv.2004.03.003 PMID: 15288617

[99] Morgenstern, B.; Michaelis, M.; Baer, P.C.; Doerr, H.W.; Cinatl, J., Jr Ribavirin and interferon-β synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines. *Biochem. Biophys. Res. Commun.*, **2005**, *326*(4), 905-908. http://dx.doi.org/10.1016/j.bbrc.2004.11.128 PMID: 15607755

[100] Falzarano, D.; de Wit, E.; Rasmussen, A.L.; Feldmann, F.; Okumura, A.; Scott, D.P.; Brining, D.; Bushmaker, T.; Martellaro, C.; Baseler, L.; Benecke, A.G.; Katze, M.G.; Munster, V.J.; Feldmann, H. Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV–infected *rhesus macaques*. *Nat. Med.*, **2013**, *19*(10), 1313-1317. http://dx.doi.org/10.1038/nm.3362 PMID: 24013700

[101] Li, H.; Xiong, N.; Li, C.; Gong, Y.; Liu, L.; Yang, H.; Tan, X.; Jiang, N.; Zong, Q.; Wang, J.; Lu, Z.; Yin, X. Efficacy of ribavirin and interferon-α therapy for hospitalized patients with COVID-19: A multicenter, retrospective cohort study. *Int. J. Infect. Dis.*, **2021**, *104*, 641-648. http://dx.doi.org/10.1016/j.ijid.2021.01.055 PMID: 33515771

[102] Tong, S.; Su, Y.; Yu, Y.; Wu, C.; Chen, J.; Wang, S.; Jiang, J. Ribavirin therapy for severe COVID-19: A retrospective cohort study. *Int. J. Antimicrob. Agents.* **2020**, *56*(3), 106114. http://dx.doi.org/10.1016/j.ijantimicag.2020.106114 PMID: 32712334

[103] Witkowski, J.T.; Robins, R.K.; Sidwell, R.W.; Simon, L.N. Design, synthesis, and broad spectrum antiviral activity of 1-.beta.-D-ribofuranosyl-1,2,4-triazole-3-carboxamide and related nucleosides. *J. Med. Chem.*, **1972**, *15*(11), 1150-1154. https://doi.org/https://doi.org/10.1021/jm00281a014 http://dx.doi.org/10.1021/jm00281a014 PMID: 4347550

[104] Maag, D.; Castro, C.; Hong, Z.; Cameron, C.E. Hepatitis C virus RNA-dependent RNA polymerase (NS5B) as a mediator of the antiviral activity of ribavirin. *J. Biol. Chem.*, **2001**, *276*(49), 46094-46098. http://dx.doi.org/10.1074/jbc.C100349200 PMID: 11602568

[105] Crotty, S.; Cameron, C.E.; Andino, R. RNA virus error catastrophe: Direct molecular test by using ribavirin. *Proc. Natl. Acad. Sci.*, **2001**, *98*(12), 6895-6900. http://dx.doi.org/10.1073/pnas.111085598 PMID: 11371613

[106] Zhou, S.; Liu, R.; Baroudy, B.M.; Malcolm, B.A.; Reyes, G.R. The effect of ribavirin and IMPDH inhibitors on hepatitis C virus subgenomic replicon RNA. *Virology*, **2003**, *310*(2), 333-342. http://dx.doi.org/10.1016/S0042-6822(03)00152-1 PMID: 12781720

[107] Nojomi, M.; Yassin, Z.; Keyvani, H.; Makiani, M.J.; Roham, M.; Laali, A.; Dehghan, N.; Navaei, M.; Ranjbar, M. Effect of Arbidol (Umifenovir) on COVID-19: A randomized controlled trial. *BMC Infect. Dis.*, **2020**, *20*(1), 954. http://dx.doi.org/10.1186/s12879-020-05698-w PMID: 33317461

[108] Liu, D.; Zeng, X.; Ding, Z.; Lv, F.; Mehta, J.L.; Wang, X. Adverse cardiovascular effects of anti-COVID-19 drugs. *Front. Pharmacol.*, **2021**, *12*, 699949. http://dx.doi.org/10.3389/fphar.2021.699949 PMID: 34512335

[109] Zhuravel, S.V.; Khmelnitskiy, O.K.; Burlaka, O.O.; Gritsan, A.I.; Goloshchekin, B.M.; Kim, S.; Hong, K.Y. Nafamostat in hospitalized patients with moderate to severe COVID-19 pneumonia: a randomised Phase II clinical trial. *EClinicalMedicine*, **2021**, *41*, 101169. http://dx.doi.org/10.1016/j.eclinm.2021.101169 PMID: 34723164

[110] Cao, B.; Wang, Y.; Wen, D.; Liu, W.; Wang, J.; Fan, G.; Ruan, L.; Song, B.; Cai, Y.; Wei, M.; Li, X.; Xia, J.; Chen, N.; Xiang, J.; Yu, T.; Bai, T.; Xie, X.; Zhang, L.; Li, C.; Yuan, Y.; Chen, H.; Li, H.; Huang, H.; Tu, S.; Gong, F.; Liu, Y.; Wei, Y.; Dong, C.; Zhou, F.; Gu, X.; Xu, J.; Liu, Z.; Zhang, Y.; Li, H.; Shang, L.; Wang, K.; Li, K.; Zhou, X.; Dong, X.; Qu, Z.; Lu, S.; Hu, X.; Ruan, S.; Luo, S.; Wu, J.; Peng, L.; Cheng, F.; Pan, L.; Zou, J.; Jia, C.; Wang, J.; Liu, X.; Wang, S.; Wu, X.; Ge, Q.; He, J.; Zhan, H.; Qiu, F.; Guo, L.; Huang, C.; Jaki, T.; Hayden, F.G.; Horby, P.W.; Zhang, D.; Wang, C. A trial of lopinavir–ritonavir in adults hospitalized with severe covid-19. *N. Engl. J. Med.*, **2020**, *382*(19), 1787-1799. http://dx.doi.org/10.1056/NEJMoa2001282 PMID: 32187464

[111] Pfizer shares *in vitro* efficacy of novel covid-19 oral treatment against omicron variant. **2022**. Available from: https://www.pfizer.com/news/press-release/press-release-detail/pfizer-shares-vitro-efficacy-novel-covid-19-oral-treatment

[112] Aygün, İ.; Kaya, M.; Alhajj, R. Identifying side effects of commonly used drugs in the treatment of Covid 19. *Sci. Rep.*, **2020**, *10*(1), 21508. http://dx.doi.org/10.1038/s41598-020-78697-1 PMID: 33299085

[113] Fan, Q.; Zhang, B.; Ma, J.; Zhang, S. Safety profile of the antiviral drug remdesivir: An update. *Biomed. Pharmacother.*, **2020**, *130*, 110532. http://dx.doi.org/10.1016/j.biopha.2020.110532 PMID: 32707440

[114] Arribas, J.R.; Bhagani, S.; Lobo, S.M.; Khaertynova, I.; Mateu, L.; Fishchuk, R.; Park, W.Y.; Hussein, K.; Kim, S.W.; Ghosn, J.; Brown, M.L.; Zhang, Y.; Gao, W.; Assaid, C.; Grobler, J.A.; Strizki, J.; Vesnesky, M.; Paschke, A.; Butterton, J.R.; De Anda, C. Randomized trial of molnupiravir or placebo in patients hospitalized with covid-19. *NEJM Evid.* **2021**, *1*(2), 1-13. http://dx.doi.org/10.1056/EVIDoa2100044

[115] Kaur, R.J.; Charan, J.; Dutta, S.; Sharma, P.; Bhardwaj, P.; Sharma, P.; Lugova, H.; Krishnapillai, A.; Islam, S.; Haque, M.; Misra, S. Favipiravir use in COVID-19: Analysis of suspected adverse drug events reported in the who database. *Infect. Drug Resist.*, **2020**, *13*, 4427-4438. http://dx.doi.org/10.2147/IDR.S287934 PMID: 33364790

[116] Wang, Y.; Li, W.; Jiang, Z.; Xi, X.; Zhu, Y. Assessment of the efficacy and safety of Ribavirin in treatment of coronavirus-related pneumonia (SARS, MERS and COVID-19). *Medicine*, **2020**, *99*(38), e22379. http://dx.doi.org/10.1097/MD.0000000000022379 PMID: 32957417

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Formulation, Characterization and Biological Evaluation of Herbal oil for Hair Growth Potential

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Objectives
Herbs are their extracts were used form ancient time for the concept of beauty and cosmetics is as ancient as mankind and civilization. Therefore, they are using various beauty products containing herbs. In the present investigation powder plant material of Eclipta alba Linn. (Leaves), Mentha piperita Linn. (Leaves) and Vitex negundo Linn (Leaves) was taken to formulate polyherbal hair oil and was further evaluated for its efficacy. The present work was aimed to formulate herbal oil for general purpose (application in hairs) using various herbs. The formulated herbal oil was evaluated and various parameters such as viscosity, saponification value, pH etc. were determined and are reported in this paper.

Introduction

In the last few decades the use of cosmetics has increased not only among women but also among men. Hair dyes, hair oils and such creams are popular among both men and women. Most countries now have laws to regulate, manufacture, label, sell, etc. cosmetics in such a way as to prevent the use of cosmetics harmful to health. The concept of beauty and cosmetics is as old as mankind and civilization itself. Therefore, they use various beauty products that contain herbs to look attractive and young. Indian herbs and their importance are popular worldwide. Herbal cosmetics are in increasing demand in the world market and are a precious gift of nature. There is a wide range of herbal cosmetic products to satisfy the beauty regime [1-2]. Adding herbs to cosmetics is very safe for our skin. Herbal hair oil is one of the most popular hair treatments. Herbal hair oil not only moisturizes the scalp but also reverses the condition of dry scalp and dry hair. It provides numerous essential nutrients needed to maintain the normal functions of the sebaceous glands and promote natural hair growth [3-5]. Keeping this in mind, the present work was undertaken. Various herbal products have been praised for their ability to promote hair growth. A number of herbal medicines are recommended for hair growth stimulation in the traditional medical system of India, but their use is limited due to lack of scientific support and expertise. *Eclipta alba* Linn. (Leaves), *Mentha piperita* Linn. (Leaves) and *Vitex negundo* Linn (Leaves) are the plant widely used for the proper growth of hair and for healthy black and long hair in India. Keeping this fact the present study was aimed to develop a polyherbal hair oil consisting dried plant material and to evaluate polyherbal hair oil.

Material and Methods

Collection of plant part

Eclipta alba Linn. (Leaves), *Mentha piperita* Linn. (Leaves) and *Vitex negundo* Linn (Leaves) were

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collected from local area of Malwa region. The collected plant material was identified and authenticated by Dr. S.N. Dwivedi, Retd. Prof. & Head, Department of Botany, Janata PG College, APS University, Rewa, (M.P.). Voucher Specimen Number: J/Bot./2022-EA/21; J/Bot./2022-MP/22 and J/Bot./2022-VN/23; was allotted.

Formulation of polyherbal hair oil

The various ingredients used in the formulation of herbal oil are presented in Table 1. Accurately weigh all the dried and fresh herbs *Eclipta alba* Linn. (Leaves), *Mentha piperita* Linn. (Leaves) and *Vitex negundo* Linn (Leaves) and were grinded in the mixture and was mixed in 70% of til oil. The above content was boiled for 15 min. and was filtered through muslin cloth. To the filtrate coconut oil was added to make up the volume (100 mL). Finally small amount of color and flavoring agent was added to the oil and it was placed in amber colored bottle. [6-7]

Table 1: Ingredients used in formulation of polyherbal hair oil

S/No.	Ingredients	Quantity (%)
1.	*Eclipta alba* Linn. (Leaves)	1.5
2.	*Mentha piperita* Linn. (Leaves)	2.0
3.	*Vitex negundo* Linn (Leaves)	2.0
4.	Til oil	70
5.	Coconut oil	15
6.	Rang	qs
7.	Flavoring agent	qs

Evaluation of polyherbal hair oil

The formulated herbal hair oil was subjected to physical evaluation. [6-8]

Sensitivity test

The prepared herbal hair oil was applied on 1 cm skin of hand and exposed to sunlight for 4-5 min.

Acid value

Preparation of 0.1 molar solution: Weighed 0.56 g KOH pellets and dissolved in 100 mL of distilled water and stirred continuously. The prepared 0.1 molar KOH solution was filled in the burette. Preparation of sample: Measured 10 mL oil and dissolved in 25 mL of ethanol and 25 mL of ether mixture and shacked. Added 1 mL of phenolphthalein solution and titrated with 0.1 molar KOH solution.

Saponification value

Accurately weighed 1 mL of oil into a 250 mL of conical flask and 10 mL of ethanol : ether mixture (2 : 1) was added. To this flask 25 mL of 0.5 N alcoholic KOH was. Kept the flask for 30 min. and the flask was cooled. The cooled solution was titrated against 0.5 N HCl using phenolphthalein indicator. Similarly the blank titration was performed without taking oil (sample). Amount of KOH in mg used was calculated.

pH: The pH of herbal hair oil was determined using pH meter.
Viscosity: The viscosity was determined using Ostwald's viscometer.
Specific gravity: Take the specific gravity bottle, rinsed it with distilled water, dry it in oven for 15 minutes, cool, closed it with cap and weigh it (a). Now fill the same specific gravity bottle with the sample and closed it with cap and again weigh it (b). Determine the weight of sample per milliliter by subtracting the weight (b-a).

Biological Activity

Healthy Wistar Rats 180-200g ms of either sex were purchased from Veterinary College, Mhow, Indore and used for hair growth promoting activity. The experimental protocol was approved by the Institutional animal ethics committee. Animals were placed in cages and kept in standard environmental conditions, fed with standard diet ad libitum and allowed free access to drinking water.

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They were acclimated 7 days before entry into subsequent study. [8]

Primary dermal irritatiou study

Male New Zealand White Rabbits were used for dermal irritancy experiment. The experimental protocol was approved by the Institutional animal ethics committee. OECD 404 guidelines were adhered during the maintenance and experment. In dermal irritancy study herbal oil formulation was used on rabbits. Approximately 24 hours before the test, fur was removed by closely clipping the dorsal area of the trunk of the animals. Half a gram of the herbal formulation was applied to a small area (approximately 6 cm2) of skin and covered with a gauze patch, which is held in place with non-irritating tape. All animals were examined for signs of erythema and oedema, and the responses scored at 60 minutes, and then at 24, 48 and 72 hours after patch removal.

Table 2: Grading of Skin Reactions

Grading	Erythema formation
No erythema	0
Very slightly erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema	4
Maximum possible	4

Table 3: Oedema Formation

Oedema formation	Erythema formation
No Oedema	0
Very Slightly Oedema	1
Slightly Oedema	2
Moderate Oedema	3
Severe Oedema	4
Maximum possible	4

Groups

Wistar Rats were divided into three groups of 6 animals in each group. Hairs from 3 cm2 area at the dorsal portion of all the mice were shaved using electric shavers and applied with marketed hair remover to completely remove hair. **Group 1** served as a negative control was applied with simple gel where there was no drug treatment. **Group 2** was topically applied with 2 % minoxidil over the shaved area as positive control. **Group 3** was applied with herbal oil (HO).

Application of Herbal oil (HO)

Herbal oil and standard drug were applied once in a day. The treatment was continued for 30 days and hair growth pattern was observed and tabulated. Skin biopsies were taken on the 30th day for follicular observation. Increase in thickness and presence of the follicles in the subcutis layer were taken as evidence for transition of follicles from telogen to anagen phase of hair growth.

Qualitative Studies on Hair Growth

Qualitative hair growth analysis was undertaken by visual observation of two parameters: hair growth initiation time (i.e. minimum time to initiate hair growth on denuded skin region) and hair growth completion time (i.e. minimum time taken to complete cover the denuded skin region with new hair).

Hair Length Determination

Hair was plucked randomly using sterile forceps from the shaved dorsal area of mice on 10th, 20th and 30th day of treatment. Hair length was measured and the results were recorded as mean length ± SEM of 25 hairs

Hair Follicle Counting

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Digital photomicrographs were taken from representative areas of slides at a fixed magnification of 100 x. All images were cropped in a fixed area with a width of 1500 µm.

Statistical Analysis

Statistical analysis of the data was carried out by one way ANOVA in respect of the test and control groups and followed by Dunnett's test. Differences between data were considered highly significant $P < 0.05$. The software used was Graphpad Instat version 3.06 computer software. The data are reported as mean ± SEM.

Results and Conclusion

Herbal hair oil is one of the most well recognized hair treatments. Herbal hair oil not only moisturizes scalp but also reverses dry scalp and dry hair condition. It provides numerous essential nutrients required to maintain normal function of sebaceous glands and promotes natural hair growth. The herbal hair oil was prepared from various herbs (Table 1) and their importance in the formulation is presented in Table 4. The various parameters like sensitivity test, viscosity, pH, irritation test, grittiness test, saponification value and acid value of herbal hair oil was evaluated (Table 5). Hence, from the present investigation it was found that the formulated herbal hair oil has optimum standards and further standardization and biological screening establishes the efficacy of formulated herbal hair oil.

Table 4: Role of herbs in herbal oil

S/No.	Ingredients	Importance
1.	*Eclipta alba* Linn. (Leaves)	Hair growth
2.	*Mentha piperita* Linn. (Leaves)	Stimulating agent
3.	*Vitex negundo* Linn (Leaves)	Hair growth
4.	Til oil	Vehicle
5.	Coconut oil	Hair growth

Table 5: Evaluation Parameters of herbal oil

S/No.	Ingredients	Inference
1.	Specific gravity	1.010
2.	Viscosity	0.72
3.	Acid value	4.2
4.	Saponification value	111.21
5.	pH	7.0
6.	Sensitivity test	No irritation
7.	Irritation test	No irritation
8.	Grittiness	Smooth

The total scores for skin irritation in terms of erythema and oedema was calculated after 12, 24, 48 and 72 hours according to OECD scoring system. Results revealed that the developed polyherbal hair gel formulation and polyherbal oil did not cause any erythema or oedema on the intact rabbit skin when observed for 72 hours. The Primary Dermal Irritation Index (PDII) of the formulation was zero; therefore according to OECD guidelines the formulation can be classified as non - irritant to the rabbit skin. Throughout the 30 days study period, all the 3 groups of animals were observed closely to determine the hair growth initiation and completion time. This was achieved using a magnifying lens that enabled observation of minute changes in the hair growth pattern. The point at which a tiny prickle of hair growth was observed and it was noted as the initiation time. Developed herbal oil treated animals showed significant reduction in hair growth initiation and completion time as compared to control and minoxidil treated animals (Table 6). In control group animals, initiation of hair growth in denuded area was observed in 13.8 days. Hair growth initiation was noted in the first week in wistar rats of minoxidil treated standard group. The formulation exhibited hair growth initiation on 12th day. The length of the hair began to increase until the end of the treatment course. The mean hair length in positive control is 2.4 followed by herbal oil (HO) has 1.7 mm and 1.1 mm in

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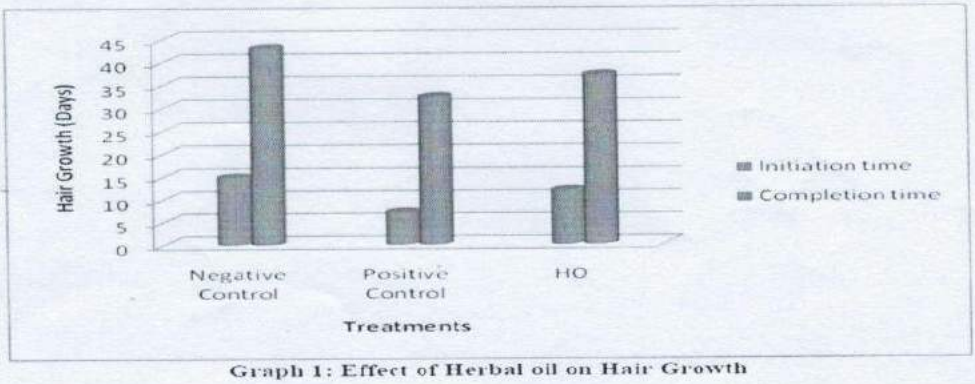
control groups which received simple gel treatment. This may be due to the premature switching of follicles from the telogen to anagen phase of hair growth cycle. Moreover, in all the groups and minoxidil treated groups, the hair looked sparsely. This denotes the presence of greater number of hair follicles in the anagen phase of the hair growth cycle in and minoxidil treated groups. Average hair length of each group at 10th day, 20th day and 30th day has been given in Table 7.

Table 6: Effect of Herbal oil on Hair Growth Initiation and Completion Time

Treatments	Hair growth in days		% Reduction in hair growth time*
	Initiation time	Completion time	
Negative Control	14.9±0.22	43.1±0.21	
Positive Control	7.15±0.20***	32.4±0.12***	25.25
HO	11.9±0.31	37.2±0.22***	25.30

*(Mean of control - Mean of treatment) / Mean of control X 100
***P < 0.05 significant when compared to control (ANOVA followed by Dunnett's test; Values are mean ± SEM



Graph 1: Effect of Herbal oil on Hair Growth

Table 7: Effect of Herbal oil on Hair Length

Treatments	Mean hair length (mm)		
	10th Day	20th Day	30th Day
Negative Control	0.0±0.0	0.2±0.0	1.1±0.18
Positive Control	0.7±0.09	1.6±0.02	2.4±0.18***
HO	0.4±0.1	0.9±0.14	1.7±0.11***

*** P < 0.05 significant when compared to control (ANOVA followed by Dunnett's test values are mean ± SEM

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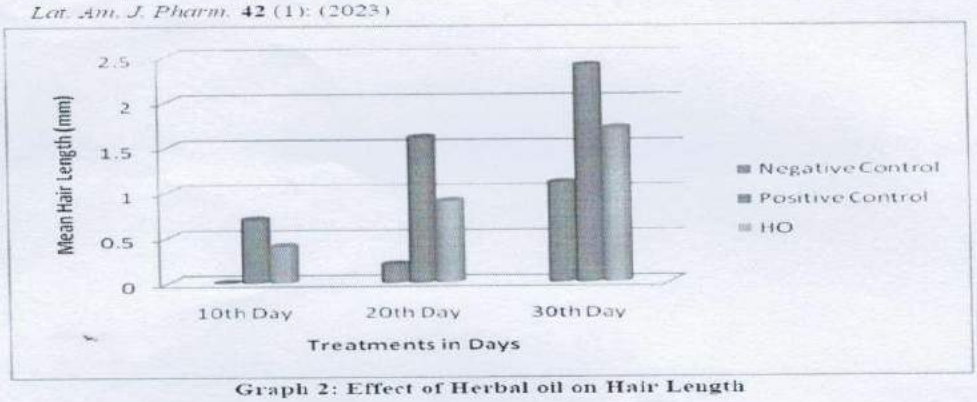
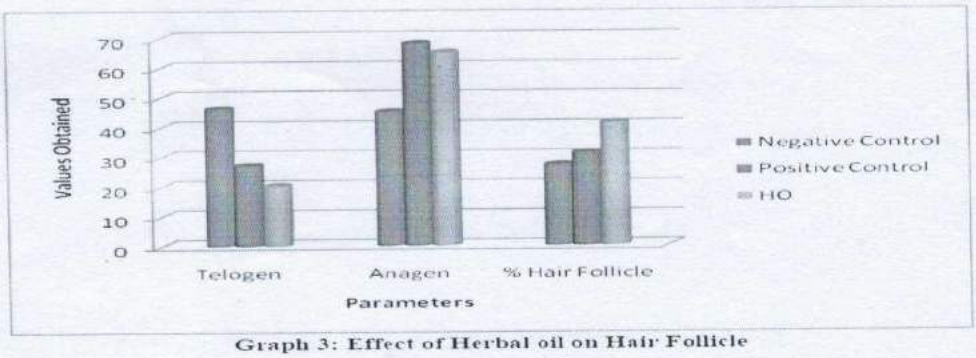


Graph 2: Effect of Herbal oil on Hair Length

Table 8: Effect of Herbal oil on Hair Follicle

Treatments	Telogen	Anagen	T/A	% Hair Follicle
Negative Control	46.2±0.21	45.2±0.11	1.02	27.24
Positive Control	27.1±.21	68.2±0.11	0.39	31.22
HO	20.3±0.42	65.2±0.02	0.31	41.38

*** P < 0.05 significant when compared to control (ANOVA followed by Dunnett's test values are mean ± SEM



Graph 3: Effect of Herbal oil on Hair Follicle

References

1. N. Sanju, N. Arun and K. K. Roop, Cosmetic Technology, 1st Edition, Birla Publications Pvt. Ltd, Delhi (2006) pp. 379-382.
2. B. M. Mithal and R. N. Shah, A Hand Book of Cosmetics, 1st Edition, Vallabh Prakashan, Delhi (2000) pp. 141-142.
3. R. Shoba Rani Hiremath Textbook of Industrial Pharmacy, 1st Edition, Orient Longaman Pvt. Ltd., Hyderabad (2007) pp. 99-102.

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Original Article

Pharmacological Evaluation of Different Extracts of *Asparagus officinalis (Asparagaceae)* as an Analgesic, Anti-Inflammatory and Anti-arthritic Agent in Rats

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ABSTRACT

Background: To treat the joint pain arial part of *Asparagus officinalis* (asparagus) has historically been used. However, its efficacy for rheumatoid arthritis has not been pharmaceutically evaluated. We explore the phytochemical analysis anti-inflammatory, analgesic and anti-arthritic activity of petroleum ether, ethanol and aqueous extracts of *Asparagus officinalis* aerial part. **Materials and Methods:** Tail- flick method was used to evaluate the analgesic activity anti-inflammatory activity was carried out using paw oedema induced with carrageenan and CFA induced arthritic model was used to evaluate the potential in anti- arthritic activity in rats. The Petroleum ether, ethanolic and aqueous extracts were dosed orally in three divided doses (75, 150 and 300 mg/kg). For anti- inflammatory and analgesic activity diclofenac sodium at 10 mg/kg was used as standard, whereas in anti-arthritic model prednisolone at 5 mg/kg and methotrexate at 0.5 mg/kg were used as standard. One-way ANOVA followed by Dunnett's multiple range test were used to analyse statistical significance between means. **Results:** The results revealed a dose-controlled anti-inflammatory, anti-arthritic effect with different extracts whereas at some extent analgesic activity was observed. Four compounds were present and confirmed by LCMS/MS. The CFA model's findings showed improved defence against arthritic lesions and changes in body weight. Additionally, *Asparagus officinalis* significantly improved rheumatoid factor, changed WBCs, and favourably altered radiographic and histological alterations. **Conclusion:** The findings indicate that *Asparagus officinalis* is a strong anti-arthritic and anti-inflammatory compound that may be suggested for the treatment of both chronic and acute inflammation.

Keywords: Carrageenan, Chemical constituents, LCMS/MS, Rheumatoid arthritis, Sparrow grass.

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INTRODUCTION

Pain and Inflammation are both prominent symptoms of Rheumatoid arthritis (RA) which is a significant worry marked by non-specific joint inflammation, articular tissue loss, and joint abnormalities. As the condition advances, the likelihood of bone degradation and cartilage breakdown increases, resulting in significant impairment.[1] An immune system response known as inflammation may be brought on by a variety of causes, such as infections, damaged cells, and toxins.[2] The creation of prostaglandins is triggered by infections (germs) such as viruses, fungi, bacteria, and illnesses, medical disorders, and external traumas such scratches or injury from foreign objects, chemicals, or radiation.[3] interleukins (IL- 1 and IL-6) and tumour necrosis factor (TNF-α) are examples of inflammatory cytokines that have been demonstrated to have a significant role in inflammation and joint destruction that occurs throughout the progression of arthritis.[4,5] Articular cartilage and the subchondral bones are degraded as a result inflammation induce in synovial membranes. Despite substantial research, the specific cause of RA is unknown. Recent research suggests that increased oxidative stress due to free radical production may be a key factor in the progression of RA.[6] Cyclooxygenase isoenzyme (COX) plays a major role in development of arthritis apart from cytokines (IL-1, IL-6, TNF-α, etc.)[7,8] Excessive cytokines (pro-inflammatory) can form a positive bridge between RA fibroblasts and macrophage-like synovial cells. Inflammatory cytokine inhibition is the most common molecular target in the treatment of RA.[9]

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Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids have been widely employed in the treatment of RA.[10] These analgesic medicines, are reported to relieve only 50% of the pain particularly NSAIDs, that too roughly 30% of people in most cases.[11] However, due to serious adverse effects shows in heart, gastrointestinal tract and kidney function, this drug is now lost their major role as first-line treatment in response to current findings.[12] In recent decades, new categorized drugs are available for this treatment such as glucocorticoids and immunosuppressant are effective anti-inflammatory medications, but they have their serious adverse effects that make long-term use difficult. As a result, we are now depending upon herbal treatment which may show lesser adverse effect and may better safety profile as compared to existing one.[13] Scientists are always in search of better alternate treatment. In that case Ayurveda is always preferred choice.

Through literature survey and endangered species existing in India especially in Southern Region we observed many plants which are still unexplored for various pharmacological activities.[14] They are traditionally use in sub local rural areas but scientific validation is not yet reported. The primary motivation for researching traditional plants is because many of the existing allopathic medications are made from plant material, and many of the structures employed in allopathy are comparable to plant constituents.

According to a recent WHO (World Health Organization) estimate, 80% of people globally rely on herbal remedies for some of their basic medical requirements. Approx 485 plant species belonging to 100 families are used for arthritis as traditional drug.[15,16] The Asparagaceae family includes Asparagus as a part. Mentioned in one of the earliest treatises of human knowledge, the Rig-Veda, was written between 4500 and 1600 BC.[17]

Added to this, very less work is available on *Asparagus officinalis* whereas other members of same class are reported for many pharmacological activities such as *Asparagus racemosus*, *Asparagus sprengeri* and *Asparagus acutifolius* are all members of the same genus.[18] *Asparagus officinalis* contains steroids, amino acids, saponins, fructans (Asparagosine and Asparagose), flavonoids and ferulic acid. Ethanolic and aqueous extracts of this plant have been use in Southern Interior region for treatment of many diseases but traditionally it is used to treat kidney and bladder stones, asthma, and cough.[19] *Asparagus officinalis* was found to have inhibitory effects on both cyclooxygenases-1 and -2, indicating that it possesses anti-inflammatory properties. Linoleic acid was one of the compound present in this plant and found active in various pharmacological activities[19] includes liver toxicity treatment,[20] anti-microbial,[21] anti-arthritic,[19] anti-oxytoxic, antiulcer, hypertensive and anticoagulant effects.[22] Ethanolic extract of *Asparagus* was reported anti-diabetic at dose of 50 mg/kg and 100 mg/kg.[23,24] Another study was also shown that 400 mg/kg of ethanolic extracts act as potential protective agent against oxidative stress in liver and kidney organs.[25] Based on our survey, we designed this study to access the pharmacological evaluation of different extracts of *Asparagus officinalis* (Asparagaceae) as an analgesic, anti-inflammatory and anti-arthritic activity in experimental rats.

MATERIALS AND METHODS

Plant material

Arial part of *Asparagus officinalis* was procured form botanical Garden of Southern region. The specimen plant was identified and authenticated by NISCAIR, New Delhi, India with reference number NISCAIR/RHMD/consult/-2021/3827-28. Fresh plant material was cleansed with tap water to remove dirt and dried for 10 days in the sun and shade before being crushed into a powder in an electric grinder.

Preparation of extracts

Powder of dried aerial plant part (2.5 kg) was added in the soxhlet's apparatus using Petroleum ether, Ethanol and water for successive extraction. The final traces of all three solvent extracts were removed using the dried vacuum method. All extracts were kept at temperature between 2- 4°C for further use.

Chemical and drugs

We bought carrageenan and Complete Freund's adjuvant from Sigma Chemicals (St Louis, USA). Ind. Swift Laboratories (Baddi, India) provided pure samples of prednisolone and diclofenac sodium, while MacLeod's Laboratory provided a gift sample of methotrexate (Mumbai, India). The study's other reagents and compounds were all of analytical grade.

Animals

Sprague dawley rats (130–150 gm) were utilised with the institutional animal ethics committee's previous consent (1125/PO/Rc/S/07/CPCSEA) with protocol no B-098. The animals were kept in normal housing with a 12:12 light-dark cycle and temperatures between 24 and 28°C. Standard feed pellet diet (Safe Diet procured from Samitek Instruments) were provided to animals and water was allowed *ad libitum*.

Drugs and dosage

75 mg/kg, 150 mg/kg, and 300 mg/kg of the extracts were administered orally as a suspension in 5 mL/kg of 1% carboxy methyl cellulose and 1.0% v/v tween 80. As an oral suspension, methotrexate (0.5 mg/kg *p.o.*) and prednisolone (5 mg/kg *p.o.*), were utilised as standards for anti- arthritic activity. Whereas diclofenac sodium (10 mg/kg *p.o.*) was used as standard in analgesic and anti-inflammatory activities.

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Experimental Design

Experiment no-1 (Analgesic activity)

Rats were slit up into eleven different groups with 6 rats in each group and treated as given; Group (1): Control vehicle treated group received 1% carboxymethylcellulose, Group (2): received diclofenac sodium (10 mg/kg *p.o.*), Group (3, 4 and 5): received petroleum ether extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (6, 7 and 8): received ethanolic extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (9, 10 and 11): received aqueous extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*.

Experiment no-2 (Anti-inflammatory activity)

Rats were split up into twelve groups (6 rats each) and treated as follows; Group (1): Control vehicle treated group received 1% carboxymethylcellulose, Group (2): received normal water Group (3): received diclofenac sodium (10 mg/kg *p.o.*), Group (4, 5 and 6): received petroleum ether extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (7, 8 and 9): received ethanolic extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (10, 11 and 12): received aqueous extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*.

Experiment no-3 (Anti-arthritic activity)

Rats were split up into thirteen different groups (6 rats each) and treated as follows; Group (1): Vehicle treated control group received 1% carboxymethylcellulose, Group (2): received normal water Group (3 and 4): received diclofenac sodium (10 mg/kg *p.o.*) and methotrexate (0.5 mg/kg. *p.o*) respectively, Group (5, 6 and 7): received petroleum ether extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (8, 9 and 10): received ethanolic extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*, Group (11, 12 and 13): received aqueous extract (75, 150 and 300 mg/kg *p.o.*) of *Asparagus officinalis*.

Tail flick method (Analgesic activity)

Analgesiometer was used to measure the analgesic effect in rats. A nichrome wire within the instrument is heated to the necessary temperature and kept there by heat regulators. The current through the bare nichrome wire was maintained at a consistent strength (4 Amps). The rat was housed in a rat cage with only its tail sticking out. The centre of the tail was positioned on the platform such that it was barely above the hot wire but not touching it. The animal's sudden, recognisable flick or tail raising in response was used to measure the animal's latency (reaction time). To prevent any tissue injury to the animal, a cut-off duration of 10 sec was planned. On the seventh day following a one-week oral medication treatment, the reaction times for each group were tested at 30, 60, 90, and 120 min.[26]

Carrageenan induced paw oedema in rats (Acute- inflammation)

After one week of oral medication treatment, all groups except the control group received 0.1 ml of 1% w/v carrageenan injections into the rat paw. After a gap of one hour, two hours, three hours, four hours, and six hours, the paw oedema volume was measured using a digital plethysmometer (Model 7140, UGO Basile, Italy). Anti-inflammatory activity was determined by calculating the proportion of oedema that was inhibited compared to the control. The formula used to get the percentage inhibition of oedema was: oedema (% inhibition) = (A-B)/A X 100, where A denotes the volume of the paws in the control group and B denotes the volume of the paws in the group that received the test medication.[27]

Complete Freunds Adjuvant induced arthritis (Chronic inflammation)

By injecting 0.1ml (0.1% w/v) of deceased Mycobacterium TB bacteria homogenised in liquid paraffin into the left hind paw, arthritis was developed. On the fifth day, a second CFA booster was administered, while the control group received an equal volume of normal saline with minor modifications as per Dar *et al.* (2019). The drug treatment programme began on day one and lasted for 21 days. The paw volume was assessed using a digital plethysmometer on days 1, 7, 14 and 21. (Model 7140, UGO Basile, Italy). The proportion of oedema that was inhibited in comparison to the control was interpreted as an anti-inflammatory response. Methotrexate (0.5 mg/kg twice weekly) and prednisolone (5 mg/kg once daily) were employed as benchmark medications for comparison. The verneir calliper device was used to measure the ankle joint diameter on the 1st, 7th, 10th, 15th, and 20th days.[28]

Sample Collection

For the analysis of rheumatoid factor, blood samples were taken on the 21st day from the retro orbital plexus, and serum was obtained by centrifuging at 3500 rpm for 30 min at 4°C and stored at -80°C. Then, animals from each group were euthanized for an ankle joint histology study and an x-ray of the femur bone.

Extracts quantification using LC-MS/MS

Bioanalytical method

Preparation of quality control and calibration samples

Main stock solutions of Stigmasterol, β-sitosterol, Rutin, Telmisartan (Internal Standard for Rutin and Quercetin) and Metoprolol (Internal Standard for Stigmasterol, β-sitosterol) were made at 1 mg/mL concentration in methanol.

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Sample preparation

Extracts were dissolved in methanol by looking at the solubility of Stigmasterol, β-sitosterol, and Rutin. And for Quercetin extracts were dissolved in DMSO. Prepared samples were diluted in acetonitrile containing internal standard + methanol + water. For LC-MS/MS (Liquid chromatography-tandem mass spectrometry) analysis, 10 µL were injected.

LC-MS/MS analysis

For Stigmasterol and β-sitosterol Q TRAP 6500+ was run in positive ion mode for the analysis. The LC system utilised was the Sciex Exion LC, which included a vacuum degasser, two isocratic pumps, an AD Multiplate Autosampler with a temperature control set to 4°C, and a thermostatic column oven with a (40°C) temperature control (Sciex Exion, AD column oven). Kinetex EVO, C_{18}, 5 µm particle size 50*4.6 mm stationary phase was utilised for the chromatography (Phenomenex, US). For the mobile phase, 0.1% formic acid in water served as the aqueous reservoir, while 0.1% formic acid in methanol served as the organic modifier. A common reverse phase gradient programme (time (min) / % B= 0.00/60, 3.0/99, 6.50/99, 7.00/99, 10.00/60 and 11.00/60) was used with run time of 11 min. Using

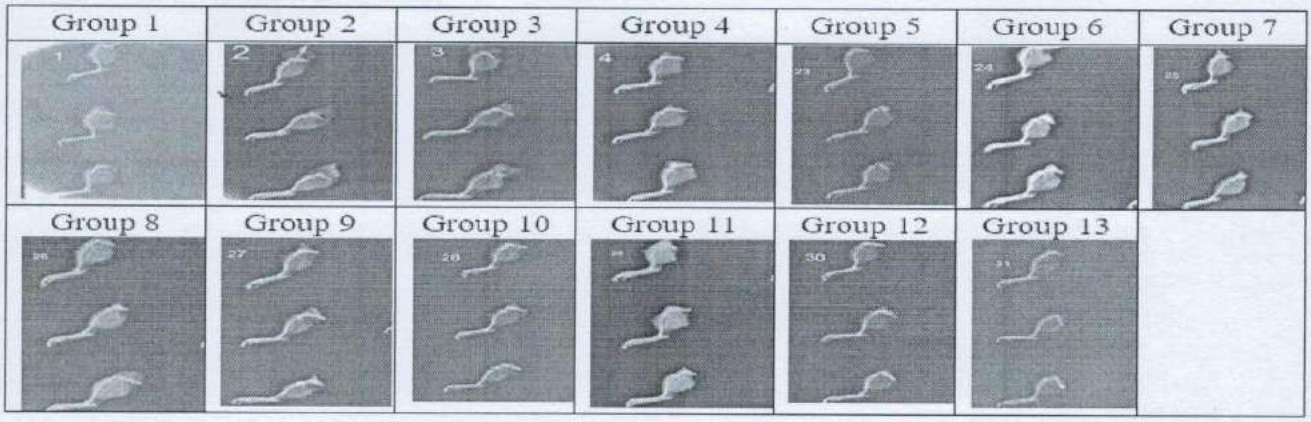

Figure 1: Effect of *Asparagus officinalis* extracts on arthritic joints in X-ray.

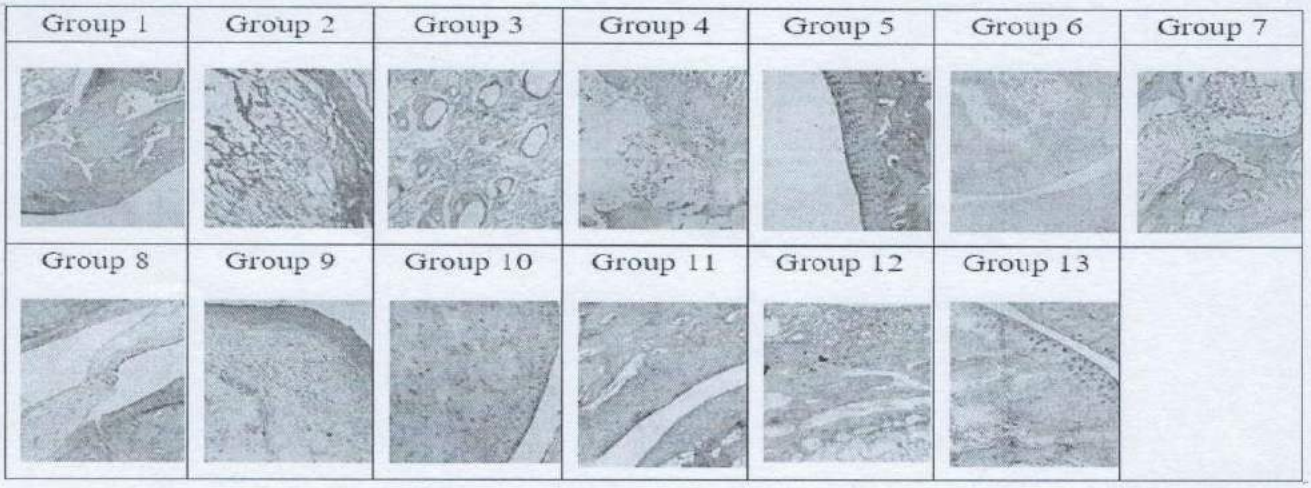

Figure 2: Histopathology of rat joints after treated with different drugs.

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Analyst software version 1.6.3, data collecting and processing for quantification were carried out (AB SCIEX). By employing a Harvard infusion pump (Harvard Apparatus, Holliston, USA) linked directly to the mass spectrometer and infusing a 500 ng/mL solution in water: methanol (50:50 *v/v*) at 10 µL/minute flow rate, the mass spectrometric settings were tuned for the substances. By using a 0.9 mL/minute flow rate of mobile phase without a column, flow injection analysis (FIA) was used to optimised the flow-dependent source parameters. The following optimised settings were used to run the Turbo V source with the ESI (Electro spray ionisation) probe: Positive polarity, 30 pressure for the curtain gas, 70 psi for the nebulizer gas, 55 psi for the heater gas, 5500 volts for the ion spray, and 550°C for the source temperature. The mass spectrometry was run in the MRM mode, which fixes both the parent ion and the fragment ion. The optimal declustering potential (DP) and collision energy (CE) for e Stigmasterol parent and fragment ions were 100 V and 5 V, respectively. For metoprolol, the parent and fragment ions' *m/z* values were 268.10 and 116.3, with DP and CE, respectively, of 110 V and 25 V (Figure 3).

For Rutin and Quercetin TRIPLE QUQD 5500+ was used. MRM for LC-MS/MS operated in positive ion mode for Quercetin and negative ion mode for rutin analysis. The LC system utilised was the Sciex Exion LC, which included a vacuum degasser, two isocratic pumps, an AD Autosampler multiplate with a temperature control at 4°C, and a temperature control thermostatic column oven with at 40°C (AD column oven. Exion, Sciex). The 50×4.6 mm Kinetex EVO, C_{18} (Phenomenex, US) was used as stationary phase used for the chromatography with 5 µm particle size. The mobile phase consisted of 10mM Ammonium Acetae with 0.1% Formic acid in Milli Q as aqueous reservoir and (100%) methanol (organic modifier) at flow rate of 1 mL/minute was used. A programme with common reverse phase gradient (time (min) / % B= 0.00/5, 1/95, 2.50/95, 2.6/5 and 11.00/5) was used for Quercetin and % B= 0.00/60, 1/99, 2.50/99, 2.6/99 and 3/60) for Rutin with short run time of 3 min. Using Analyst software version 1.6.3, data collecting and processing for quantification were carried out (AB SCIEX). By employing a Harvard infusion pump (Harvard Apparatus, Holliston, USA) linked directly to the mass spectrometer and infusing a 500 ng/mL solution in water: methanol (50:50, v/v) at 10 µL/minute flow rate, the mass spectrometric settings were tuned for the substances. By using flow injection analysis (FIA) and a 1 mL/min flow rate of mobile phase without a column, flow dependant source characteristics were optimised. The Turbo V source with the ESI (Electro spray ionisation) probe was run for quercetin with

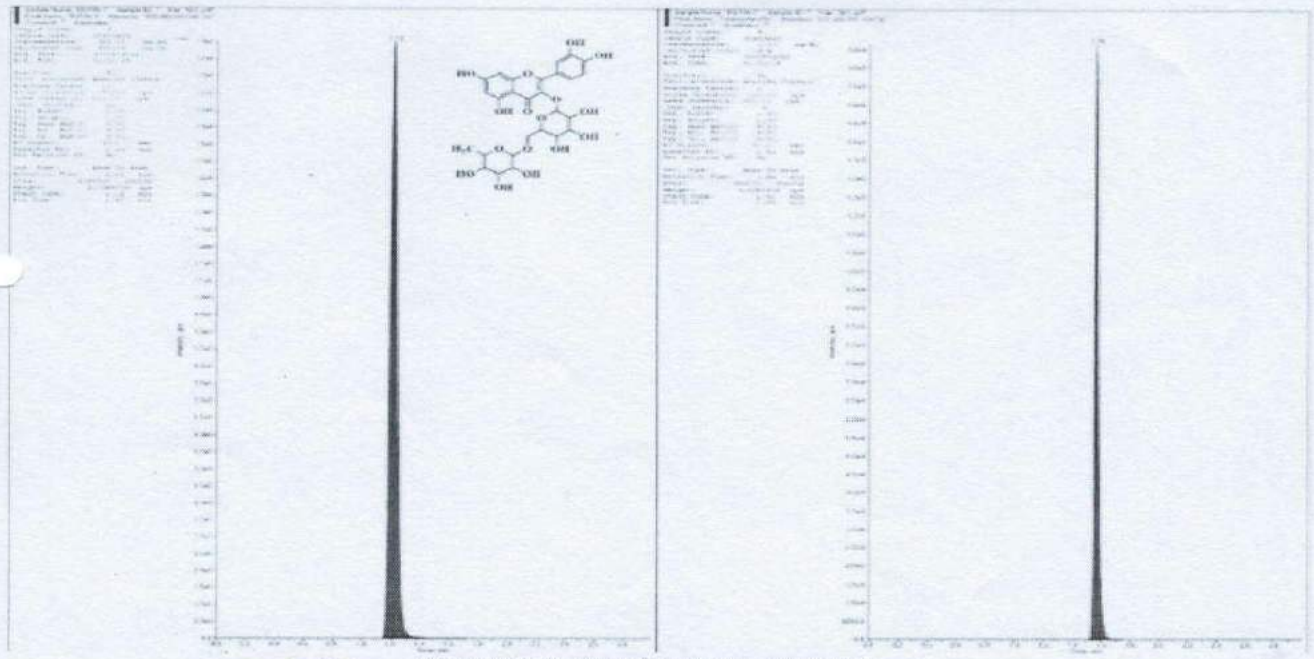

Figure 3a: LC-MS picture of Standard compound Rutin.

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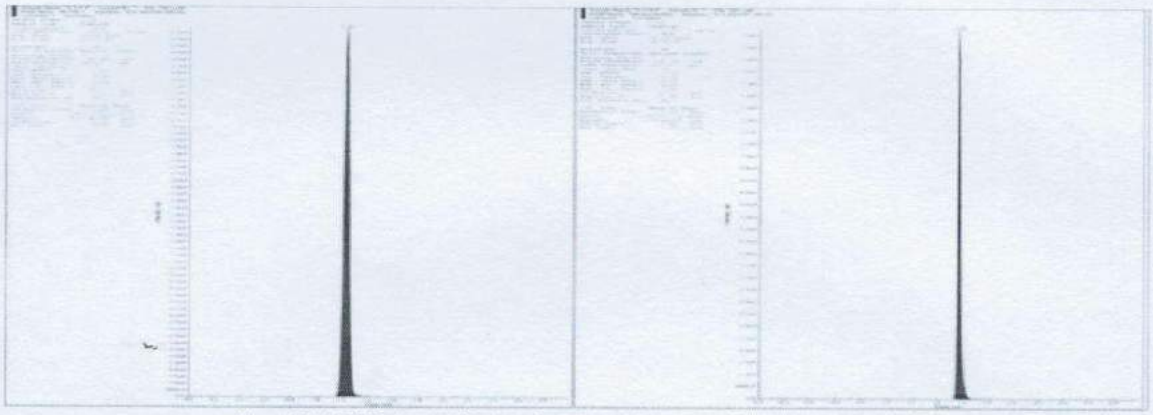

Figure 3b: LC-MS picture of Rutin in Petroleum ether extract of *Asparagus officinalis*.

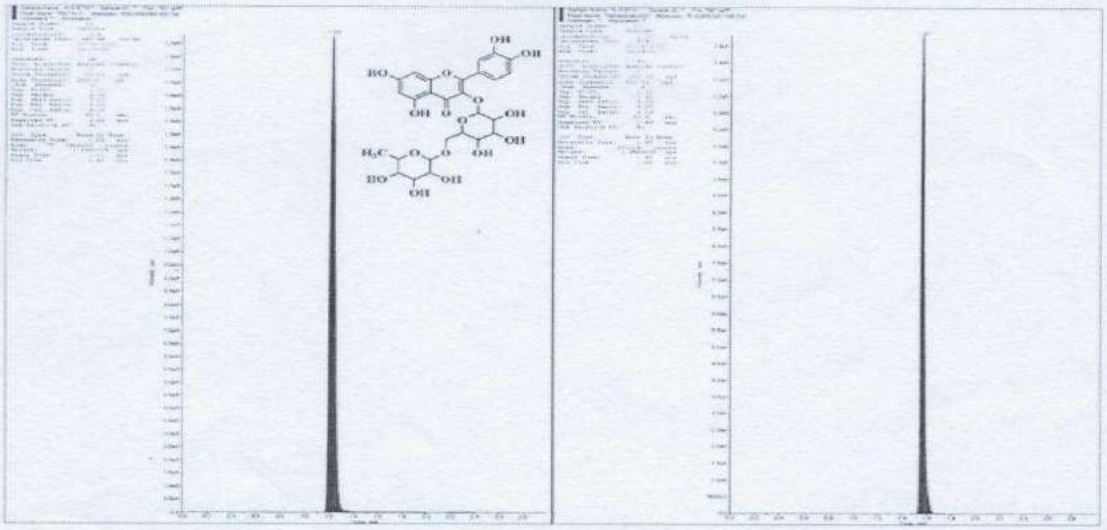

Figure 3c: LC-MS picture of Rutin in ethanolic extract of Asparagus officinalis.

the following optimised settings: Positive polarity, 45 pressure for the curtain gas, 60 psi for the heater gas, 55 psi for the nebulizer gas, 550°C for the source temperature and 5500 volts for the ion spray. The mass spectrometry was run in the MRM mode, which fixes both the parent ion and the fragment ion. The parent and fragment ions of quercetin were measured at *m/z* values of 303.20 and 229.00, respectively, with an ideal collision energy (CE) and declustering potential (DP) of 37 V and 77 V. The *m/z* values for parent and fragment ions for telmisartan were 515.30 and 276.00, with CE and DP of 50 V and 10 V, respectively.

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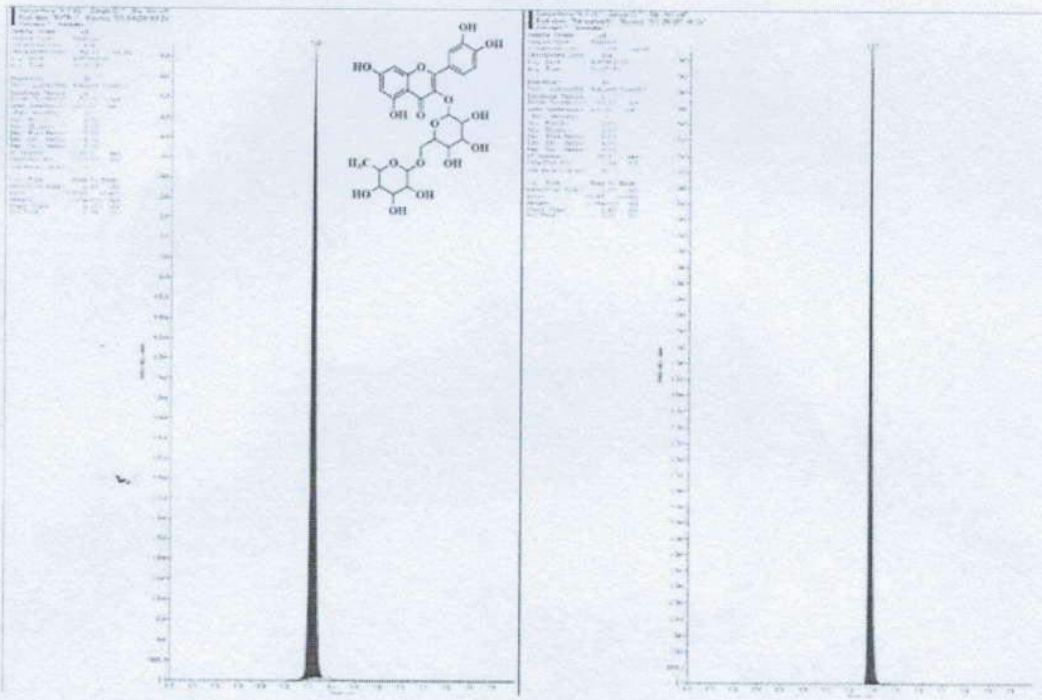
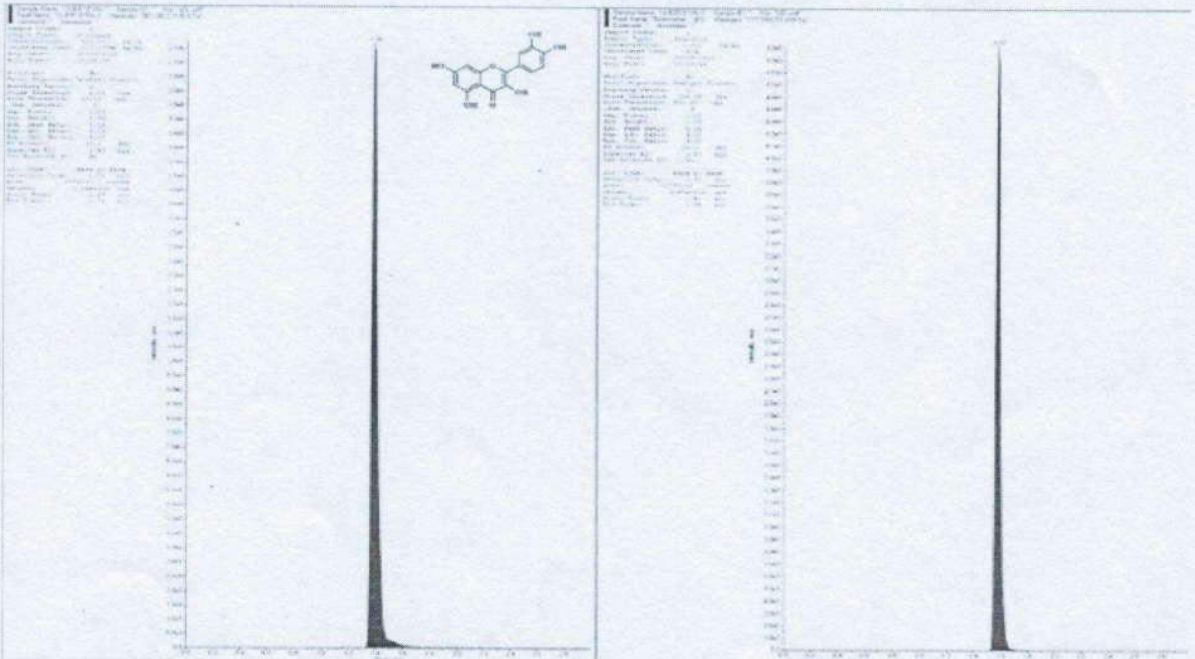


Figure 3d: LC-MS picture of Rutin in Aqueous extract of *Asparagus officinalis*.



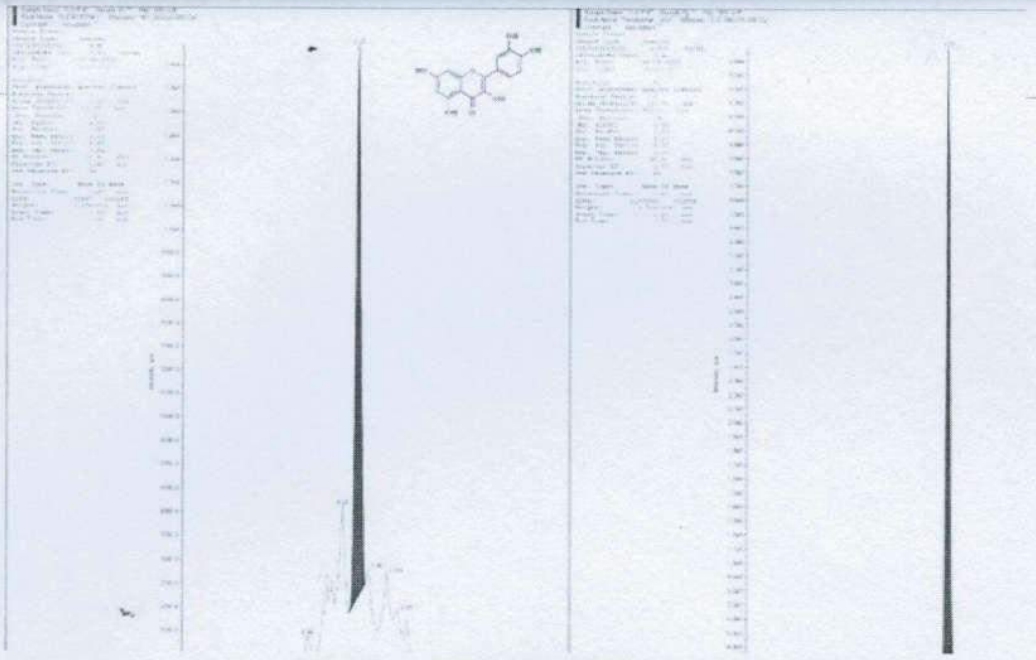


Figure 3f: LC-MS picture of Quercetin in Petroleum ether extract of *Asparagus officinalis*.

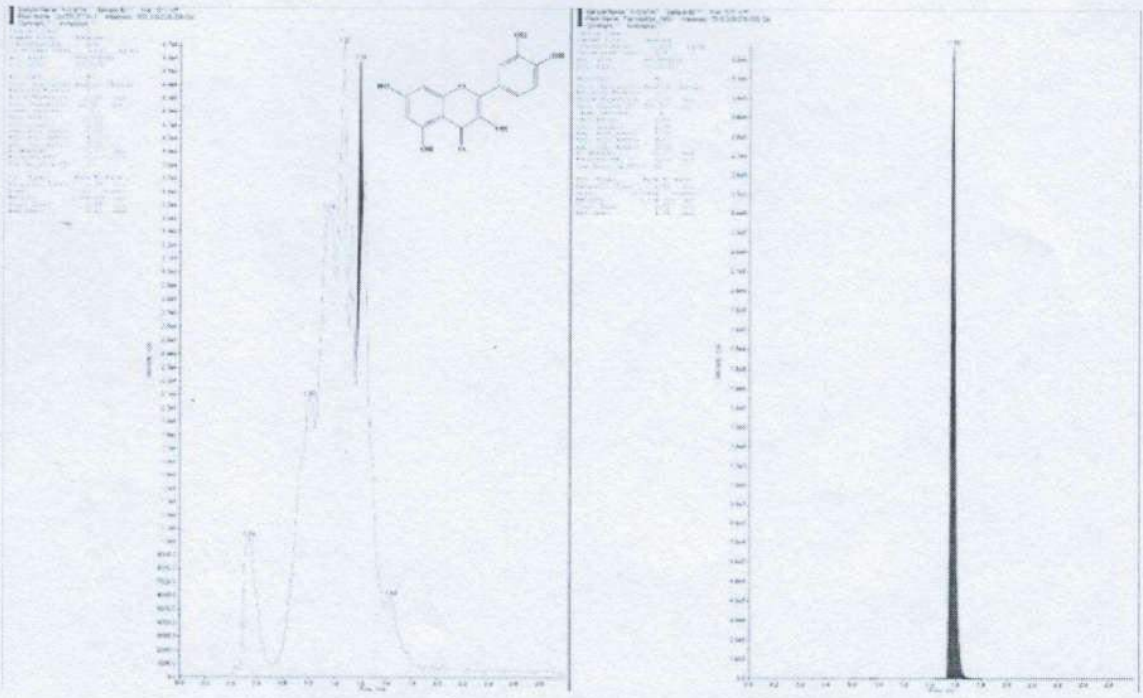


Figure 3g: LC-MS picture of Quercetin in Ethanolic extract of *Asparagus officinalis*.

potential (DP) were -49V and -122 V. For telmisartan, the error. $^{a}P<0.0001$, $^{b}P<0.001$, $^{c}P<0.05$ was considered statistically

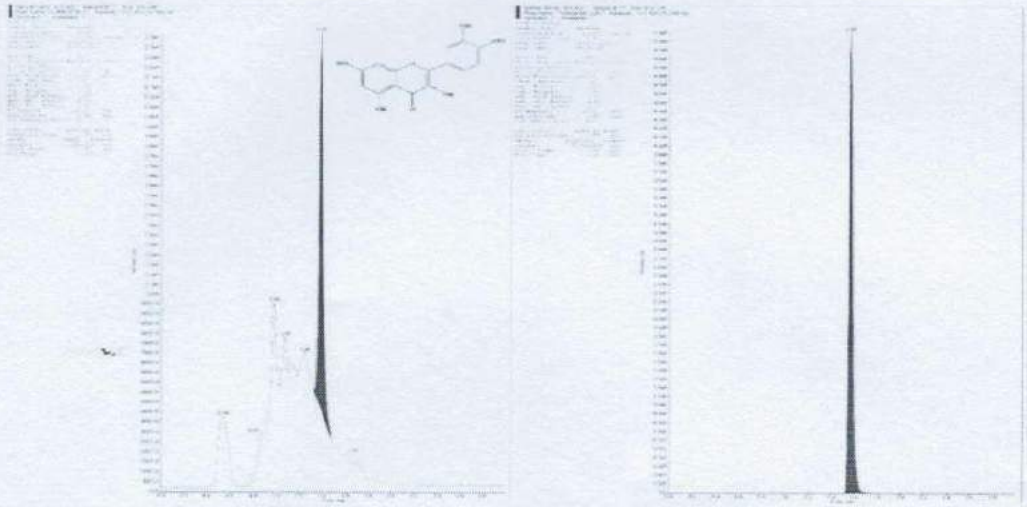


Figure 3h: LC-MS picture of Quercetin in aqueous extract of *Asparagus officinalis*.

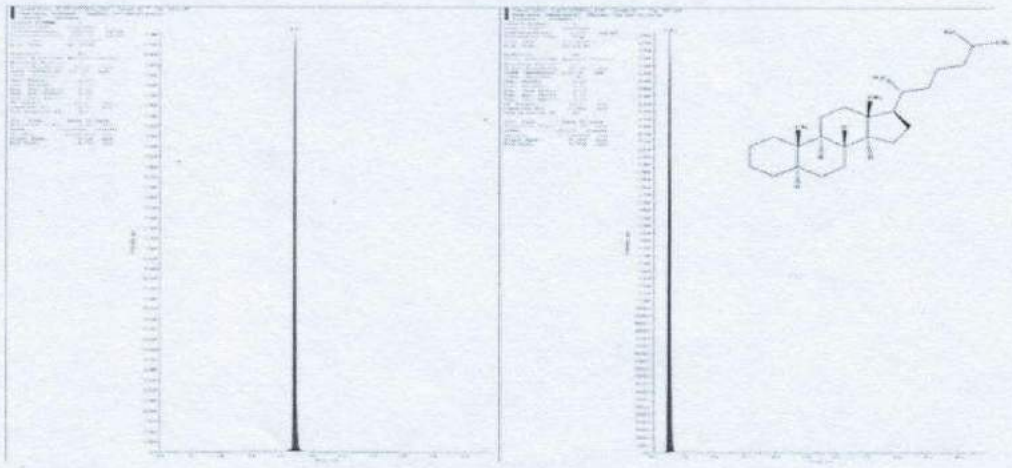


Figure 3i: LC-MS picture of Standard compound *β-sitosterol*.

Analgesic Activity

In this study, highly significant ($p<0.0001$) effect was found at 120 min with dose of 300 mg/kg of petroleum ether extract (83.49%), followed by aqueous extract (80.5%) and ethanolic extract (78.22%). Moderate analgesic effect noted with ethanolic extract, aqueous extract and petroleum ether extracts at 75 mg/kg and 150 mg/kg. Response time was increased 124% to 204% with diclofenac sodium (Table 1).

Anti-inflammatory activity

The results (Table 2) were found extremely statistical ($p<0.0001$) significant difference with all extracts at a higher dose from 2hr

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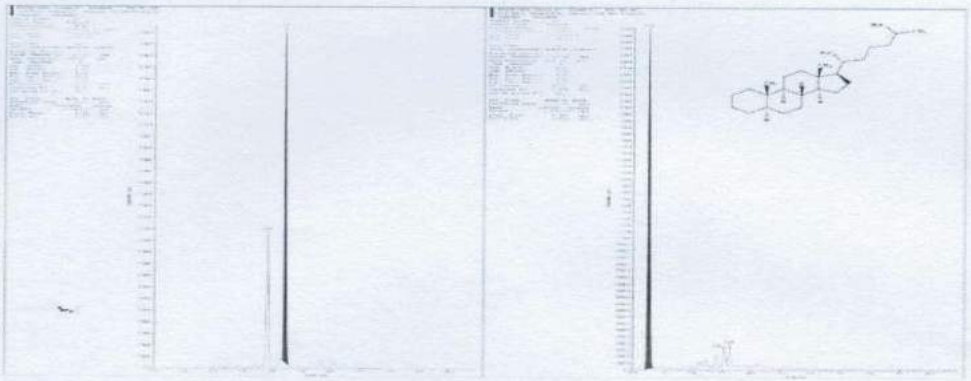

Figure 3j: LC-MS picture of β-sitosterol in pet ether extract of *Asparagus officinalis*.

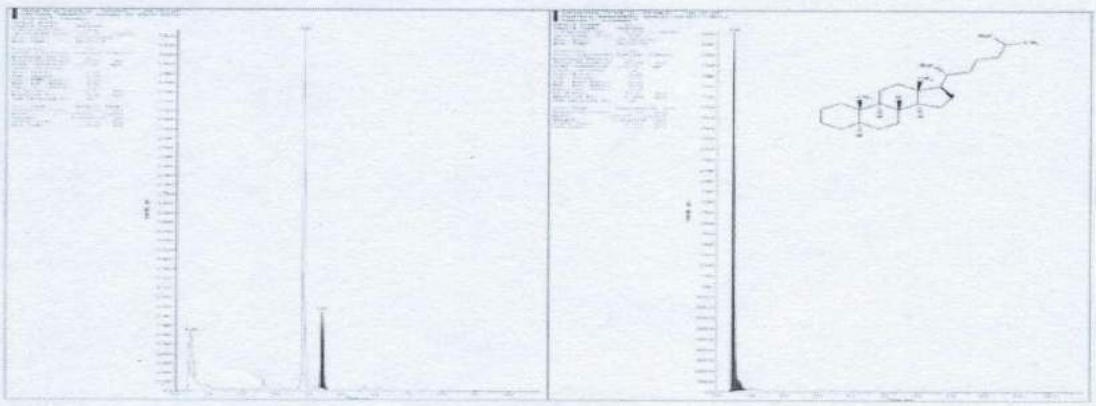

Figure 3k: LC-MS picture of β-sitosterol in ethanolic extract of *Asparagus officinalis*.

to 6 hr and less significant effect at lower doses also. Diclofenac sodium treated group showed 17.33% to 21.53% inhibition and petroleum ether extract was observed with the highest significant range of 13.14% to 22.95% inhibition followed by aqueous extract 9.90% to 20.74% inhibition and at last ethanolic extract was observed with a significant range of 11.17% to 20.46% inhibition.

Anti-arthritic Activity

Effect on body weight

Body weight of positive control group was decreased at different time intervals in comparison with normal control group. Higher dose of all three different extracts showed slightly changes in the body weights as compared to positive control group at 15th day

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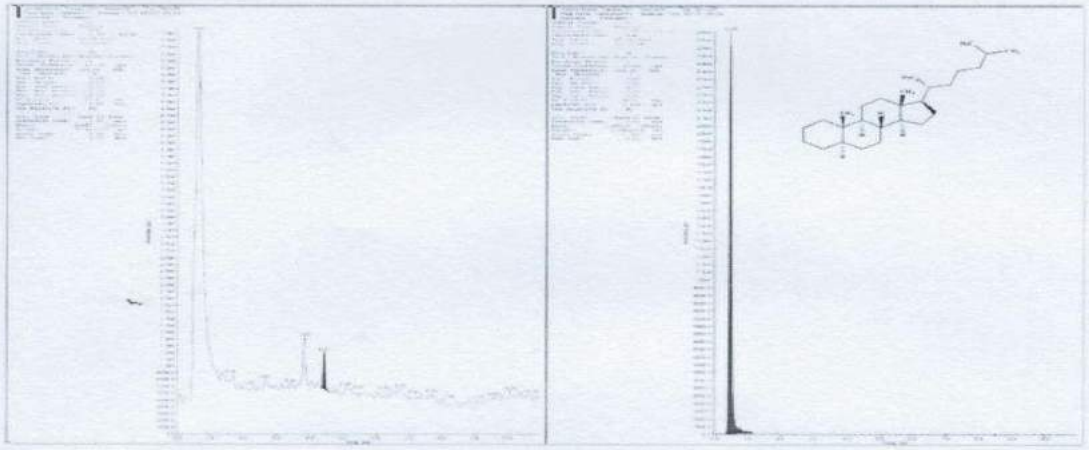

Figure 3l: LC-MS picture of β-sitosterol in aqueous extract of *Asparagus officinalis*.

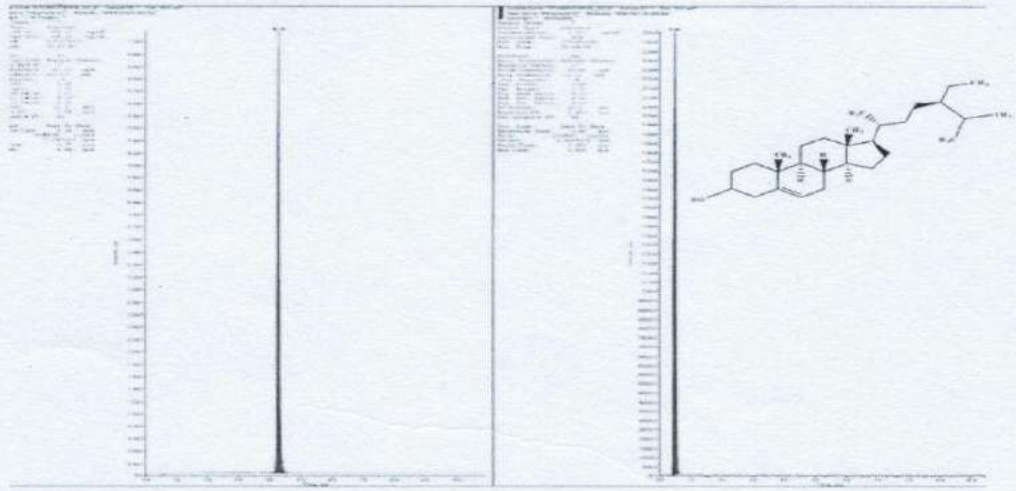

Figure 3m: Stigmasterol: Standard.

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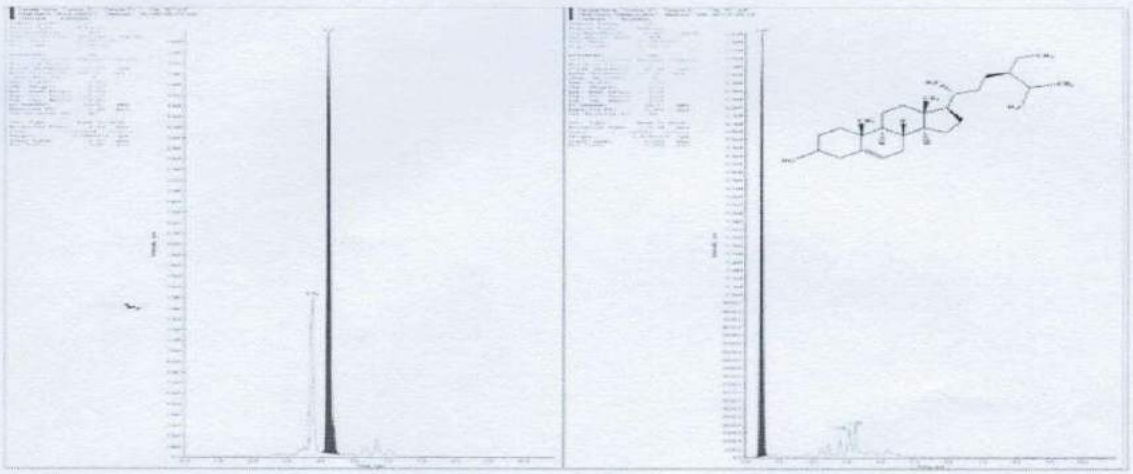

Figure 3n: Stigmasterol: *Asparagus officinalis*, petroleum ether extract.

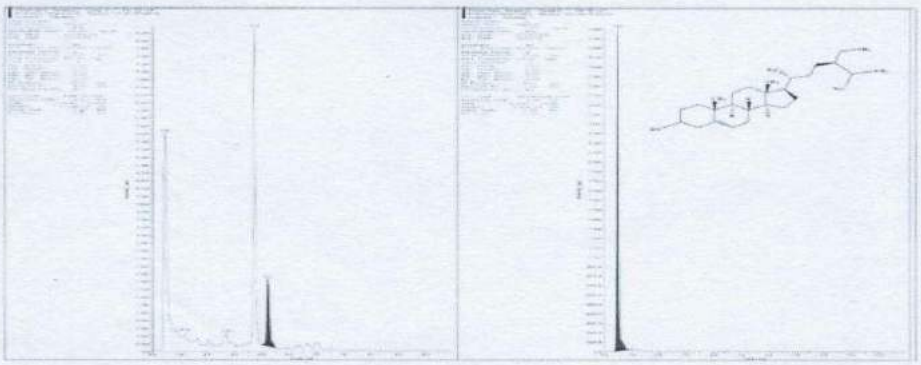

Figure 3o: Stigmasterol: *Asparagus officinalis*, ethanolic extract.

and 21st day but there was no statistical significant difference. Standard treated group (Methotrexate and Prednisolone) were able to keep the animal body weight in normal range. It was increased from 3.71% to 7.39% with petroleum ether extract and in aqueous extract treated group, it was noted from 0.79% to 14.76% (Table 3).

Effect on paw oedema

From 14th day and 21st day, the percentage of inflammation inhibition was found increase and statistically significant difference at all three doses of all three different extracts as compared with a positive control group. It was observed that

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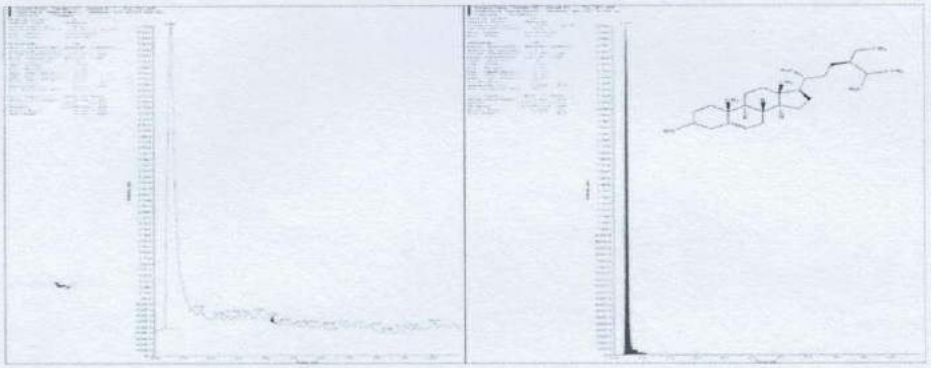

Figure 3p: Stigmasterol: *Asparagus officinalis*, aqueous extract.

Table 1: Effect of *Asparagus officinalis* extracts in analgesic activity on rats.

Groups ($n = 6$)	0 min (s)	After 30 min (s)	After 60 min (s)	After 90 min (s)	After 120 min (s)	After 180 min (s)
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	1.84 ± 0.35	1.76 ± 0.42	1.87 ± 0.38	1.85 ± 0.38	1.93 ± 0.24	1.62 ± 0.42
Group 2 (Diclofenac sodium, 10 mg/kg)	1.69 ± 0.18	1.84 ± 0.19 (4.74)	2.71 ± 0.52 [c] (45.05)	4.54 ± 0.37 [a] (145.76)	5.84 ± 0.44 [a] (203.29)	3.63 ± 0.42 [a] (124.97)
Group 3 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	1.73 ± 0.38	1.83 ± 0.32 (3.79)	1.86 ± 0.40 (-0.62)	2.09 ± 0.24 (13.18)	2.21 ± 0.41 (14.62)	1.91 ± 0.18 (18.27)
Group 4 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	1.66 ± 0.23	1.99 ± 0.18 (13.08)	2.13 ± 0.19 (14.18)	2.17 ± 0.49 (17.33)	2.51 ± 0.32 [c] (30.19)	2.60 ± 0.34 [a] (60.68)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	1.73 ± 0.25	1.73 ± 0.22 (-1.71)	1.96 ± 0.21 (4.91)	2.08 ± 0.19 (12.55)	2.54 ± 0.32 [c] (31.66)	2.96 ± 0.46 [a] (83.49)
Group 6 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	1.76 ± 0.23	1.79 ± 0.10 (1.80)	1.95 ± 0.38 (4.19)	2.20 ± 0.18 (19.13)	2.16 ± 0.24 (12.11)	2.23 ± 0.35 [c] (37.87)
Group 7 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	1.74 ± 0.34	1.91 ± 0.14 (8.34)	2.07 ± 0.41 (10.97)	2.40 ± 0.26 [c] (29.78)	2.29 ± 0.21 (18.60)	2.66 ± 0.37 [a] (64.60)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	1.77 ± 0.34	2.02 ± 0.16 (14.88)	2.19 ± 0.19 (16.95)	2.19 ± 0.25 (18.50)	2.35 ± 0.28 (21.80)	2.88 ± 0.26 [a] (78.22)
Group 9 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	1.70 ± 0.22	1.94 ± 0.19 (10.33)	1.97 ± 0.50 (5.26)	2.04 ± 0.27 (10.65)	2.17 ± 0.39 (17.82)	2.36 ± 0.27 [c] (46.23)
Group 10 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	1.67 ± 0.32	1.95 ± 0.24 (11.00)	2.00 ± 0.17 (6.87)	2.19 ± 0.18 (18.32)	2.27 ± 0.20 (17.99)	2.60 ± 0.23 [a] (61.20)
Group 11 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	1.74 ± 0.17	1.86 ± 0.14 (5.97)	2.08 ± 0.35 (11.42)	2.57 ± 0.41 [c] (39.08)	2.43 ± 0.28 (25.87)	2.92 ± 0.19 [a] (80.50)
F Value	0.1938	1.056	2.598	34.03	71.89	16.49
P Value	0.9960	0.4112	0.0117	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6). [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

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Table 2: Effect of *Asparagus officinalis* extracts in Acute Inflammation (Using plethysmometer) activity on rats.

Groups ($n = 6$)	Pre-Induction hour	1st hour	2nd hour	3rd hour	4th hour	6th hour
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.690 ± 0.023 (-2.73)	0.695 ± 0.019 (1.65)	0.672 ± 0.015 [a] (23.24)	0.667 ± 0.022 (26.74)[a]	0.663 ± 0.023 (29.18)[a]	0.663 ± 0.019 [a] (23.90)
Group 2 (Carrageenan induced Inflammation Control)	0.672 ± 0.028	0.707 ± 0.022	0.875 ± 0.019	0.910 ± 0.011	0.937 ± 0.022	0.872 ± 0.028
Group 3 (Diclofenac sodium 10 mg/kg)	0.682 ± 0.023 (-1.49)	0.705 ± 0.018 (0.24)	0.723 ± 0.016 [a] (17.33)	0.752 ± 0.038 [a] (17.40)	0.735 ± 0.019 [a] (21.53)	0.702 ± 0.017 [a] (19.50)
Group 4 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.678 ± 0.033 (-0.99)	0.707 ± 0.010 (0.00)	0.732 ± 0.026 [a] (16.38)	0.768 ± 0.029 [a] (15.57)	0.753 ± 0.024 [a] (19.57)	0.737 ± 0.019 [a] (15.49)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.682 ± 0.029 (-1.49)	0.713 ± 0.020 (-0.94)	0.760 ± 0.011 [a] (13.14)	0.785 ± 0.019 [a] (13.74)	0.740 ± 0.023 [a] (21.00)	0.717 ± 0.016 [a] (17.78)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.675 ± 0.027 (-0.50)	0.715 ± 0.005 (-1.18)	0.742 ± 0.015 [a] (15.24)	0.798 ± 0.042 [a] (12.27)	0.722 ± 0.029 [a] (22.95)	0.733 ± 0.022 [a] (15.87)
Group 7 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.667 ± 0.027 (0.74)	0.702 ± 0.015 (0.71)	0.743 ± 0.024 [a] (15.05)	0.778 ± 0.033 [a] (14.47)	0.745 ± 0.018 [a] (20.46)	0.713 ± 0.022 [a] (18.16)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.678 ± 0.019 (-0.99)	0.715 ± 0.016 (-1.18)	0.740 ± 0.021 [a] (15.43)	0.788 ± 0.023 [a] (13.37)	0.748 ± 0.015 [a] (20.11)	0.725 ± 0.019 [a] (16.83)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.668 ± 0.029 (0.50)	0.713 ± 0.022 (-0.94)	0.757 ± 0.029 [a] (13.52)	0.808 ± 0.012 [a] (11.17)	0.752 ± 0.015 [a] (19.75)	0.732 ± 0.020 [a] (16.06)
Group 10 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.668 ± 0.025 (0.50)	0.713 ± 0.012 (-0.94)	0.755 ± 0.019 [a] (13.71)	0.793 ± 0.016 [a] (12.82)	0.773 ± 0.016 [a] (17.44)	0.715 ± 0.019 [a] (17.97)
Group 11 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.672 ± 0.031 (0.50)	0.718 ± 0.020 (-1.65)	0.740 ± 0.014 [a] (15.43)	0.753 ± 0.015 [a] (17.22)	0.743 ± 0.012 [a] (20.74)	0.702 ± 0.018 [a] (19.50)
Group 12 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.675 ± 0.029 (-0.50)	0.723 ± 0.018 (-2.36)	0.788 ± 0.017 [a] (9.90)	0.818 ± 0.017 [a] (10.07)	0.782 ± 0.019 [a] (16.55)	0.715 ± 0.015 [a] (17.97)
F Value	0.3819	1.186	35.30	29.37	60.09	37.85
P Value	0.9582	0.3162	<0.0001	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6). [a] $P<0.0001$, [b] $P<0.001$, [c] $P<0.05$ compared with negative control group. ns: non-significant.

aqueous, ethanolic and petroleum ether extracts have statistically high significant anti-arthritic effects from day 14th to 21st day at all doses. Methotrexate showed 28.33% inhibition of oedema, Prednisolone showed 32.18% inhibition of oedema. Aqueous extract was observed with highest significant range of 16.71% to 29.66% inhibition followed by ethanolic extract 16.19% to 26.34% followed by petroleum ether extract 11.41 to 23.95% inhibition (Table 4).

Effect on Paw diameter (Using Vernier caliper)

It was observed that aqueous, ethanolic and petroleum ether extracts have statistically high significant anti-arthritic effects from day 14th to 21st day at all doses. Methotrexate showed 30.78% inhibition of oedema, Prednisolone showed 32.97% inhibition of oedema. The aqueous extract was observed with highest significant ($p<0.0001$) range of 24.45 to 34.21% inhibition followed by ethanolic extract 22.19 to 29.91% followed by petroleum ether extract 18.69 to 26.87% inhibition (Table 5).

Effect on arthritis symptoms (Using Visual Scoring)

It was observed that aqueous, alcoholic and petroleum ether extracts have statistically high significant anti-arthritic effect from day 14 to day 21 at all doses. Visual scorings were given based on walking, inflammation, joint deformities and pain

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Table 3: Effect of *Asparagus officinalis* extracts on body weights of Arthritic animals.

Groups ($n = 6$)	Day 1	Day 7	Day 15	Day 21
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	128.96±10.72 (-0.03)	179.67±10.99 (-5.46)	244.19±13.66 (4.25)	299.30±12.00 [c] (9.74)
Group 2 (CFA induced, Arthritic Control)	128.99±10.20	190.05±13.41	234.24±13.40	272.74±8.44
Group 3 (Methotrexate, 0.5 mg/kg)	129.10±9.75 (0.08)	181.75±12.26 (-4.37)	236.55±15.15 (0.98)	294.53±12.33 (7.99)
Group 4 (Prednisolone, 5 mg/kg)	129.43±9.06 (0.34)	182.93±9.67 (-3.75)	236.80±10.34 (1.09)	286.80±12.49 (5.16)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	129.70±6.97 (0.55)	189.53±7.84 (-0.27)	242.50±10.91 (3.53)	292.88±12.89 (7.39)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	129.71±7.00 (0.56)	188.57±8.57 (-0.77)	238.25±15.54 (1.71)	282.87±20.50 (3.71)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	129.68±6.96 (0.53)	187.54±10.12 (-1.32)	234.47±17.94 (0.10)	292.39±18.28 (7.21)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	129.75±6.83 (0.59)	189.76±11.09 (-0.15)	240.12±20.13 (2.51)	289.28±20.00 (6.07)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	129.77±6.83 (0.60)	192.10±5.46 (1.08)	243.53±6.39 (3.97)	299.04±9.20 [c] (9.65)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	129.73±6.89 (0.57)	189.80±8.50 (-0.13)	242.64±11.59 (3.59)	289.56±14.32 (6.17)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	129.76±6.84 (0.59)	177.73±11.82 (-6.48)	230.76±15.92 (1.48)	272.39±22.40 (0.13)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	129.84±6.95 (0.66)	199.14±6.05 (4.79)	254.41±10.06 [c] (8.61)	294.82±18.86 (8.10)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	129.90±6.97 (0.71)	190.37±8.10 (0.17)	240.08±13.13 (2.49)	312.99±11.16 [b] (14.76)
F Value	0.01032	2.070	1.109	2.957
P Value	>0.9999	0.0315	0.3687	0.0025

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6). [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Assessment using Grimace scale (0= Normal to 5= Severe). Methotrexate showed 51.85% recovery, Prednisolone showed 81.48% recovery. Aqueous extract was observed with highest significant (p<0.0001) range of 18.52 to 66.67% recovery followed by ethanolic extract 14.81 to 55.56% recovery followed by petroleum ether extract22.22 to 48.15% recovery of arthritic symptoms (Table 6).

Effect on Spleen weight

It is very common during arthritis spleen weight increases. It was observed that aqueous, alcoholic and petroleum ether extracts were able to statistically normalise the spleen weight which shows the anti-arthritic effect. Methotrexate showed 18.28% recovery, Prednisolone showed 43.38% recovery. Ethanolic extract was observed with highest significant(p<0.0001) range of 22.09 to 32.05% recovery followed by aqueous extract 17.37 to 26.94% recovery followed by Petroleum ether extract10.30 to 23.59% recovery of arthritic symptoms (Table 7).

Effect on RA Factor

An immune system protein called rheumatoid factor protein has the ability to assault the body's healthy tissues. The most typical association between elevated blood levels of rheumatoid factor and autoimmune illnesses. It was observed that aqueous, alcoholic and petroleum ether extracts were able to statistically decrease the RA factor value in arthritic animals. Methotrexate showed 38.43% recovery, Prednisolone showed 49.32% recovery. Ethanolic extract was observed with highest significant (p<0.0001) range of 30.11 to 63.99% recovery followed by aqueous extract 23.60 to 57.34% recovery followed by Petroleum ether extract 36.31 to 55.98% recovery of RA factor (Table 8).

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Table 4: Effect of *Asparagus officinalis* extracts in Arthritis (Using plethysmometer) activity on rats.

Groups ($n = 6$)	1st Day	7th Day	14th Day	21st Day
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.74 ± 0.02	0.94 ± 0.02 [a] (49.09)	1.26 ± 0.06 [a] (48.11)	1.50 ± 0.09 [a] (40.41)
Group 2 (CFA induced, Arthritic Control)	0.75 ± 0.03	1.84 ± 0.03	2.42 ± 0.33	2.51 ± 0.26
Group 3 (Methotrexate, 0.5 mg/kg)	0.74 ± 0.05	1.84 ± 0.05 (0.09)	1.92 ± 0.17 [a] (20.65)	1.80 ± 0.16 [a] (28.33)
Group 4 (Prednisolone, 5 mg/kg)	0.73 ± 0.01	1.82 ± 0.10 (0.73)	1.77 ± 0.12 [a] (26.77)	1.70 ± 0.15 [a] (32.18)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.73 ± 0.02	1.82 ± 0.04 (1.13)	2.20 ± 0.07 (9.36)	2.23 ± 0.03 [c] (11.41)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.73 ± 0.02	1.84 ± 0.03 (0.00)	2.02 ± 0.08 [a] (16.66)	1.91 ± 0.08 [a] (23.95)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.74 ± 0.02	1.83 ± 0.02 (0.54)	1.98 ± 0.09 [a] (18.44)	1.92 ± 0.12 [a] (23.56)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.74 ± 0.02	1.82 ± 0.03 (1.18)	2.22 ± 0.16 (8.40)	2.08 ± 0.11 [a] (17.05)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.75 ± 0.02	1.84 ± 0.02 (0.09)	2.10 ± 0.11 [b] (13.42)	2.12 ± 0.19 [a] (16.19)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.74 ± 0.03	1.80 ± 0.03 (1.91)	1.87 ± 0.06 [a] (22.64)	1.85 ± 0.08 [a] (26.34)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.74 ± 0.02	1.83 ± 0.04 (0.36)	2.17 ± 0.07 [c] (10.25)	2.09 ± 0.10 [a] (16.72)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.73 ± 0.03	1.84 ± 0.04 (0.00)	2.03 ± 0.15 [a] (16.10)	1.94 ± 0.17 [a] (22.89)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.73 ± 0.02	1.83 ± 0.04 (0.27)	1.86 ± 0.09 [a] (23.33)	1.77 ± 0.09 [a] (29.66)
F Value	0.4824	204.9	24.41	20.68
P Value	0.9180	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Effect on WBC's in Arthritic animals

As rheumatic arthritis is autoimmune diseases WBC's count increase is very common observation. The most common link between elevated WBC numbers is autoimmune disorders. It was observed that aqueous, alcohol and petroleum ether extracts were able to statistically decrease the WBCs value in arthritic animals. Methotrexate showed 43.32%, Prednisolone showed 65.21% decrease in WBC's count. The ethanol extract was observed with highest significant (p<0.0001) range of 52.07 to 77.19% decrease followed by petroleum ether extract41.94 to 68.43% recovery followed by aqueous extract 43.09 to 64.98% decrease in WBC's count (Table 9).

Effect on Joint Diameter in X-Ray

X-ray is good tool to see the deformities in bones and tissues around the same. It was observed that aqueous, alcohol and petroleum ether extracts were able to statistically cure the arthritis. Methotrexate showed 18.52%, Prednisolone showed 18.86% decrease joint diameter. Aqueous extract was observed with highest significant (p<0.0001) 8.13 to 22.36% decrease followed by ethanolic extract 13.53 to 18.34% decrease followed by petroleum ether extract 9.87 to 17.15% decrease joint diameter (Table 10, Figure 1).

Histopathological Study

A histological investigation of the joints after CFA injection is shown in Figure 2. The joint architecture appears normal in the non-arthritic control group; The synovial membrane, a sizable joint gap, and articular cartilage as well as bone are all destroyed in the arthritic control group. On the other hand, treatments using aqueous, ethanol, and petroleum ether extracts of *Asparagus officinalis* stopped the degeneration of the articular architecture in the treated animals. The healthy control knee joint underwent a histological investigation and showed no signs of bone erosion, inflammation, cartilage degeneration, or cellular infiltration. The

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Table 5: Effect of *Asparagus officinalis* extracts in Arthritis (Using Vernier calliper) activity on rats.

Groups ($n = 6$)	1st Day (mm)	7th Day (mm)	10th Day (mm)	15th Day (mm)	20th Day (mm)
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	4.33 ± 0.23	4.45 ± 0.26 [a] (48.32)	5.15 ± 0.40 [a] (48.27)	5.37 ± 0.21 [a] (46.14)	5.54 ± 0. 13 [a] (43.82)
Group 2 (CFA induced, Arthritic Control)	4.29 ± 0.12	7.84 ± 0.87	9.95 ± 0.59	9.98 ± 0.27	9.86 ± 0.40
Group 3 (Methotrexate, 0.5 mg/kg)	4.31 ± 0.28	7.48 ± 0.44 (4.65)	8.14 ± 0.82 [a] (18.14)	7.22 ± 0.450 [a] (27.68)	6.83 ± 0.21 [a] (30.78)
Group 4 (Prednisolone, 5 mg/kg)	4.40 ± 0.26	7.55 ± 0.40 (3.76)	7.90 ± 0.24 [a] (20.58)	6.84 ± 0.41 [a] (31.44)	6.61 ± 0.50 [a] (32.97)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	4.17 ± 0.26	7.43 ± 0.33 (5.21)	8.75 ± 0.71 [c] (12.05)	8.24 ± 0.58 [a] (17.46)	8.02 ± 0.44 [a] (18.69)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	4.21 ± 0.07	7.41 ± 0.16 (5.46)	8.65 ± 0.27 [c] (13.06)	7.65 ± 0.58 [a] (23.35)	7.27 ± 0.27 [a] (26.25)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	4.27 ± 0.27	7.48 ± 0.16 (4.59)	8.80 ± 0.78 [c] (11.51)	7.52 ± 0.51 [a] (24.61)	7.21 ± 0.52 [a] (26.87)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	4.22 ± 0.09	7.51 ± 0.49 (4.23)	8.45 ± 0.90 [b] (15.02)	7.52 ± 0.64 [a] (24.66)	7.67 ± 0.42 [a] (22.19)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	4.21 ± 0.10	7.34 ± 0.37 (6.44)	8.86 ± 0.74 [c] (10.93)	7.35 ± 0.42 [a](26.34)	7.41 ± 0.48 [a] (24.84)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	4.22 ± 0.12	7.37 ± 0.32 (5.99)	8.05 ± 0.59 [a] (19.02)	7.11 ± 0.40 [a] (28.75)	6.91 ± 0.34 [a] (29.91)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	4.21 ± 0.15	7.55 ± 0.29 (3.72)	8.26 ± 0.40 [b] (16.98)	7.50 ± 0.39 [a] (24.79)	7.45 ± 0.34 [a](24.45)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	4.19 ± 0.17	7.68 ± 0.52 (2.13)	8.17 ± 0.50 [a] (17.86)	7.55 ± 0.42 [a] (24.29)	7.44 ± 0.59 [a](24.52)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	4.21 ± 0.14	7.43 ± 0.40 (5.27)	8.31 ± 0.63 [a](16.44)	7.44 ± 0.71 [a] (25.41)	6.49 ± 0.33 [a] (34.21)
F Value	0.7459	24.77	18.18	26.36	36.83
P Value	0.7018	<0.0001	0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6). [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

joint architecture of the animals in the arthritis control group displayed cellular infiltration, bone erosion, and cartilage loss; however, animals dosed with different plant extracts (75, 150, and 300 mg/kg), prednisolone (5 mg/kg) and methotrexate (0.5 mg/kg) demonstrated significant joint architecture protection by bone erosion reduction, cartilage destruction reduction, and decrease in cellular infiltration.

Extracts quantification using LC-MS/MS

All three extracts were analysed using LC-MS/MS. Results depicted the presence of rutin, quercetin, β-sitosterol, stigmasterol (Table 11, Figure 3). Highest concentration of rutin was present in the ethanol extract i.e. 650.45 µg/gm whereas in other two extracts showed less than 100ug/gm. In case of quercetin, highest concentration was found in aqueous extract than other two extracts. The concentration of β-sitosterol and stigmasterol were present in higher quantity in pet ether extract and very less in ethanolic extract. Both compounds were absent in aqueous extracts.

DISCUSSION

Arthritis is nothing but the presence of swelling, redness, pain or inflammation in a joint. The word "disease of the joints" in Greek is the origin of the word "arthritis." Acute or persistent joint inflammation is what's known as it, and discomfort and structural damage are frequently present alongside it. Arthralgia, or joint pain without inflammation, can be brought on by disease in the joint itself or in the nearby soft tissues, ligaments, and tendons. Subcutaneous nodules on the surfaces of the extensor muscles and soft tissue edoema around the affected joints are also observed.[29]

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Table 6: Effect of *Asparagus officinalis* extracts in Arthritis (Using Arthritis Visual Score) activity on rats.

Groups ($n = 6$)	1st Day	5th Day	10th Day	15th Day	20th Day
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.0000	0.00 ± 0.00 [a] (100)	0.00 ± 0.00 [a] (100)	0.00 ± 0.00 [a] (100)	0.00 ± 0.00 [a] (100)
Group 2 (CFA induced, Arthritic Control)	0.0000	2.67 ± 0.52	4.50 ± 0.55	4.50 ± 0.55	4.50 ± 0.55
Group 3 (Methotrexate, 0.5 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.33 ± 0.52 (3.70)	2.67 ± 0.52 [a] (40.74)	2.17 ± 0.75 [a] (51.85)
Group 4 (Prednisolone, 5 mg/kg)	0.0000	2.67 ± 0.52 (0.00)	3.50 ± 0.55 [c] (22.22)	2.17 ± 0.41 [a] (51.85)	0.83 ± 0.75 [a] (81.48)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.0000	2.67 ± 0.52 (0.00)	4.67 ± 0.52 (-3.70)	4.17 ± 0.75 (7.41)	3.50 ± 0.55 (22.22)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.67 ± 0.52 (-3.70)	3.33 ± 0.52 [c] (25.93)	2.67 ± 0.52 [a] (40.74)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.67 ± 0.52 (-3.70)	2.83 ± 0.41 [a] (37.04)	2.33 ± 0.82 [a] (48.15)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.0000	2.67 ± 0.52 (0.00)	4.83 ± 0.41 (-7.40)	3.67 ± 0.82 (18.52)	3.83 ± 0.75 (14.81)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.83 ± 0.41 (-7.40)	3.17 ± 0.41 [b] (29.63)	3.50 ± 0.55 (22.22)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.67 ± 0.52 (-3.70)	2.33 ± 0.52 [a] (48.15)	2.00 ± 0.63 [a] (55.56)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.83 ± 0.41 (-7.40)	3.17 ± 0.41 [b] (29.63)	3.67 ± 0.52 (18.52)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.0000	2.83 ± 0.41 (-6.25)	4.50 ± 0.55 (0.00)	3.33 ± 0.52 [c] (25.93)	2.83 ± 0.75 [b] (37.04)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.0000	3.00 ± 0.00 (-12.50)	4.67 ± 0.52 (-3.70)	2.50 ± 0.55 [a] (44.44)	1.50 ± 0.55 [a] (66.67)
F Value		28.27	44.18	26.71	25.19
P Value		<0.0001	<0.0001	<0.0001	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group ($n=6$). [a] $P<0.0001$.

Elevated WBC count represents the change in immunity. A minor to moderate rise in WBC counts is brought on by the production of the IL-Ib inflammatory response during arthritic circumstances. Granulocyte production and macrophage colony-stimulating factor both rise in response to IL-Ib.[1] Same was observed in our study arthritic control (14466 Cells/cumm) was observed with maximum count whereas it was significantly decreased in treated groups (4566.67 to 8400 Cells/cumm).

Rheumatoid factor (RF) was measured in serum collected on the terminal day indicates that as compare to arthritic control (22IU/mL) significant decrease was observed in treatment groups. An immunoglobulin molecule known as serum rheumatoid factor (RF) is regarded as a "non-self" molecule that has the ability to activate the immune system. A major indication of RA in the RA aetiology may be abnormal variations in the blood levels of RF and CRP.[30]

Paw oedema and joint swelling is an indicator of the anti-arthritis activity of various medicines. Determining foot swelling is a simple, sensitive, and rapid way to assess and assess the degree of inflammation.[1] With the help of plethysmometer and vernier calliper oedema and swelling was observed. Treatment groups were observed with significant decrease in swelling as compare to inflammation control group.

Same way X-ray and histopathology suggest the treatment as compare to arthritic group. Radiographic changes in the condition of arthritis are a useful diagnostic tool to get to know the severity of the arthritis. Soft tissue swelling is an early sign of radiology, but marked radiological changes such as bone erosion and narrowing of the joint space can only be observed during development.[31]

Immediately after the completion of experiment, the spleen was removed from the animal and the organ weight was measured. Animal organ weight was calculated. Spleen weight increase is very common symptom during arthritis.[30,32] Systemic autoimmune disorders are marked by an extremely high prevalence and destructiveness of splenic long-lived plasma

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Table 7: Effect of *Asparagus officinalis* extracts in Arthritis Animal Spleen Weight

Groups ($n = 6$)	Spleen Weight (g)
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	0.73 ± 0.08 [c] (19.67)
Group 2 (CFA induced, Arthritic Control)	0.91 ± 0.08
Group 3 (Methotrexate, 0.5 mg/kg)	0.75 ± 0.09 [c] (18.28)
Group 4 (Prednisolone, 5 mg/kg)	0.52 ± 0.08 [a] (43.38)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	0.82 ± 0.09 (10.30)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	0.70 ± 0.14 [c] (23.59)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	0.72 ± 0.06 [c] (20.80)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	0.69 ± 0.11 [c] (25.08)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	0.71 ± 0.09 [c](22.09)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	0.62 ± 0.07 [a] (32.03)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	0.76 ± 0.13 (17.37)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	0.73 ± 0.10 [c] (20.20)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	0.67 ± 0.08 [b] (26.94)
F Value	5.702
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Table 8: Effect of *Asparagus officinalis* extracts on RA Factor in Arthritis.

Groups ($n = 6$)	IU/mL
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	7.60 ± 1.15 [a] (65.51)
Group 2 (CFA induced, Arthritic Control)	22.03 ± 0.59
Group 3 (Methotrexate, 0.5 mg/kg)	13.57 ± 4.19 [b] (38.43)
Group 4 (Prednisolone, 5 mg/kg)	11.17 ± 5.35 [a] (49.32)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	14.03 ± 2.01 [c] (36.31)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	9.70 ± 3.05 [a] (55.98)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	13.63 ± 4.34 [b] (38.12)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	11.23 ± 2.06 [a] (49.02)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	15.40 ± 3.65 [c] (30.11)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	7.93 ± 2.67 [a] (63.99)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	16.83 ± 0.06 (23.60)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	12.93 ± 7.01 [b] (41.30)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	9.40 ± 1.81 [a] (57.34)
F Value	7.776
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

cells. The research has shown that spleen-derived CD11b+Gr-1+ myeloid cells (SDMCs) play a pathogenic role in the deposition of splenic long-lived plasma cells in the sanroque lupus-prone mouse and that SDMCs can be accumulate.[33]

Form qualitative phytochemical assays it is clear that *Asparagus officinalis* is reach in terpenoids and flavonoids. Various routes of action are already proven for mode of action against inflammation and arthritis by both flavonoids and terpenoids. Results of LC/MS proved that the extracts contain higher concentration of rutin, quercetin, β-sitosterol and stigmasterol and the protective effect may be due to the molecular action of these compounds in obstacle of rheumatoid arthritis.

The central effects of the extract in inducing anti- nociception were assessed using tail flick experiments. The tests can also be characterised by their proclivity to respond to pain stimuli delivered via various neural pathways. The tail flick method is thought to be a supraspinally organised reflex and requires higher brain activity. It looks like that flavones extracted from diverse plants, such as quercetin and rutin, produce considerable anti-nociceptive reactions. It's possible that flavonoids are responsible for the extract's analgesic action.[30] The excessive expression of TRPV1 can be inhibited by quercetin, which indicates that quercetin might relieve thermal hyperalgesia.[34]

In the case of rheumatoid arthritis, several inflammatory cytokines, such as TNF, IL6, JAK, and the TNFR1 receptor, were discovered to be actively engaged in the signalling resulting in the inflammatory response that causes the destruction of bones and adjacent tissues of the synovial joints. The inflammatory response that is involved in the course of rheumatoid arthritis can be directly inhibited by disrupting the signalling pathways involved

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Table 9: Effect of *Asparagus officinalis* extracts on WBC's in Arthritis.

Groups ($n = 6$)	Cells/cumm
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	6066.67 ± 1795.36 [a] (58.06)
Group 2 (CFA induced, Arthritic Control)	14466.67 ± 750.56
Group 3 (Methotrexate, 0.5 mg/kg)	8200.00 ± 2271.56 [a] (43.32)
Group 4 (Prednisolone, 5 mg/kg)	5033.33 ± 702.38 [a] (65.21)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	8400.00 ± 3508.56 [a] (41.94)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	4566.67 ± 1553.49 [a] (68.43)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	5333.33 ± 1123.98 [a] (63.13)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	6933.33 ± 2064.78 [a] (52.07)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	6133.33 ± 680.69 [a] (57.60)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	3300.00 ± 173.21 [a] (77.19)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	8233.33 ± 862.17 [a] (43.09)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	5600.00 ± 1135.78 [a] (61.29)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	5066.67 ± 2369.25 [a] (64.98)
F Value	16.02
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Table 10: Effect of *Asparagus officinalis* extracts on Arthritic Joints in X-Ray.

Groups ($n = 6$)	mm
Group 1 (Normal Control, Tween 80 (1%v/v) + 0.5%w/v CMC in Aqueous extract (99%v/v))	6.29 ± 0.35 [a] (38.43)
Group 2 (CFA induced, Arthritic Control)	10.21 ± 0.64
Group 3 (Methotrexate, 0.5 mg/kg)	8.32 ± 0.68 [a] (18.52)
Group 4 (Prednisolone, 5 mg/kg)	8.29 ± 0.51 [a] (18.86)
Group 5 (*Asparagus officinalis*, Petroleum ether extract, 75 mg/kg)	9.21 ± 0.44 [c] (9.87)
Group 6 (*Asparagus officinalis*, Petroleum ether extract, 150 mg/kg)	8.54 ± 0.57 [a] (16.38)
Group 7 (*Asparagus officinalis*, Petroleum ether extract, 300 mg/kg)	8.46 ± 0.20 [a] (17.15)
Group 8 (*Asparagus officinalis*, Ethanolic extract, 75 mg/kg)	9.73 ± 0.42 (4.72)
Group 9 (*Asparagus officinalis*, Ethanolic extract, 150 mg/kg)	8.83 ± 0.37 [a] (13.53)
Group 10 (*Asparagus officinalis*, Ethanolic extract, 300 mg/kg)	8.34 ± 0.40 [a] (18.34)
Group 11 (*Asparagus officinalis*, Aqueous extract, 75 mg/kg)	9.38 ± 0.63 [c] (8.13)
Group 12 (*Asparagus officinalis*, Aqueous extract, 150 mg/kg)	8.32 ± 0.31 [a] (18.51)
Group 13 (*Asparagus officinalis*, Aqueous extract, 300 mg/kg)	7.93 ± 0.49 [a] (22.36)
F Value	23.75
P Value	<0.0001

Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's multiple range test. The values are expressed as mean ± SD for each group (n=6), [a] P<0.0001, [b] P<0.001, [c] P<0.05 compared with negative control group. ns: non-significant.

Table 11: Extracts quantification using LC-MS/MS.

Extracts	Rutin (µg/g)	Quercetin (µg/g)	β-sitosterol (µg/g)	Stigmasterol (µg/g)
Asparagus officinalis, petroleum ether extract	91.07	3.51	941.986	860.932
Asparagus officinalis, ethanolic extract	650.45	3.63	34.36	26.196
Asparagus officinalis, aqueous extract	41.18	4.48	-	-

in the control of the inflammatory response, either by suppressing the aforementioned cytokines or by antagonisting the TNFR1 receptor. As a result, the TNFR1 receptor and the cytokines TNF, IL6, and JAK potentially serve as significant therapeutic targets for the therapy of rheumatoid arthritis.

Flavonoids have anti-inflammatory, analgesic, and antioxidant properties. These effects are associated with the suppression of NF-B-dependent pro-inflammatory cytokines, VEGF, ICAM-1, and STAT3, as well as the activation of the antioxidant transcription factor Nrf2.

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The PI3K/Akt signalling pathway is thought to be a link between FLS cell proliferation and apoptosis. PI3K and Akt are highly expressed in RA-FLS cells and have an impact on FLS cell migration. Furthermore, many inflammatory cytokines, such as IL-17 and IL-21, can endorse FLS cell inflammatory proliferation by provoking and activating PI3K flavonoids, which can inhibit p-Akt expression in B cells.[35] Likewise, flavonoids can restrict the MAPK signalling pathway, thereby reducing RA symptoms.[36] In RA, NF-B and STAT are profoundly and sustainably activated, which flavonoids improve.[37] Triterpene decreases pro-inflammatory cytokines such IL-6, IL-1, TNF-α and increases the anti-inflammatory cytokine IL-10 in rats with arthritis caused by complete Freund's adjuvant (CFA).[38]

According to certain studies, the useful pharmacological effects of terpenes for arthritis are linked to the regulation of many intracellular signalling pathway proteins, including RANKL, the MAPK family, NFkB, PGE-2, COX-2, iNOS, matrix metalloproteinases, MPO, and c –FOS.[39] Angiogenesis is primarily mediated by VEGF/VEGFR2 signalling, which is a key target for anti-angiogenic therapies. When VEGF and VEGFR2 interact, VEGFR2 is phosphorylated on multiple tyrosine residues, triggering signalling cascades that promote EC proliferation, migration, survival, and permeability. Thus, VEGFR2 activation is a critical step in the angiogenesis process, and blocking VEGF/VEGFR2 signalling with VEGFR2 inhibitors can suppress angiogenic responses. β-Sitosterolis has already reported the improvement in RA by VEGF pathway.[40]

By lowering MMP-8 secretion and synthesis, Stigmasterol therapy is said to ameliorate joint-related pathology to levels close to normal. This drop in the levels of metalloproteinase MMP-8 and inflammatory mediators is most likely brought on by a decrease in the activity of the transcription factors MAPKs and NF-kBp65.[41]

CONCLUSION

According to the findings of this study, *Asparagus officinalis* is a plant that contains various chemical component groups that have anti-inflammatory, analgesic and anti-arthritic activities. *In vivo* tests have been done to assess these qualities. These findings support the plant's use in the conventional management of chronic inflammatory illnesses, and also suggest that it may be a candidate for the discovery of novel anti-inflammatory, analgesic and/or anti-arthritic compounds. We have identified the presence of rutin, quercitine, stigmasterol and β- sitosteroid. But during our analysis we observed many other molecular weight peaks which shows the presence of many more ingredient availability. To determine the precise mechanism of action of *Asparagus officinalis* on rheumatoid arthritis, more research can be done.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LC-MS/MS: Liquid Chromatography with tandem mass spectrometry; **µL:** Microliter; **mL:** Millilitre; **ng:** Nanogram; **IL:** Interleukins; **TNF-α:** Tumour necrosis factor α; **COX:** Cyclooxygenase isoenzyme; **CFA:** Complete Freund's adjuvant; **kg:** Kilogram; **mg:** Milligram; **p.o.:** Per oral; **Amps:** Amperes; **DP:** Declustering potential; **CE:** Collision energy; **MRM:** Multiple reaction monitoring; **V:** Volt; **WBC's:** White blood cells; **gm:** Gram; **RF:** Rheumatoid factor; **IU:** International units; **SDMCs:** Spleen-derived CD11b+Gr-1+ myeloid cells; **TRPV:** Transient receptor potential cation channel subfamily V; **VEGF:** Vascular endothelial growth factor; **ICAM:** Intercellular Adhesion Molecule; **MAPK:** Mitogen-activated protein kinases; **RANKL:** Receptor activator of nuclear factor kappa-B ligand; **MPO:** Myeloperoxidase.

SUMMARY

Asparagus officinalis is one of the plant originate from region of South India. The plant has a potential of various pharmacological activities. Traditionally, this plant reported to treat inflammation. Extracts of *Asparagus officinalis* showed anti inflammatory and anti arthritic activity.

Authors' Contribution

All the authors contributed equally for the design of concept, execution of research work and drafting of the manuscript. SG and PS designed the concept, while the execution of studies was performed by SK. All SK, PS and SG equally contributed for the drafting and finalization of the manuscript.

Ethical Approval

This study was approved by the Deemed University Animal and Ethical Committee.

REFERENCES

1. Kshirsagar AD, Panchal PV, Harle UN, Nanda RK, Shaikh HM. Anti-inflammatory and antiarthritic activity of anthraquinone derivatives in rodents. Int J Inflam. 2014;2014:690596. doi: 10.1155/2014/690596, PMID 25610704.
2. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al.* Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204-18. doi: 10.18632/oncotarget.23208, PMID 29467962.
3. Cologne, Germany: Institute for quality and efficiency in health care (IQWiG). p. 2006-; 2010. What is an inflammation? Inform Educ. Health.org; [updated Feb 22 2018] [internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279298.

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4. Yuksel N. Meet Our Editorial Board Member. Curr Drug Deliv. 2017;14(1):1- doi: 10.2174/156720181401170109115332.
5. Yeom MJ, Lee HC, Kim GH, Lee HJ, Shim I, Oh SK, *et al.* Anti-arthritic effects of Ephedra sinica STAPF herb-acupuncture: Inhibition of lipopolysaccharide-induced inflammation and adjuvant-induced polyarthritis. J Pharmacol Sci. 2006;100(1):41-50. doi: 10.1254/jphs.fp0050637, PMID 16404132.
6. Peng S, Hu C, Liu X, Lei L, He G, Xiong C, *et al.* Rhoifolin regulates oxidative stress and proinflammatory cytokine levels in Freund's adjuvant-induced rheumatoid arthritis via inhibition of NF-κB. Braz J Med Biol Res. 2020;53(6):e9489. doi: 10.1590/1414-431x20209489. PMID 32401927, PMCID PMC7233197.
7. Marnett LJ, Rowlinson SW, Goodwin DC, Kalgutkar AS, Lanzo CA. Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. J Biol Chem. 1999;274(33):22903-6. doi: 10.1074/jbc.274.33.22903, PMID 10438452.
8. Giménez-Bastida JA, Shibata T, Uchida K, Schneider C. Roles of 5-lipoxygenase and cyclooxygenase-2 in the biosynthesis of hemiketals E2 and D2 by activated human leukocytes. FASEB J. 2017;31(5):1867-78. doi: 10.1096/fj.201601136R, PMID 28096231.
9. Gou H, Ye J, Wang Y, Xu X, Shen Q, Xue J, *et al.* The anti-inflammatory activity of Toonaciliatin K against adjuvant arthritis. BioMed Res Int. 2017;2017:9436280. doi: 10.1155/2017/9436280, PMID 29181410.
10. Gupta S, Mehla K, Chauhan D, Nair A. Anti-inflammatory activity of leaves of Michelia champaca investigated on acute inflammation induced rats. 2010.
11. Fan SH, Ali NA, Basri DF. Evaluation of Analgesic Activity of the Methanol Extract from the Galls of Quercus infectoria (Olivier) in Rats. Evid Based Complement Alternat Med. 2014;2014:976764. doi: 10.1155/2014/976764. PMID 25254062.
12. Zhang L, Zhu M, Li M, Du Y, Duan S, Huang Y, *et al.* Ginsenoside Rg1 attenuates adjuvant-induced arthritis in rats via modulation of PPAR-γ/NF-κB signal pathway. Oncotarget. 2017;8(33):55384-93. doi: 10.18632/oncotarget.19526, PMID 28903427.
13. Toonen EJM, Laskewitz AJ, Van Dijk TH, Bleeker A, Grefhorst A, Schouten AE, *et al.* Glucose kinetics in the collagen-induced arthritis model: An all-in-one model to assess both efficacy and metabolic side effects of glucocorticoids. PLOS ONE. 2014;9(9):e98684. doi: 10.1371/journal.pone.0098684, PMID 25181348.
14. Kala CP, Dhyani PP, Sajwan BS. Developing the medicinal plants sector in northern India: Challenges and opportunities. J Ethnobiology Ethnomedicine. 2006;2(1):32. doi: 10.1186/1746-4269-2-32.
15. Choudhary M, Kumar V, Malhotra H, Singh S. Medicinal plants with potential anti-arthritic activity. J Intercult Ethnopharmacol. 2015;4(2):147-79. doi: 10.5455/jice.20150313021918, PMID 26401403.
16. Adhikari PP, Paul SB. History of Indian traditional medicine: A medical inheritance. Asian J Pharm Clin Res. 2018;11(1):421-6. doi: 10.22159/ajpcr.2018.v11i1.21893.
17. Tewari D. Traditional medicine an Indian Perspective: History and Outlook. Eur J Mol Clin Med. 2020;07:07.
18. Battu GR, Kumar BM. Anti-inflammatory activity of leaf extract of *Asparagus racemosus* Willd. Int J Chem Sci. 2010;8(2):1329-38.
19. Al-Snafi AE. Arabian medicinal plants with anti-inflammatory effects- plant based review (part 1). IOSR J Pharm. 2018;8(7):55-100.
20. Kim BY, Cui ZG, Lee SR, Kim SJ, Kang HK, Lee YK, *et al.* Effects of *Asparagus officinalis* extracts on liver cell toxicity and ethanol metabolism. J Food Sci. 2009;74(7):H204-8. doi: 10.1111/j.1750-3841.2009.01263.x, PMID 19895471.
21. Khorasani A, Sani W, Philip K, Tah RM, Rafat A. Antioxidant and antibacterial activities of ethanolic extracts of *Asparagus officinalis* cv. Mary Washington: Comparison of *in vivo* and *in vitro* grown plant bioactivities. Afr J Biotechnol. 2010;9(49):8460-6.
22. Minh NP, Them LT, Dang NC, Ho TP, Hieu NT, Luan LQ. Investigation of *Asparagus officinalis* Shoot size and drying affecting to herbal tea production. J Pharm Sci Res. 2019;11(2):267-72.
23. Hafizur RM, Kabir N, Chishti S. *Asparagus officinalis* extract controls blood glucose by improving insulin secretion and β-cell function in streptozotocin-induced type 2 diabetic rats. Br J Nutr. 2012;108(9):1586-95. doi: 10.1017/S0007114511007148, PMID 22221560.
24. Navya PE, Allenki V. Evaluation of *Asparagus officinalis* for anti- diabetic activity in rats. IJIPSR. 2017;5(07):1-9.
25. Poormoosavi SM, Najafzadehvarzi H, Behmanesh MA, Amirgholami R. Protective effects of *Asparagus officinalis* extract against bisphenol A- induced toxicity in Wistar rats. Toxicol Rep. 2018;5:427-33. doi: 10.1016/j.toxrep.2018.02.010, PMID 29854613, PMCID PMC5977383.
26. Lalan BK, Hiray RS, Ghongane BB. Evaluation of Analgesic and Anti-Inflammatory Activity of Extract of Holoptelea integrifolia and *Argyreia speciose* in Animal Models. J Clin Diagn Res. 2015;9(7):FF01-4. doi: 10.7860/JCDR/2015/12059.6200. PMID 26393140.
27. Onasanwo SA, Fabiyi TD, Oluwole FS, Olaleye SB. Analgesic and anti-inflammatory properties of the leaf extracts of Anacardium occidentals in the laboratory rodents. Niger J Physiol Sci. 2012;27(1):65-71. PMID 23235310.
28. Kumar S, Hitendra, Bhatia M, Garg LN, Gupta S. Acute and chronic inflammation studies of *Strobilanthes callosus* leaves extract on rat model. Inflammopharmacology. 2013;21(3):233-9. doi: 10.1007/s10787-012-0150-8, PMID 22983841.
29. Senthelal S, Li J, Ardeshirzadeh S, *et al.* Updated 2022 Jun 19. Arthritis [cited 6/12/2022]. In: StatPearls [Internet]. Treasure Island, (FL): StatPearls Publishing; 2022 Jan-. Available from, Available from: https://www.ncbi.nlm.nih.gov/books/NBK518992 [cited 6/12/2022].
30. Mbiantcha M, Almas J, Shabana SU, Nida D, Aisha F. Anti-arthritic property of crude extracts of *Piptadeniastrum africanum* (*Mimosaceae*) in complete Freund's adjuvant-induced arthritis in rats. BMC Complement Altern Med. 2017;17(1):111. doi: 10.1186/s12906-017-1623-5, PMID 28202019.
31. Chinnasamy V, Subramaniyan V, Chandiran S, Kayarohanam S, Kanniyan DC, Velaga VSSR, *et al.* Antiarthritic activity of Achyranthes aspera on formaldehyde - induced arthritis in rats. Open Access Maced J Med Sci. 2019;7(17):2709-14. doi: 10.3889/oamjms.2019.559, PMID 31844425.
32. Cui X, Wang R, Bian P, Wu Q, Seshadri VDD, Liu L. Evaluation of antiarthritic activity of nimbolide against Freund's adjuvant induced arthritis in rats. Artif Cells Nanomed Biotechnol. 2019;47(1):3391-8. doi: 10.1080/21691401.2019.1649269, PMID 31394949.
33. Jang E, Cho S, Pyo S, Nam JW, Youn J. An inflammatory loop between spleen-derived myeloid cells and CD4+ T cells leads to accumulation of long-lived plasma cells that exacerbates lupus autoimmunity. Front Immunol. 2021;12:631472. doi: 10.3389/fimmu.2021.631472, PMID 33643317.
34. Gao W, Zan Y, Wang ZJ, Hu XY, Huang F. Quercetin ameliorates paclitaxel-induced neuropathic pain by stabilizing mast cells, and subsequently blocking PKCε-dependent activation of TRPV1. Acta Pharmacol Sin. 2016;37(9):1186-77. doi: 10.1038/aps.2016.58, PMID 27498772.
35. Feng FB, Qiu HY. Effects of artesunate on chondrocyte proliferation, apoptosis and autophagy through the PI3K/AKT/mTOR signaling pathway in rat models with rheumatoid arthritis. Biomed Pharmacother. 2018;102:1209-20. doi: 10.1016/j.biopha.2018.03.142, PMID 29710540.
36. Sun G, Xing C, Zeng L, Huang Y, Sun X, Liu Y. *Flemingia philippinensis* flavonoids relieve bone erosion and inflammatory mediators in CIA mice by downregulating NF-κB and MAPK pathways. Mediators Inflamm. 2019;2019:5790291. doi: 10.1155/2019/5790291, PMID 30906224, PMCID PMC6397971.
37. Bai L, Bai Y, Yang Y, Zhang W, Huang L, Ma R, *et al.* Baicalin alleviates collagen induced arthritis and suppresses TLR2/MYD88/NF κB p65 signaling in rats and HFLS RAs. Mol Med Rep. 2020;22(4):2833-41. doi: 10.3892/mmr.2020.11369, PMID 32945496.
38. Chen JY, Wu H, Li H, Hu SL, Dai MM, Chen J. Anti-inflammatory effects and pharmacokinetics study of *geniposide* on rats with adjuvant arthritis. Int Immunopharmacol. 2015;24(1):102-9. doi: 10.1016/j.intimp.2014.11.017, PMID 25434608.
39. Carvalho AMS, Heimfarth L, Santos KA, Guimarães AG, Picot L, Almeida JRGS, *et al.* Terpenes as possible drugs for the mitigation of arthritic symptoms – A systematic review. Phytomedicine. 2019;57:137-47. doi: 10.1016/j.phymed.2018.10.028, PMID 30668316.
40. Qian K, Zheng XX, Wang C, Huang WG, Liu X8, Xu SD, *et al.* β-sitosterol Inhibits Rheumatoid synovial angiogenesis through Suppressing VEGF Signaling Pathway. Front Pharmacol. 2021;12:816477. doi: 10.3389/fphar.2021.816477, PMID 35295740.
41. Ahmad Khan M, Sarwar AHMG, Rahat R, Ahmed RS, Umar S. Stigmasterol protects rats from collagen Induced arthritis by Inhibiting proinflammatory cytokines. Int Immunopharmacol. 2020;85:106642. doi: 10.1016/j.intimp.2020.106642, PMID 32470883

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J. Integr. Sci. Technol. 2023, 11(1), 427 Review

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Edible Mushroom assisted synthesis and applications of metal nanoparticles: A comprehensive review

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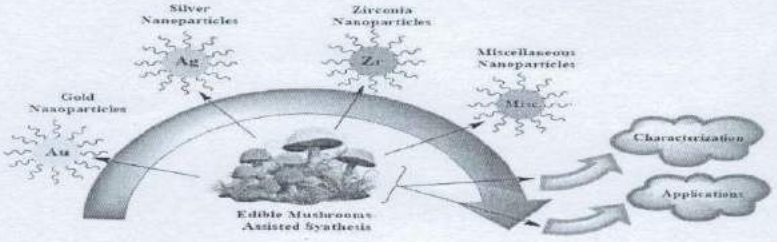
ABSTRACT

Edible mushrooms have been used as either reducing or stabilizing agents in the biological process of synthesis of nanoparticles. The mushroom-assisted synthesis has been reported to produce large quantities of proteins and has the characteristic large yield and low toxicity issues. The nanoparticles derived from them are coated with special coatings that protect them from external environment, thus improving their life span and stability. This review describes the various mushrooms that assisted in the synthesis of nanoparticles such as silver, gold, zirconia and their tentative use in various biological aspects have been discussed. Moreover, the characterization of various nanoparticles using analytical techniques has also been highlighted.



Keywords: Edible mushrooms, nanoparticles, gold nanoparticles, silver nanoparticles, zirconia nanoparticles, biosynthesis.

INTRODUCTION

Natural products are the hub for various bioactives that possess various medicinal properties.[1–5] Natural products are shown to possess anticancer, antimalarial, antimicrobial, analgesic, anti-Alzheimer, anti-Parkinson, and wound healing effect.[6–12] Out of them, mushrooms are one of the rich sources of bioactives. Mushrooms have been used for therapeutic interventions as well as asource of food. They have been reported in earlier texts such as *Materia Medica* for their use in diseases and ailments. They are rich in polysaccharides and have active moieties needed for immune response. They contain high nutritional value-based ingredients such as vitamins, protein, etc. but it lacks cholesterol.[13] Mushrooms as such do not belong to any taxonomic category. But researchers had tried to name it as the macro fungus that has a budding fruitful body that can be seen through the naked eye and can be sensed through organoleptic evaluation. There are over 20,000 varieties of mushrooms but only 10% of them have been explored. The edible class of mushrooms that have shown therapeutic potential included the *Lentinus, Auricularia, Hericium, Grifola, Flammulina, Pleurotus, Ganoderma, Trametesand Tremella*. The mushroom known as *Pleurotus* has been reported to possess medicinal properties such as anticancer, antioxidant, and antitumor properties.

Owing to the diverse range of active substances that can be derived from mushrooms, they have been browbeaten by investigators for the amalgamation of nanoparticles from them. The proteins of mushrooms had been utilized in the synthesis of metallic nanoparticles such as Ag, Au, etc. Many researchers have developed nanoparticles using mushrooms for therapeutic applications as shown in Table 1. The main benefit of using mushrooms as factories of Nanoparticle synthesis is due to the presence and release of a higher number of extracellular enzymes that further act as a stabilizing agent for synthesis.[14–19] The chemicals secreted by mushrooms during nanoparticle synthesis are also able to reduce the toxicity arising from it. When nanoparticle synthesis is carried out in bacterial cell the nanoparticles does not

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remain localized, instead, they interact with the bacterial cell wall and create toxicity to cells.[20,21]

A mushroom has high metal binding capacity in comparison to bacteria and hence has gained wider exposure. Various mushrooms with various types of nanoparticles produced from them have been tabulated as per Table 1.

Table 1. Literature survey of various edible mushrooms and nanoparticles produced from them.

Mushrooms	Medicinal property	Type of nanoparticles	Ref.
Pleuroussajor-caju	Antibacterial	AgNPs	22
Pleurotusflorida	Antibacterial	AgNPs	23
Agaricusbisporus	Antibacterial	AgNPs	24
Calocybe indica	Antibacterial	AgNPs	
Ganoderma lucidum	Antibacterial	AgNPs	
Pleurotusostreatus	Antibacterial	AgNPs	
Pycnoporussanguineus	Antibacterial	AgNPs	25
Schizophyllum commune	Antibacterial	AgNPs	
Pycnoporussanguineus	Antibacterial	AgNPs	
Schizophyllum commune	Antibacterial	AgNPs	26
Pleurotusflorida	Antibacterial	AgNPs	27
Ganoderma lucidum	Antibacterial	AgNPs	28
Pleurotus giganteus	Antibacterial	AgNPs	29
Pleurotusostreatus	Antifungal	AgNPs	30
Pleurotuscornucopiae var. citrinopileatus	Antifungal	AgNPs	31
Polyporus rhinoceros	Anticancer	SeNPs	32
Ganoderma neo-japonicum	Anticancer	AgNPs	33
Pleurotusdjamor var. roseus	Anticancer	AgNPs	34

SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) are formerly used as an active drug moiety in gene therapy, targeted drug transport, and artificial implants, as well as the diagnostic agent for sensing and imaging in the early stages of various diseases.[35–38] They may be produced efficaciously via several synthesis techniques, including heating methods, ionizing radiation, laser irradiation, and radiolysis. These approaches for the processing of nanomaterials are expensive and environmentally unfriendly. The use of *Pleurotusflorida* extract, edible mushroom, and silver nitrate ($AgNO_3$) salt inthe synthesis of stable biofunctionalized AgNPs via photo-irradiation andreaction mixture exposed directly to the sunlight, is an attempt to establish an environmentally friendly process for AgNPs synthesis.[27] The mycosynthetic system usedto biosynthesize the Ag nanoparticles has numerous benefits over the chemical approaches, including higher biosafety, non-toxicity, and being eco-friendly to the environment.[39]

The *Pleurotus sp.* is among the most popular medicinal and edible mushrooms, as it comprises of several active composites. *P. ostreatus, P. florida,* and *P. sajorcaju* are generally used in AgNPs synthesis. The oyster mushroom engaged as first amongst the macrofungi in nanoparticle synthesis and their utilizations. Among all metallic nanoparticles, AgNPs are the most common which are synthesized from this mushroom. These silver nanoparticles exhibit inhibitory effects towards several pathogenic microorganisms, yeasts, and molds and are used to confiscate textile dyes, as well as in cancer treatment.[40]

The initial research on nanoparticle synthesis via edible mushrooms with proteins from the spent mushroom substrate to form AgNPs, which is accompanied by different extracts from mycelium and mushroom fruiting bodies. A modest application for the formation of AgNPs in core-shell via SMS (spent mushroom substrate) exposed to an organic surface, which reduces the Ag^+ and form the AgNPs stabilization via secreted mushroom protein. After 24 hours, the $AgNO_3$ solution incubated with SMS turned yellow, suggesting the development of stable Ag-NPs (Silver nanoparticles).[41]

Although the mechanism of AgNPs synthesis is unknown, recent researches suggest that the intracellular synthesis of silver nanoparticles is linked to nitrate reductase, which releases an electron from NADH that converts Ag^+ to Ag^0, resulting inthe creation of NAD^+. The extracellular synthesis of silver nanoparticles is linked to the reduction of Ag^+ via natural organic mycomaterials e.g., proteins, enzymes, polysaccharides, amino acids, etc.[40] as illustrated in Figure 1.

Roy et al. synthesized silver nanoparticles using the enoki mushroom water extract and integrated them with Starch/agar-based functional films for active packing applications.[42] The integration of nanoparticles showed strong antibacterial action against food-borne bacteria such as *L.monocytogenes* and *E.Coli*. Also due to the presence of silver nanoparticles, the water vapor barrier and hydrophobicity of films got enhanced.Similarly, Li et al. studied the extraction of silver nanoparticles from *Aspergillus terreus*.[43] The formation of silver nanoparticles was monitored using UV Spectroscopy as shown in Figure 2.

GOLD NANOPARTICLES

The synthesis of gold nanoparticles has been fascinated as a major consideration because of their incipient utilizations in various fields such as biomedicines, biosensors, and bioimaging.[44,45] Because of their high Fermi potential, metal nanoparticles, especially AuNPs have excellent catalytic properties. The yellowish color of the solution of gold ions worn as the color of mushroom stripe turned to the pinkish-redcolor, suggesting the development of intracellular gold nanoparticles(AuNPs) and the aqueous solution gradually turned out to be colorless, demonstrating no formation of extracellular gold nanoparticles.[46]

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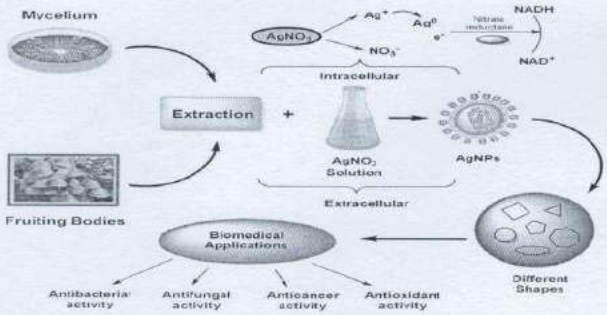

Figure 1. Synthesis of silver nanoparticles (AgNPs) by using the edible mushrooms.

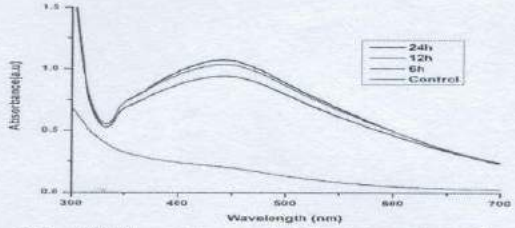

Figure 2. The UV-Vis spectra recorded for the reaction of fungal cell filtrate with $AgNO_3$ solution. Reproduced with permission from Ref [43](under creative common licence).

They display surface plasmon resonance (SPR), Rayleigh scattering, and surface-enhanced Raman scattering (SERS), making them useful in medicinal fields, catalysis, and optoelectronics.[47]

Mushrooms are widely recognized as a high proteinaceous food, containing more than 75% protein. Oyster mushroom(*Pleurotus Sp.*) is a virtuous source of riboflavin, in addition to proteins.[48] Riboflavin plays a vital role in bound coenzymes to form flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which aid as the catalysts for severaloxidation and reduction reactions. It is known that flavins (i.e., flavoproteins) existing in the extract of mushrooms are reliable for reducing Au ions into AuNPs. When exposed to sunlight, the reaction mixture absorbs the photons of energy, and flavins in the reaction mixture become excited and act as oxidizers or electron donors. This supplies a robust indication for the renovation of Au^+ to Au^0. In addition,the presence of protein isalleged to cap the AuNPs produced, making them biofunctionalized and stable.[49]

Gold nanoparticles (AuNPs) have been manufactured by the reduction of chloroauric acid with glucan, eluted from *Pleurotus florida*, an edible mushroom. Glucan serves as the stabilizing as well as the reducing agent. This synthesis specified that the size distribution of AuNPs altered with a variation in the concentration of chloroauric acid ($HAuCl_4$).[50] The representation of the pathway for gold nanoparticles (AuNPs) synthesis is shown in Figure 3.

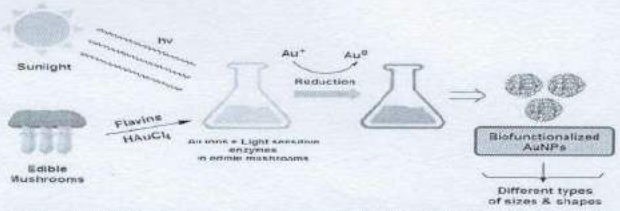

Figure 3. Synthesis of gold nanoparticles (AuNPs) by using edible mushrooms.

To determine the existence of Au-NPs, tinny slices of fungal mycelium have been examined underneath a field emission-scanning electron microscope (FE-SEM) with subordinate electron detectors at 15 kV voltage. The fundamental investigation of mushroom mycelia has been done by energy-dispersive X-ray spectrometry (EDS) that is coupled with scanning electron microscopy (SEM). The amount of gold deposited within mushroom mycelia is determined by inductively coupled plasma-optical emission spectrometry (ICP-OES).[51]

The core-shell morphology of Au-NPs is determined by using FTIR spectroscopy. The AFM technique is employed to investigate the surface morphology of AuNPs. The size and exact location of accumulated AuNPs in mushroom mycelium are determined via transmission electron microscopy (TEM), indicating that the formed nanoparticles are of uneven shape. The size and shape of gold nanoparticles (AuNPs) are regulated via deviations in temperature conditions and the relative concentration of extract about metal ions.[52]

Zirconia Nanoparticles

Zirconia is a technologically significant material with excellent natural color, transformation durability, higher strength and chemical stability, corrosion resistance, and chemical, and microbial resistance. Zirconia is an amphoteric element, which means it can be both acidic and basic. The more notable feature of zirconia is its steadiness under reducing circumstances, making it a valuable material in the catalytic region.[53]

Zirconium dioxide (ZrO_2), also known as zirconia, is a white color powder, that exists in three different polymorphic forms i.e., monoclinic, cubic, and tetragonal. The monoclinic phase of zirconia is constant below 1170 °C, and exists in the tetragonal phase between 1170 °C and 2370 °C, whereas above 2370 °C, zirconia renovates into the cubic phase.[54] Zirconium is used in a variety of applications including, structural reinforcement, adsorption, photodegradation, and antimicrobial agents.[55] ZrO_2 NPs have sparked a lot of research interest among transition metal oxide nanoparticles because of their specific catalytic, thermal, electrical,

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mechanical, optical, sensing, and biocompatible properties.[56] They have been used in solid oxide fuel cells, solar cells, bone implants, nitrogen oxide, and oxygen gas sensors. Because of their long-term stability and strong oxygen ion transport capabilities, the stabilized zirconia nanoparticles are well-equipped for higher-temperature energy conversion systems.[53]

The usage of zirconium NPs in biological fields is rapidly growing. They are generally used as the drug delivery carriers for several medications such aspenicillin, itraconazole, alendronate, and zoledronate as well as gene delivery carriers with target specificity.[57,58] Zirconia nanoparticles have been synthesized by using several physicochemical approaches including, sol-gel synthesis, hydrothermal methods, and aqueous precipitation methods, and require the conditions of higher pressure and temperature. Biological methods for the synthesis of zirconia nanoparticles have more benefits because they use an environmentally friendly approach at mild pH, temperature, and pressure, as well as at significantly lower costs without the explosion of any toxic waste to the environment.[59]

Zirconia NPs might be formedvia challenging the fungus i.e., *Fusarium oxysporum* with aqueous ZrF_6^{2-} anions; extracellularly protein-mediated hydrolysis of anionic complexes outcomes in the simplistic synthesis of nano-crystalline zirconia at room temperature.[60] Furthermore, zirconium NPs have been synthesized vialeaf extract of *Eucalyptus globulus* with spherical morphology in the range of 9-11 nm. These zirconia nanoparticles displayed a higher antioxidant potential, strong antibacterial activity against using the disc diffusion method as well as remarkable anticancer activity towardshuman lung (A-549)cancer cell lines and human colon (HCT-116)cancer cell lines by using MTT and DPPH assays, respectively.[57,61] The figurative demonstration of the pathway for zirconia nanoparticle synthesis is depicted in Figure 4.

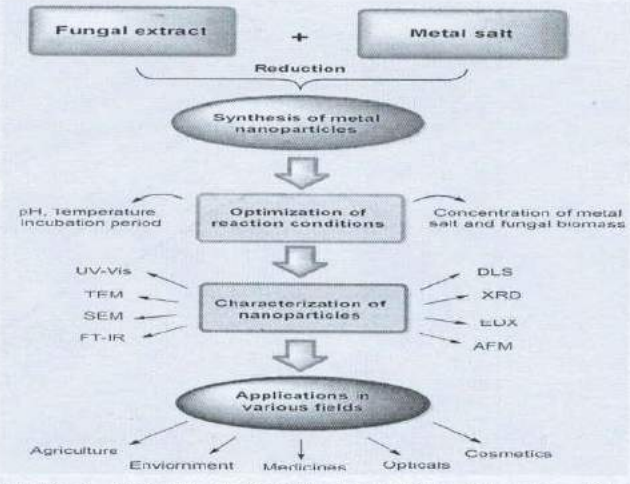

Figure 4. Synthesis of Zirconia nanoparticles by using edible mushrooms

Miscellaneous Nanoparticles

The "green" methodology for the synthesis of nanoparticles, which is promptly substituting conventional chemical synthesis, is of prodigious curiosity due to its environmental-friendly nature, economical views, feasibility,and applications in numerous areas including, nano-medicines and catalysis medicines.[16]

The myogenesis of nanoparticles has been discovered to be an effective and appropriate method for the production of several nanoparticles with considerable potential and their utilities in various fields such as food, medicines, agriculture, textiles, optics, electronics, and cosmetics.[15] Since fungi and yeasts are efficient extracellular enzyme secretors and numerous varieties develop quickly; culturing and maintaining them in the laboratories is facile and simple. By using intracellular or extracellular reducing enzymes, edible mushrooms may produce metal-NPs and nanostructures.[16] Several mushroom-active materials are capable of forming the nanoparticles. Many investigators have examined several mushroom elements including, enzymes, proteins, polysaccharides, and complexes of polysaccharide-proteins as the sources for metal reduction and nanoparticle stabilization in addition to the mushroom extract.[62] Roy et al. synthesized sulfur nanoparticles from the facile acid hydrolysis process of enoki mushrooms with a size range of 20nm.[63] The nanoparticles were further integrated with the Gelatin-cellulose nanofiber for food-packing applications. The presence of sulfur nanoparticles enhanced the mechanical and UV protection activity of the film. The films were further characterized using the FESEM, FTIR, Mechanical properties, and vapor barrier activities. Green synthesis of multifunctional and spherical Palladium nanoparticles (Pd NPs) using *Agaricus bisporus* (mushroom) fungus was recently described.[64] Until a brown hue formed, indicative of the development of Pd NPs, the mushroom was mixed with Pd (II) ions at a ratio of 1:9 at room temperature to synthesize the NPs. The loss of UV/Vis absorbance at 405 nm, corresponding to Pd (II) ions, was used as evidence for the production of Pd NPs. The synthesized Pd NPs had a size of 13 nm and a zeta potential of 24.3 mV, making them very stable. Both Gram-positive Streptococcus pyogenes and Gram-negative Enterobacter aerogenes were killed by these compounds to a substantial degree. The manufactured NPs were also shown to be biocompatible with RBCs and to exhibit antioxidant and anti-inflammatory properties.

Recently, the biological synthesis of NPs has gained a lot of consideration via several biological tools like extracts of plants and microorganisms as the stabilizing and reducing agents. These synthesized nanoparticles have been characterized by using Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FT-IR),Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS),and Energy Dispersive X-ray (EDX) method.

In mushroom studies, two species i.e., *Coriolus versicolor* and *Pleurotus ostratus* are utilized prominently to produce the cadmium nanoparticles(Cd-NPs). Cd-NPs are successfully formed by using a solution of extracellular biomass of *Caribena versicolor* and

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cadmium sulfide.[65] Selenium nanoparticles (SeNPs) have recently become a new goal of research since, they exhibit excellent bioavailability, lower toxicity, and remarkable anticancer activity.[66] Highly stable SeNPs have been efficaciously produced via mushroom polysaccharide-protein complexes (PSPs) that are eluted from the sclerotia of *Pleurotus tuber-regium*. Furthermore, these novel SeNPs are significantly inhibiting the growth of tumor progression concomitantly in patients of breast carcinoma and MCF-7 cancer cell lines by inducing apoptosis in a dose-dependent mode, without showing any cytotoxicity to the normal cells which signify that their cytotoxicity is cancer-specific.[67]

Table 2. Literature survey of various techniques enlisted with property to be determined for nanoparticle characterization.

Property of Nanoparticles	Techniques employed	Ref.
Particle morphology	Transmission electron microscopy	68,69
	Scanning electron microscopy	70,71
	Freeze fracture electron microscopy	72,73
	High-resolution transmission electron microscopy	74
Surface hydrophilicity	Hydrophobic interaction chromatography	75,76
	Zeta potential measurement	77,78
Molecular weight and crystallinity	High-resolution mass spectroscopy	79,80
	Gas chromatography-mass spectrometry analysis	81,82
	Raman spectroscopy	83–85
	X-ray diffraction	86,87
Surface chemistry	Fourier transform infrared spectroscopy	88
	Nuclear magnetic resonance	89,90
	Mass spectroscopy	91,92
Thermal stability	Differential scanning calorimetric	93,94
	Thermogravimetric analysis	95,96

VARIOUS APPLICATIONS OF NANOPARTICLES

Applications of Silver Nanoparticles

Silver nanoparticles derived from edible mushrooms have been used for their antibacterial and antimicrobial action. The nanoparticles derived from *Ganoderma lucidum* extract showed DNA cleavage activity.[97] It becomes determined that the silver nanoparticles have been capable of reason the single stress DNA cleavage for 30 and 60min at distinction dilutions. The nanoparticles also showed strong antibacterial action against gram-positive microbes such as *S.aureus, E.hirae,* and *B.cereus.* Also, it was able to inhibit the microbes belonging to the gram-negative class of bacteria and *C. albicans* fungus. Similarly, Arun et al. described the antimicrobial action of AgNPs synthesized *Schizophyllum commune* for their antimicrobial activity against the *E.Coli, B.subtilis, Klebsiella pneumoniae,* and *Pseudomonas fluorescens.*[26] The NPs were also able to inhibit the growth of *Trichophytonsimii, Trichophyton mentagrophytes,* and *Trichophytonrubrum* significantly.

Researchers developed silver nanoparticles from the *fomitopsis pinicola* and it was found that they possess anticancer properties.[98] The silver nanoparticles caused deformities in cellular morphology. The nucleus of cells also got disintegrated and condensed as many of the cells were found to be dead. The dose-dependent action on tumor cells was observed as confirmed by the colorimetric assay. The main reason behind the cell death can be attributed due to the presence of program cell death, as the nucleus undergoes disintegration and DNA fragments after nanoparticle introduction.[99] Sanpui et al. described the mechanism of AgNPs that it interferes with the regular activity of cells and interferes with the equilibrium of the membrane inducing the formation of apoptotic signaling genes leading to cell death. Similarly, another group of researchers also reported the anticancer application of silver nanoparticles.[100,101]

The silver nanoparticles derived from *Agaricus bisporus* fungi are also reported to possess photocatalytic, antioxidant, and anti-inflammatory activity.[102] The nanoparticles showed better anti-inflammatory activity compared to aceclofenac. The silver nanoparticles have been found to impede the growth of biofilm formation and reduce the death rate of *Ruditapes philippinarum.*[103]

Researchers synthesized silver nanoparticles derived from *Ganoderma lucidum* for the remedy of drug-resistant *E.coli* remoted from the catheter used for urinary tract infections.[104] The DPPH and ARP results showed comparable results in terms of the potency of free radical scavenging activity, in comparison to Quercetin. The AgNPs showed a reducing effect on tumor cell lines such as MDA-MB-231. Similarly, silver nanoparticles derived from *Ganoderma neo-japonicum Imazeki* showed anticancer activity.[33] Silver nanoparticles can produce Reactive oxygen species. Accumulation of a larger amount of ROS leads to oxidative damage.[105] The cells were equally treated with Doxorubicin and silver nanoparticles. As the intracellular production of ROS increased the levels of ROS generation in silver nanoparticles treated cells also increased. The results showed that ROS is an important factor for apoptosis in yeast cells. Silver nanoparticles are said to possess the antibacterial property of *S.aureus* and showed good inhibition characteristics.[24] Moreover, they also were exploited for their antibacterial and anti-inflammatory action on wounds.[106] Researchers synthesized silver nanoparticles using the *Pleurotus florida* mushroom extract.[107] The nanoparticles showed significant antibacterial activity against *Streptococcus pyogenes (22.17 ± 0.66 mm), Enterococcus faecalis (16.54 ± 0.88 mm), Klebsiella pneumoniae (26.32 ± 0.88 mm), Shigella flexneri (27.21 ± 0.66 mm), Candida albicans (15.13 ± 0.33 mm) and Aspergillus fumigatus (14.89 ± 0.33 mm).* The bactericidal activity was found to increase significantly with increasing synergistic AgNPs with mushroom extract concentration. Differences in cell

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wall composition explain why AgNPs have different antibacterial actions on Gram-negative and Gram-positive bacteria. Because Ag+ ions are released from NPs and interact with bacterial enzymes, AgNPs exhibited a larger zone of inhibition. By interacting with thiol, carboxyl, hydroxyl, amino, phosphate, and imidazole groups in proteins and enzymes on bacterial membranes, green-synthesized AgNPs exert antibacterial action. This results in severe structural deformation of the cell membrane. Then, the AgNPs enter the cells through the porous membranes and inactivate the enzymes, causing the cells to suffocate, stop replicating, and die.[108]

Applications of Gold Nanoparticles

Researchers prepared gold nanoparticles from *Agaricus bisporus* mushroom.[109] The nanoparticles thus prepared were able to degrade the decolorizing activity of Methylene blue and the decrease was showing a dose-dependent effect with decolorization at 97.98%. Similarly, another group of researchers prepared nanoparticles from the mushroom. The nanoparticles were able to reduce the methylene blue dye with a decolorization efficiency of 75.35% after 4h of treatment.[110] The gold nanoparticles also showed inhibitory action against the various cancer lines such as A-549, K-562, and HeLa.[49] But no effect was observed against the Vero cell lines. Another group of researchers also showed the cytotoxic effect of mushrooms produced from *Inonotusobliquus* on the cancer cell lines such as MCF-1 and NCI-N87.[111] The anticancer activity of gold nanoparticles was also observed for the HepG2 and HCT-116 cells.[112] The nanoparticles showed alterations in the morphology of cells. The anticancer effect of AuNPs may be attributed due to irregular shape and functional groups attached to the surface. The gold nanoparticles, derived from *Pleurotus sajor-caju* showed ant proliferative properties in the case of colon cancer cell lines.[113] The nanoparticles caused morphological alterations in the HCT-116 cell lines. The cells treated showed a loss in shape and cell adhesion capacity and got shrunk. Many studies supported evidence of the antiproliferative and anticancer effects on the HeLa cells, Hep-2 cell lines, and sarcoma 180.[114–116] The nanoparticles derived from *Pleurotus florida* and *Hericium erinaceus* have been used as anticancer agents.[117]

Applications of other metallic nanoparticles

Dias et al., synthesized zinc nanoparticles based on *Cordyceps militaris*. The nanoparticles thus obtained showed 70-90% survival rate in lethality assay and possessed α-amylase and α-glucosidase inhibitory effects. Along with this the nanoparticles also showed antibacterial action on *P.aeruginosa, Shigella flexneri, P.vulgaris*.[118] Zeng et al. studied the antiproliferative effect of selenium nanoparticles decorated with water-soluble polysaccharides of various mushrooms. The nanoparticles induced caspases and mitochondria-mediated apoptosis but doesn't affect neighboring organs.[119] Similarly, Ali et al. also decorated selenium nanoparticles using proteins derived from protein precipitation of *Lentinus edodes* mushroom. The nanoparticles showed antifibrinolytic activity *invitro*.[120] *Liu et al.* studied the antifatigue effect of selenium nanoparticles derived from polysaccharides of *Lyciumbarbarum(LBP)*.[121] The effectiveness of LBP-decorated SeNPs in fighting tiredness was measured with a forced swimming test. After 30 days of storage, the findings revealed that LBP1-SeNPs maintained an average particle size of around 105.4 nm, which is lower than that of LBP2-SeNPs and LBP3-SeNPs. All LBP1-SeNPs dose groups examined had a longer fatigue swimming time compared to the control group (p 0.05), with the high-dose group's duration being even noticeably longer than the positive group. LBP1-SeNPs alleviated tiredness by boosting glycogen reserve, raising antioxidant enzyme levels, and modulating metabolic process, as evidenced by measurements of glycogen, blood urea nitrogen (BUN), blood lactic acid (BLA), superoxide dismutase (SOD), and malondialdehyde (MDA).

CONCLUSION

Though the nanoparticles produced from myco-source are quite effective for their antibacterial and other medicinal action. But still,the toxicity level needs to be evaluated. There have been no extract guidelines for evaluating their effectiveness such that they can be standardized for normal human usage. Thus, there is a need to develop some regulatory framework for the development of evaluation techniques. The rate of production of nanoparticles depends on environmental factors too. One cannot say if mushrooms are grown from the same species, the place and quality of nutrition given also influences nanoparticle production. Thus, still, more clinical aspects need to be evaluated in the future time.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this article.

REFERENCES

1. R.K. Singla, C.S. Sai, H. Chopra, et al. Natural Products for the Management of Castration-Resistant Prostate Cancer: Special Focus on Nanoparticles Based Studies. *Frontiers in cell and developmental biology* **2021**, 9.
2. H. Bansal, R.K. Singla, S. Behzad, et al. Unleashing the Potential of Microbial Natural Products in Drug Discovery: Focusing on Streptomyces as Antimicrobials Goldmine. *Current Topics in Medicinal Chemistry* **2021**, 21 (26), 2374–2396.
3. R.K. Singla, S. Behzad, J. Khan, et al. Natural Kinase Inhibitors for the Treatment and Management of Endometrial/Uterine Cancer: Preclinical to Clinical Studies. *Frontiers in Pharmacology* **2022**, 13 (February), 1–15.
4. R.K. Singla, X. He, H. Chopra, et al. Natural Products for the Prevention and Control of the COVID-19 Pandemic: Sustainable Bioresources. *Frontiers in pharmacology* **2021**, 12.
5. H. Chopra, S. Bibi, F. Islam, et al. Emerging Trends in the Delivery of Resveratrol by Nanostructures: Applications of Nanotechnology in Life Sciences. *ene* **2022**, 4, 13.
6. I. Singh Bakshi, H. Chopra, M. Sharma, et al. Herbal bioactives for wound healing application. *Herbal Bioactive-Based Drug Delivery Systems* **2022**, 259–282.
7. H. Chopra, A.K. Mishra, A.A. Baig, et al. Narrative Review: Bioactive Potential of Various Mushrooms as the Treasure of Versatile Therapeutic Natural Product. *Journal of Fungi* **2021**, 7 (9), 728.
8. F. Islam, S. Bepary, M.H. Nafady, et al. Review Article Polyphenols Targeting Oxidative Stress in Spinal Cord Injury : Current Status and Future Vision. **2022**, 2022.
9. T. Bhattacharya, G.A.B.E. Soares, H. Chopra, et al. Applications of Phyto-Nanotechnology for the Treatment of Neurodegenerative Disorders. *Materials* **2022**, 15 (3), 804.
10. J.C. Bose, H. Chopra, S. Bibi, et al. Preparation and Evaluation of Chitosan/PVA Based Hydrogel Films Loaded with Honey for Wound Healing Application. *Gels* **2022**, 8 (2), 111.

INDORE INSTITUTE OF PHARMACY IQAC INDORE
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INDORE (M.P.)

11. H. Chopra, S. Bibi, I. Singh, et al. Green Metallic Nanoparticles: Biosynthesis to Applications. *Frontiers in Bioengineering and Biotechnology* **2022**, 0, 548.
12. J. Kour, H. Chopra, S. Bukhari, et al. Nutraceutical-A deep and profound concept. *Nutraceuticals and Health Care* **2022**, 1–28.
13. H. Chopra, A.K. Mishra, A.A. Baig, et al. Narrative review: Bioactive potential of various mushrooms as the treasure of versatile therapeutic natural product. *Journal of Fungi* **2021**, 7 (9).
14. M. Ovais, A.T. Khalil, M. Ayaz, et al. Biosynthesis of metal nanoparticles via microbial enzymes: A mechanistic approach. *Int. J. Mol. Sci.* 2018.
15. P. Khandel, S.K. Shahi. Mycogenic nanoparticles and their bio-prospective applications: current status and future challenges. *J. Nanostructure in Chemistry* **2018**, 8 (4), 369–391.
16. A.B. Moghaddam, F. Namvar, M. Moniri, et al. Nanoparticles biosynthesized by fungi and yeast: A review of their preparation, properties, and medical applications. *Molecules*. **2015**, 6540–16565.
17. M. Guilger-Casagrande, R. de Lima. Synthesis of Silver Nanoparticles Mediated by Fungi: A Review. *Front. Bioengineer. Biotechnol.* **2019**, 287.
18. H. Bahrulolum, S. Nooraei, N. Javanshir, et al. Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector. *J. Nanobiotechnology*. **2021**, 86.
19. X. Li, H. Xu, Z.S. Chen, G. Chen. Biosynthesis of nanoparticles by microorganisms and their applications. *J. Naaomaterials*. **2011**.
20. L. Wang, C. Hu, L. Shao. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int. J. Nanomedicine*. **2017**, 1227–1249.
21. Y.N. Slavin, J. Asnis, U.O. Häfeli, H. Bach. Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. *J. Nanobiotechnol.* **2017**, 65.
22. N. Vigneshwaran, A.A. Kathe, P. V. Varadarajan, R.P. Nachane, R.H. Balasubramanya. Silver-protein (core-shell) nanoparticle production using spent mushroom substrate. *Langmuir* **2007**, 23 (13), 7113–7117.
23. I.K. Sen, A.K. Mandal, S. Chakraborti, et al. Green synthesis of silver nanoparticles using glucan from mushroom and study of antibacterial activity. *Int. J. Biological Macromolecules* **2013**, 62, 439–449.
24. S. Mirunalini, v. Arulmozhi, k. Deepalakshmi, m. Krishnaveni. Intracellular Biosynthesis and Antibacterial Activity of Silver Nanoparticles Using Edible Mushrooms. *Notulae Scientia Biologicae* **2012**, 4 (4), 55–61.
25. Y.S. Chan, M. Mat Don. Biosynthesis and structural characterization of Ag nanoparticles from white rot fungi. *Materials Science and Engineering C* **2013**, 33 (1), 282–288.
26. G. Arun, M. Eyini, P. Gunasekaran. Green synthesis of silver nanoparticles using the mushroom fungus Schizophyllum commune and its biomedical applications. *Biotechnol. Bioprocess Engineering* **2014**, 19 (6), 1083–1090.
27. R. Bhat, R. Deshpande, S. V. Ganachari, D.S. Huh, A. Venkataraman. Photo-irradiated biosynthesis of silver nanoparticles using edible mushroom Pleurotus florida and their antibacterial activity studies. *Bioinorganic Chemistry and Applications* **2011**, 2011.
28. A. Karwa, S. Gaikwad, M. Rai. Mycosynthesis of silver nanoparticles using lingzhi or reishi medicinal mushroom, ganoderma lucidum (W. Curt.:Fr.) P. karst. and their role as antimicrobials and antibiotic activity enhancers. *International Journal of Medicinal Mushrooms* **2011**, 13 (5), 483–491.
29. G. Debnath, P. Das, A.K. Saha. Green Synthesis of Silver Nanoparticles Using Mushroom Extract of Pleurotus giganteus: Characterization, Antimicrobial, and α-Amylase Inhibitory Activity. *BioNanoScience* **2019**, 9 (3), 611–619.
30. R.S. Yehia, H. Al-Sheikh. Biosynthesis and characterization of silver nanoparticles produced by pleurotus ostreatus and their anticandidal and anticancer activities. *World Journal of Microbiology and Biotechnology* **2014**, 30 (11), 2797–2803.
31. M.N. Owaid, J. Raman, H. Lakshmanan, et al. Mycosynthesis of silver nanoparticles by Pleurotus comucopiae var. citrinopileatus and its inhibitory effects against Candida sp. *Materials Letters* **2015**, 153, 186–190.
32. H. Wu, H. Zhu, X. Li, et al. Induction of apoptosis and cell cycle arrest in A549 human lung adenocarcinoma cells by surface-capping selenium nanoparticles: An effect enhanced by polysaccharide-protein complexes from Polyporus rhinocerus. *Journal of Agricultural and Food Chemistry* **2013**, 61 (41), 9859–9866.
33. S. Gurunathan, J. Raman, S.N. Abd Malek, P.A. John, S. Vikineswary. Green synthesis of silver nanoparticles using Ganoderma neo-japonicum Imazeki: A potential cytotoxic agent against breast cancer cells. *Int. J. Nanomedicine* **2013**, 8, 4399–4413.
34. J. Raman, G.R. Reddy, H. Lakshmanan, et al. Mycosynthesis and characterization of silver nanoparticles from Pleurotus djamor var. roseus and their in vitro cytotoxicity effect on PC3 cells. *Process Biochemistry* **2015**, 50 (1), 140–147.
35. B.S. Chhikara. Current trends in nanomedicine and nanobiotechnology research. *J. Materials NanoScience* **2017**, 4 (1), 19–24.
36. N. Gupta, C. Gupta, S. Sharma, R.K. Sharma, H.B. Bohidar. Comparative study of antibacterial activity of standard antibiotic with silver nanoparticles synthesized using ocimum tenuiflorum and garcinia mangostana leaves. *Chem. Biol. Lett.* **2015**, 2 (2), 41–44.
37. A. Albasher Omar, N. Abdunzq Ahmad, M. Moftah Rajab, et al. Biosynthesis of Silver nanoparticles using Olive Wastewater. *J. Materials NanoScience* **2021**, 8 (1), 11–15.
38. G. Saini, K. Thakur, M.D. Gnaneswari. Preparation of NanoSilver coated Cotton Fabric and its bactericidal evaluation. *J. Materials NanoScience* **2019**, 6 (1), 13–18.
39. M.N. Owaid, I.J. Ibraheem. Mycosynthesis of nanoparticles using edible and medicinal mushrooms. *Eur. J. Nanomedicine*. **2017**, 5–23.
40. M.N. Owaid. Green synthesis of silver nanoparticles by Pleurotus (oyster mushroom) and their bioactivity: Review. *Environmental Nanotechnology, Monitoring and Management*. **2019**, 100256.
41. S. Sudheer, R.G. Bai, K. Muthoosamy, et al. Biosustainable production of nanoparticles via mycogenesis for biotechnological applications: A critical review. *Environmental Research* **2022**, 204.
42. S. Roy, J.W. Rhim. Starch/agar-based functional films integrated with enoki mushroom-mediated silver nanoparticles for active packaging applications. *Food Bioscience* **2022**, 49, 101867.
43. G. Li, D. He, Y. Qian, et al. Fungus-Mediated Green Synthesis of Silver Nanoparticles Using Aspergillus terreus. *Int. J. Mol. Sci.* **2011**, 13 (1), 466–476.
44. S. Rastogi. Controlled Aqueous Phase Synthesis of Gold Nanoparticles using Fruit extract of Momordica charantia. *J. Materials NanoScience* **2016**, 3 (1), 11–13.
45. B.S. Chhikara, R. Kumar, Poonam, P. Bazard, R.S. Varma. Viral infection mitigations using advanced nanomaterials and tools: lessons from SARS-CoV-2 for future prospective interventions. *J. Materials NanoScience* **2021**, 8 (2), 64–82.
46. J. Sarkar, S. Kalyan Roy, A. Laskar, D. Chattopadhyay, K. Acharya. Bioreduction of chloroaurate ions to gold nanoparticles by culture filtrate of Pleurotus sapidus Quél. *Materials Letters* **2013**, 92, 313–316.
47. S.S. Shankar, A. Rai, B. Ankamwar, et al. Biological synthesis of triangular gold nanoprisms. *Nature Materials* **2004**, 3 (7), 482–488.
48. C. Sánchez. Cultivation of Pleurotus ostreatus and other edible mushrooms. *Applied Microbiology and Biotechnology*. **2010**, 1321–1337.
49. R. Bhat, V.G. Sharanabasava, R. Deshpande, et al. Photo-bio-synthesis of irregular shaped functionalized gold nanoparticles using edible mushroom Pleurotus florida and its anticancer evaluation. *Journal of Photochemistry and Photobiology B: Biology* **2013**, 125, 63–69.
50. I.K. Sen, K. Maity, S.S. Islam. Green synthesis of gold nanoparticles using a glucan of an edible mushroom and study of catalytic activity. *Carbohydrate Polymers* **2013**, 91 (2), 518–528.
51. K.B. Narayanan, H.H. Park, S.S. Han. Synthesis and characterization of biomatrixed-gold nanoparticles by the mushroom Flammulina velutipes and its heterogeneous catalytic potential. *Chemosphere* **2015**, 141, 169–175.
52. D. Philip. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* **2009**, 73 (2), 374–381.
53. A.S. Keiteb, E. Saion, A. Zakaria, N. Soltani. Structural and optical properties of zirconia nanoparticles by thermal treatment synthesis. *J. Nanomaterials*, **2016**.

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54. T.Y. Luo, T.X. Liang, C.S. Li. Stabilization of cubic zirconia by carbon nanotubes. *Materials Science and Engineering A* **2004**, 366 (1), 206–209.
55. A.F.V. da Silva, A.P. Fagundes, D.L.P. Macuvele, et al. Green synthesis of zirconia nanoparticles based on Euclea natalensis plant extract: Optimization of reaction conditions and evaluation of adsorptive properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2019**, 583, 123915.
56. A. Nikam, T. Pagar, S. Ghotekar, K. Pagar, S. Pansambal. A Review on Plant Extract Mediated Green Synthesis of Zirconia Nanoparticles and Their Miscellaneous Applications. *Journal of Chemical Reviews* **2019**, 1 (3), 154–163.
57. A.R. Golnaraghi Ghomi, M. Mohammadi-Khanaposhti, H. Vahidi, et al. Fungus-mediated extracellular biosynthesis and characterization of zirconium nanoparticles using standard penicillium species and their preliminary bactericidal potential: A novel biological approach to nanoparticle synthesis. *Iranian J. Pharmaceutical Res.* **2019**, 18 (4), 2101–2110.
58. S. Tanga, B. Maji. Recent trends in precision drug and gene delivery. *Applied NanoMedicine* **2022**, 22 (2), 429.
59. M. Kumaresan, K. Vijai Anand, K. Govindaraju, S. Tamilselvan, V. Ganesh Kumar. Seaweed Sargassum wightii mediated preparation of zirconia (ZrO 2) nanoparticles and their antibacterial activity against gram positive and gram negative bacteria. *Microbial Pathogenesis* **2018**, 124, 311–315.
60. V. Bansal, D. Rautaray, A. Ahmad, M. Sastry. Biosynthesis of zirconia nanoparticles using the fungus Fusarium oxysporum. *J. Materials Chemistry* **2004**, 14 (22), 3303–3305.
61. J.B. Fathima, A. Pugazhendhi, R. Venis. Synthesis and characterization of ZrO2 nanoparticles-antimicrobial activity and their prospective role in dental care. *Microbial Pathogenesis* **2017**, 110.
62. G.Q. Chen, Z.J. Zou, G.M. Zeng, et al. Coarsening of extracellularly biosynthesized cadmium crystal particles induced by thioacetamide in solution. *Chemosphere* **2011**, 83 (9), 1201–1207.
63. S. Roy, J.W. Rhim. Gelatin/cellulose nanofiber-based functional films added with mushroom-mediated sulfur nanoparticles for active packaging applications. *J. Nanostructure Chem.* **2022**, 12 (5), 979–990.
64. S. Mohana, S. Sumathi. Multi-Functional Biological Effects of Palladium Nanoparticles Synthesized Using Agaricus bisporus. *J. Cluster Science* **2020**, 31 (2).
65. R. Sanghi, P. Verma. Biomimetic synthesis and characterisation of protein capped silver nanoparticles. *Bioresource Technology* **2009**, 100 (1), 501–504.
66. M.P. Ganeshkar, M.R. Mirjankar, A.T. Gaddigal, et al. Selenium Nanoparticles: Synthesis using Averrhoa carambola extract, characterization and its biological applications. *Applied NanoMedicine* **2022**, 22 (1), 324.
67. D. Zeng, J. Zhao, K.H. Luk, et al. Potentiation of in Vivo Anticancer Efficacy of Selenium Nanoparticles by Mushroom Polysaccharides Surface Decoration. *J. Agricultural and Food Chemistry* **2019**, 67 (10), 2865–2876.
68. D.J. Smith. Characterization of nanomaterials using transmission electron microscopy. In *RSC Nanoscience and Nanotechnology*; Royal Society of Chemistry, **2015**; Vol. 2015-January, pp 1–29.
69. M.A. Asadabad, M.J. Eskandari. Transmission electron microscopy as best technique for characterization in nanotechnology. *Synthesis and Reactivity in Inorganic, Metal-Organic and Nano-Metal Chemistry* **2015**, 45 (3), 323–326.
70. V.D. Hodoroaba, S. Rades, T. Salge, et al. Characterisation of nanoparticles by means of high-resolution SEM/EDS in transmission mode. In *IOP Conference Series: Materials Science and Engineering*; Institute of Physics Publishing, **2016**; Vol. 109, p 012006.
71. P.J. Kempen, C. Hitzman, L.S. Sasportas, S.S. Gambhir, R. Sinclair. Advanced characterization techniques for nanoparticles for cancer research: Applications of SEM and NanoSIMS for locating au nanoparticles in cells. In *Materials Research Society Symposium Proceedings*; Materials Research Society, **2013**; Vol. 1569.
72. J.L. Carson. Fundamental technical elements of freeze-fracture/freeze-etch in biological electron microscopy. *J. Visualized Experiments* **2014**, No. 91.
73. L. Isa, F. Lucas, R. Wepf, E. Reimhult. Measuring single-nanoparticle wetting properties by freeze-fracture shadow-casting cryo-scanning electron microscopy. *Nature Communications* **2011**, 2 (1), 1–9.
74. G.C. Ruben, L.W. Hrubesh, T.M. Tillotson. High resolution transmission electron microscopy nanostructure of condensed-silica aerogels. *Journal of Non-Crystalline Solids* **1995**, 186, 209–218.
75. L.E. Crandon, K.M. Boenisch, B.J. Harper, S.L. Harper. Adaptive methodology to determine hydrophobicity of nanomaterials in situ. *PLoS ONE* **2020**, 15 (6), e0233844.
76. A. Valsesia, C. Desmet, I. Ojea-Jiménez, et al. Direct quantification of nanoparticle surface hydrophobicity. *Communications Chemistry* **2018**, 1 (1), 1–11.
77. M.K. Rasmussen, J.N. Pedersen, R. Marie. Size and surface charge characterization of nanoparticles with a salt gradient. *Nature Communications* **2020**, 11 (1), 1–8.
78. G. V. Lowry, R.J. Hill, S. Harper, et al. Guidance to improve the scientific value of zeta-potential measurements in nanoEHS. *Environmental Science: Nano* **2016**, 3 (5), 953–965.
79. S. Nicolardi, Y.E.M. Van Der Burgt, J.D.C. Codée, et al. Structural Characterization of Biofunctionalized Gold Nanoparticles by Ultrahigh-Resolution Mass Spectrometry. *ACS Nano* **2017**, 11 (8), 8257–8264.
80. C. Chang, J. Zhang, Z. Li, et al. Applications of nanoparticles in mass spectrometry for highly sensitive analysis. In *Handbook of Nanoparticles*; Springer International Publishing, **2015**; pp 1313–1337.
81. S. Patil, R. Chandrasekaran. Biogenic nanoparticles: a comprehensive perspective in synthesis, characterization, application and its challenges. *Journal of Genetic Engineering and Biotechnology*. Springer Science and Business Media Deutschland GmbH December 1, 2020.
82. V. Karthick, V.G. Kumar, T.S. Dhas, et al. Biosynthesis of gold nanoparticles and identification of capping agent using gas chromatography-mass spectrometry and matrix assisted laser desorption ionization-mass spectrometry. *J. Nanoscience and Nanotechnology* **2015**, 15 (6), 4052–4057.
83. N.C. Lindquist, C.D.L. de Albuquerque, R.G. Sobral-Filho, I. Paci, A.G. Brolo. High-speed imaging of surface-enhanced Raman scattering fluctuations from individual nanoparticles. *Nature Nanotechnology* **2019**, 14 (10), 981–987.
84. J. Lu, Z. Cai, Y. Zou, et al. Silver Nanoparticle-Based Surface-Enhanced Raman Spectroscopy for the Rapid and Selective Detection of Trace Tropane Alkaloids in Food. *ACS Applied Nano Materials* **2019**, 2 (10), 6592–6601.
85. R.E. Darienzo, J. Wang, O. Chen, et al. Surface-Enhanced Raman Spectroscopy Characterization of Breast Cell Phenotypes: Effect of Nanoparticle Geometry. *ACS Applied Nano Materials* **2019**, 2 (11), 6960–6970.
86. S. Mourdikoudis, R.M. Pallares, N.T.K. Thanh. Characterization techniques for nanoparticles: Comparison and complementarity upon studying nanoparticle properties. *Nanoscale*, **2018**, 12871–12934.
87. S.M. Londoño-Restrepo, R. Jeronimo-Cruz, B.M. Millán-Malo, E.M. Rivera-Muñoz, M.E. Rodriguez-Garcia. Effect of the Nano Crystal Size on the X-ray Diffraction Patterns of Biogenic Hydroxyapatite from Human, Bovine, and Porcine Bones. *Scientific Reports* **2019**, 9 (1), 1–12.
88. S. Suresh, S. Karthikeyan, K. Jayamoorthy. FTIR and multivariate analysis to study the effect of bulk and nano copper oxide on peanut plant leaves. *Journal of Science: Advanced Materials and Devices* **2016**, 1 (3), 343–350.
89. T. Gutmann, G. Buntkowsky. Solid-state NMR studies of supported transition metal catalysts and nanoparticles. In *Modern Magnetic Resonance*; **2018**.
90. L.E. Marbella, J.E. Millstone. NMR techniques for noble metal nanoparticles. *Chemistry of Materials* **2015**, 27 (8), 2721–2739.
91. C.K. Chiang, W.T. Chen, H.T. Chang. Nanoparticle-based mass spectrometry for the analysis of biomolecules. *Chemical Society Reviews* **2011**, 40 (3), 1269–1281.
92. C.T. Chen, Y.C. Chen. Fe3O4/TiO2 core/shell nanoparticles as affinity probes for the analysis of phosphopeptides using TiO2 surface-assisted laser desorption/ionization mass spectrometry. *Analytical Chemistry* **2005**, 77 (18), 5912–5919.

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93. F. Castelli, C. Puglia, M.G. Sarpietro, L. Rizza, F. Bonina. Characterization of indomethacin-loaded lipid nanoparticles by differential scanning calorimetry. *Int. J. Pharmaceutics* **2005**, 304 (1–2), 231–238.
94. H. Bunjes, T. Unruh. Characterization of lipid nanoparticles by differential scanning calorimetry, X-ray and neutron scattering. *Adv Drug Deliv Rev*, **2007**, 379–402.
95. K.B. Sebby, E. Mansfield. Determination of the surface density of polyethylene glycol on gold nanoparticles by use of microscale thermogravimetric analysis. *Analytical and Bioanalytical Chemistry* **2015**, 407 (10), 2913–2922.
96. E. Mansfield, K.M. Tyner, C.M. Poling, J.L. Blacklock. Determination of nanoparticle surface coatings and nanoparticle purity using microscale thermogravimetric analysis. *Analytical Chemistry* **2014**, 86 (3), 1478–1484.
97. A. Aygün, S. Özdemir, M. Gülcan, K. Cellat, F. Şen. Synthesis and characterization of Reishi mushroom-mediated green synthesis of silver nanoparticles for the biochemical applications. *Journal of Pharmaceutical and Biomedical Analysis* **2020**, 178, 112970.
98. S. Rehman, R. Jermy, S. Mousa Asiri, et al. UsingFomitopsis pinicolafor bioinspired synthesis of titanium dioxide and silver nanoparticles, targeting biomedical applications. *RSC Advances* **2020**, 10 (53), 32137–32147.
99. K. Kansara, A. Kumar, D. Bhatia. In vivo sojourn of DNA nanodevices: Taking stock of the past and perspective for future challenges & applications. *Applied NanoMedicine* **2022**, 22 (2), 337.
100. Y.H. Hsin, C.F. Chen, S. Huang, et al. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. *Toxicology Letters* **2008**, 179 (3), 130–139.
101. S. Rehman, R. Farooq, R. Jermy, et al. A wild fomes fomentarius for biomediation of one pot synthesis of titanium oxide and silver nanoparticles for antibacterial and anticancer application. *Biomolecules* **2020**, 10 (4).
102. M. Sriramulu, S. Sumathi. Photocatalytic, antioxidant, antibacterial and anti-inflammatory activity of silver nanoparticles synthesised using forest and edible mushroom. *Advances in Natural Sciences: Nanoscience and Nanotechnology* **2017**, 8 (4).
103. L. Zhang, Y. Wei, H. Wang, et al. Green Synthesis of Silver Nanoparticles Using Mushroom Flammulina velutipes Extract and Their Antibacterial Activity Against Aquatic Pathogens. *Food and Bioprocess Technology* **2020**, 13 (11), 1908–1917.
104. M.M. Al-Ansari, P. Dhasarathan, A.J.A. Ranjitsingh, L.A. Al-Humaid. Ganoderma lucidum inspired silver nanoparticles and its biomedical applications with special reference to drug resistant Escherichia coli isolates from CAUTI. *Saudi Journal of Biological Sciences* **2020**, 27 (11), 2993–3002.
105. R. Naik, S.A. Shivashankar, P.J. Bindu. In-vitro antioxidant and anticancer activities of MnFe2O4 nanoparticles synthesized using spinach leaves extract. *Applied NanoMedicine* **2022**, 22 (1), 330.
106. M. Fu, Q. Li, D. Sun, et al. Rapid preparation process of silver nanoparticles by bioreduction and their characterizations. *Chinese Journal of Chemical Engineering* **2006**, 14 (1), 114–117.
107. K. Chandran Priyadarshni, R. Krishnamoorthi, C. Mumtha, P. Ulagan Mahalingam. Biochemical analysis of cultivated mushroom, Pleurotus florida and synthesis of silver nanoparticles for enhanced antimicrobial effects on clinically important human pathogens. *Inorganic Chemistry Communications* **2022**, 142, 109673.
108. I. Ocsoy, M.L. Paret, M.A. Ocsoy, et al. Nanotechnology in plant disease management: DNA-directed silver nanoparticles on graphene oxide as an antibacterial against Xanthomonas perforans. *ACS Nano* **2013**, 7 (10), 8972–8980.
109. M.A. Dheyab, M.N. Owaid, M.A. Rabeea, A.A. Aziz, M.S. Jameel. Mycosynthesis of gold nanoparticles by the Portabello mushroom extract, Agaricaceae, and their efficacy for decolorization of Azo dye. *Environmental Nanotechnology, Monitoring and Management* **2020**, 14, 100312.
110. M.A. Rabeea, M.N. Owaid, A.A. Aziz, M.S. Jameel, M.A. Dheyab. Mycosynthesis of gold nanoparticles using the extract of Flammulina velutipes, Physalacriaceae, and their efficacy for decolorization of methylene blue. *J. Environmental Chemical Engineering* **2020**, 8 (3), 103841.
111. K.D. Lee, P.C. Nagajyothi, T.V.M. Sreekanth, S. Park. Eco-friendly synthesis of gold nanoparticles (AuNPs) using Inonotus obliquus and their antibacterial, antioxidant and cytotoxic activities. *Journal of Industrial and Engineering Chemistry* **2015**, 26, 67–72.
112. E.B. El, E.B. El Domany, T.M. Essam, A.E. Ahmed, A.A. Farghali. Biosynthesis Physico-Chemical Optimization of Gold Nanoparticles as Anti-Cancer and Synergetic Antimicrobial Activity Using Pleurotus ostreatus Fungus. *J. Applied Pharmaceutical Sci.* **2018**, 8 (05), 119–128.
113. V.K. Chaturvedi, N. Yadav, N.K. Rai, et al. Pleurotus sajor-caju-Mediated Synthesis of Silver and Gold Nanoparticles Active against Colon Cancer Cell Lines: A New Era of Herbonanoceutics. *Molecules* **2020**, 25 (13), 3091.
114. C. Zhuang, T. Mizuno, A. Shimada, et al. Antitumor Protein-containing Polysaccharides from a Chinese Mushroom Fengweigu or Houbitake, Pleurotus sajor-caju(Fr.) Sings. *Bioscience, Biotechnology, and Biochemistry* **1993**, 57 (6), 901–906.
115. T.C. Finimundy, G. Gambato, R. Fontana, et al. Aqueous extracts of Lentinula edodes and Pleurotus sajor-caju exhibit high antioxidant capability and promising in vitro antitumor activity. *Nutrition Research* **2013**, 33 (1), 76–84.
116. C.B.S. Telles, D.A. Sabry, J. Almeida-Lima, et al. Sulfation of the extracellular polysaccharide produced by the edible mushroom Pleurotus sajor-caju alters its antioxidant, anticoagulant and antiproliferative properties in vitro. *Carbohydrate Polymers* **2011**, 85 (3), 514–521.
117. J. Raman, H. Lakshmanan, P.A. John, et al. Neurite outgrowth stimulatory effects of mycosynthesized AuNPs from Hericium erinaceus (Bull.: Fr.) Pers. on pheochromocytoma (PC-12) cells. *International Journal of Nanomedicine* **2015**, 10, 5853–5863.
118. C. Dias, M. Ayyanar, S. Amalraj, et al. Biogenic synthesis of zinc oxide nanoparticles using mushroom fungus Cordyceps militaris: Characterization and mechanistic insights of therapeutic investigation. *J. Drug Delivery Sci. Technology* **2022**, 73, 103444.
119. D. Zeng, J. Zhao, K.H. Luk, et al. Potentiation of in Vivo Anticancer Efficacy of Selenium Nanoparticles by Mushroom Polysaccharides Surface Decoration. *Journal of Agricultural and Food Chemistry* **2019**, 67 (10), 2865–2876.
120. S.M. Ali, J. Raman, H. Lakshmanan, et al. Surface Decoration of Selenium Nanoparticles by Proteins from the Culinary-Medicinal Shiitake Mushroom, Lentinus edodes (Agaricomycetes), for Enhanced Fibrinolytic Activity. *Int. J. Med. mushrooms* **2018**, 20 (11), 1021–1030.
121. G. Liu, X. Yang, J. Zhang, et al. Synthesis, stability and anti-fatigue activity of selenium nanoparticles stabilized by Lycium barbarum polysaccharides. *Int. J. Biological Macromolecules* **2021**, 179, 418–428.

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Recent advancement of Polymeric transdermal drug delivery system for emetic drug therapy

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Abstract

Thisworkdescribetheformulation and characterization of Ondansetron HCL transdermal patchestomanage and deliverdrugcontentinappropriatemanner. The purpose of this research study focused on the development of polymer base transdermal films containing Ondansetron HCL by solvent evaporation technique and analysis of dissolution kinetics. Auxiliary substances represented an important role in pharmaceutical forms, in first stage, drug content and excipients compatibility were performed and verifiedbetween TDS1 9.80 ± 0.125 $\mu g \cdot cm^{-2} \cdot h^{-1}$TDS3 20.12 ± 0.125 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and TDS8 22.11 ± 0.125 $\mu g \cdot cm^{-2} \cdot h^{-1}$. Release studies were done at36°C transdermal drug delivery system ex-vivo drug permeation study was found optimized results. In-vitro drug release kinetics were done at 32 °C ± 1 °C with a Franz diffusion cell. The results obtained were analysed and confirms the active substances with two matrix-forming polymers of FT-IR. At pH 5.5 to 7.4, flux values were between 15.22 ± 0.125 $\mu g \cdot cm^{-2} \cdot h^{-1}$, 20.12 · 0.380 $\mu g \cdot cm^{-2} \cdot h^{-1}$; 30.12 ± 0.380 $\mu g \cdot cm^{-2} \cdot h^{-1}$; 40.2 ± 0.380 $\mu g \cdot cm^{-2} \cdot h^{-1}$; 22.11 ± 0.380 $\mu g \cdot cm^{-2} \cdot h^{-1}$; 30.14 ± 0.380 $\mu g \cdot cm^{-2} \cdot h^{-1}$; 50.11 ± 0.380 $\mu g \cdot cm^{-2} \cdot h^{-1}$. Thisstudyconfirmed therate of penetration and creatingnovelapproaches of Ondansetron HCL deliverywithpolymersolution. Thisnovelapproaches of Ondansetron HCL loaded transdermal patcheshasincreasedtheapplication of antiemeticdrug of theirconventionalroutes of drugadministrationviaskindelivery.

Keywords: Drugdeliverysystem, Anti-emetics, Polymericpatch, Skinpenetration.

1. Introduction

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Antiulcer effect of *Euphorbia neriifolia* Linn leaf extract

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Introduction: Ulcer is a common gastrointestinal disorder which is seen among many people. It is basically an inflamed break in the skin or the mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance.

Aim & Objective: Present study to evaluate the antiulcer potential of *Euphorbia neriifolia* linn. Leaf extract

Methods: The 70% v/v hydro-alcoholic extract of dried leaves of *Euphorbia neriifolia* was evaluated for its antiulcer activity using two models. Models are pylorus ligation induced gastric ulcers model and ethanol induced gastric ulcer model in mice. It was found that the hydro-alcoholic extract of leaves have significant antiulcer activity in dose dependent manner where 3 different oral doses prepared (100 mg/kg of body weight, 200 mg/kg of body weight and 400 mg/kg of body weight). Evaluation was done on both models comparing with reference standard Omeprazole (20 mg/Kg/ p. o.).

Result: The compounds like sugar, tannins, flavonoids, alkaloids, 24-methylene cycloartenol, triterpennoidal and saponins were detected by usual chemical test in hydro-alcoholic extract.

Summary & Conclusion: The above result shows that *Euphorbia neriifolia* leaves probably contains some active ingredients that could be developed for above mentioned abnormal condition as have been claimed by traditional system of medicine.

Key words: *Euphorbia neriifolia, gastric ulcer, ethanol, pylorus.*

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INTRODUCTION

Many groups and civilizations throughout the world have used herbal remedies from the ancient era. Since a few decades ago, the number of individuals using herbal remedies without a prescription has increased. Since they come from natural sources, they are typically seen to be safe. Herbal preparations, such as anti-diabetics, anti-arthritics, aphrodisiacs, hepatoprotective,

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REVIEW ARTICLE

Natural Products-based Drugs: Potential Drug Targets Against Neurological Degeneration

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Abstract: Phytochemicals or natural products have been studied extensively for their potential in the treatment of neurodegenerative diseases (NDs) like Parkinson's disease, Alzheimer's disease, etc. The neuronal structure loss and progressive dysfunction are the main characteristics of these diseases. In spite of impressive and thorough knowledge of neurodegenerative molecular pathways, little advancement has been found in the treatment of the same. Moreover, it was proved that natural products can be used efficiently in the treatment of NDs while certain issues regarding the patient's safety and clinical data are still existing. As ND is a bunch of diseases and it will start the myriad of pathological processes, active targeting of the molecular pathway behind ND will be the most efficient strategy to treat all ND-related diseases. The targeting pathway must prevent cell death and should restore the damaged neurons. In the treatment of ND and related diseases, natural products are playing the role of neuroprotective agents. This review will target the therapeutic potential of various phytochemicals which shows neuroprotective action.

Keywords: Neurodegeneration, phytochemicals, Alzheimer's disease, targeting, Parkinson's disease, neuroprotective action.

1. INTRODUCTION

Neurodegenerative disorders (NDs) are a group of chronic, progressive central nervous system disorders, characterized by the degradation, and subsequent loss of neurons. NDs are an increasing source of death and morbidity worldwide, especially among the elderly, making them one of the most promising public health issues. Because of their irreversibility, lack of effective treatment, and associated social and economic consequences, age-related diseases such as NDs are becoming increasingly relevant. Acute neurodegeneration is a disorder in which neurons are injured rapidly and frequently die as a result of traumatic events including, strokes, head injury, traumatic brain injury, ischemic brain damage, and cerebral or subarachnoid hemorrhage. Whereas, chronic neurodegeneration is a long-term condition in which neurons in the nervous system experience a neurodegenerative process that begins slowly and worsens over time due to a variety of factors, resulting in the irreversible death of explicit neuron inhabitants. Chronic neurodegenerative disorders include Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, and Huntington's disease [1].

Alzheimer's disease is an age-related, chronic, and progressive neurological illness that causes memory and cognitive impairments, as well as behavioral changes. It is characterized by two important neuropathological characteristics such as production and deposition of the extracellular amyloid-beta (Aβ) plaques in the brain, and protein accretion of intracellular hyperphosphorylated tau proteins termed neurofibrillary tangles. Parkinson's disease is a chronic and progressive neurological illness that affects motor functioning by causing a progressive loss of dopaminergic nigrostriatal neurons. It causes bradykinesia, postural imbalance, resting tremor, and muscular rigidity. Parkinson's disease is distinguished by the accretion of Lewy bodies, Lewy neurites, and intracellular protein aggregates, which are primarily composed of the misfolded and aggregated forms of presynaptic protein alpha (α)-synuclein, as well as the progressive loss of dopaminergic nigrostriatal neurons [2, 3].

Amyotrophic lateral sclerosis (ALS) is another progressive neurodegenerative disorder that is characterized by the progressive degeneration and death of upper and lower motor neurons, leading to paralysis and death from respiratory failure.

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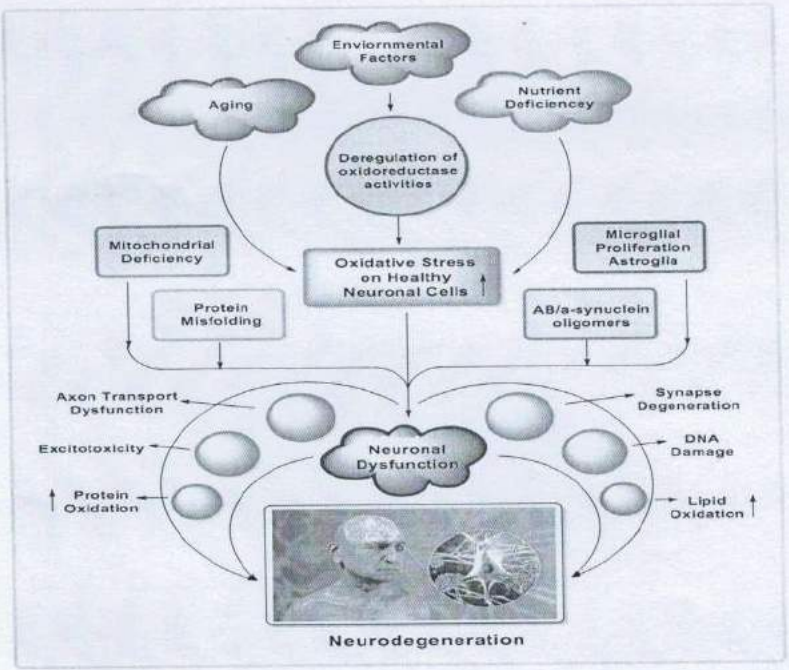

Fig. (1). Therapeutic drug targets and their mechanisms associated with neurodegeneration. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

The mechanisms underlying amyotrophic lateral sclerosis are unknown, but several factors such as genetic factors, oxidative stress, excitotoxicity, impaired axonal transport, autoimmune response, environmental factors, mitochondrial dysfunction, and neurofilament aggregation have been considered. Amyotrophic lateral sclerosis is allied to a mutation in the gene that codes for the copper/zinc superoxide dismutase-1 (SOD1) enzyme. Whereas, Huntington's disease is a condition of neurological illness that is pathologically defined by enhanced dopaminergic activity and reduced gamma-aminobutyric acid (GABA) functioning in the basal ganglia, and clinically defined by psychiatric disturbances, cognitive deficits, and abnormal movements. It is triggered by a trinucleotide reiteration development of the nucleotide's cytosine, adenine, and guanine (a CAG expansion) in the Huntingtin (HTT) gene, which is located on the petite arm of chromosome-4 [4-8].

2. POTENTIAL DRUG TARGETS OF NATURAL PRODUCTS FOR NEURO REGENERATION AND THEIR MECHANISM OF ACTION

Neurodegenerative disorders are characterized by protein aggregation, inflammation, as well as oxidative stress (OS) in the central nervous system. Several biological progressions are associated with neurodegenerative disorders including neurotransmitter diminution or inadequate synthesis, oxidative stress, and atypical ubiquitination. The mechanisms underlying neurodegenerative diseases are intricate and multifactorial. NDs exhibit some communal characteristics such as abnormal cellular transport, abnormal protein deposition, inflammation, mitochondrial deficits, intracellular Ca^{2+} overload, excitotoxicity, and uncontrolled generation of ROS, implying the existence of converging neurodegeneration pathways and emphasizing the characteristics of these pathways as common targets for intervention tactics. The therapeutic drug targets and their mechanisms associated with the neurodegeneration that are implicated in the progression and pathogenesis of neurodegenerative disorders are described in Fig. (**1**).

Investigations based on natural products and their metabolites have been demonstrated to be an effective methodology in the development of newer, novel, physiologically active, and innovative medications. Natural product-based drug discoveries have sparked a lot of interest over the last three decades. According to an investigation, at least one-third of the marketed drugs have originated or derived from various natural resources. Natural products and their isolated natural

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Table 1. Natural products and their bioactive compounds with neuroprotective activities in the treatment of neurodegenerative disorders.

S. No.	Phytochemicals/ Natural Products	Treatment of Diseases	Neuroprotective Activities	References
1.	*Capsicum annuum* (Solanaceae)	Parkinson's disease	Prevented the neuronal degeneration in substantial nigra, cerebral cortex, and hippocampus by attenuating brain 5-lipoxygenase activity, inhibited the increase of brain malondialdehyde and nitric oxide levels, restored the brain GSH level, cholinesterase activity, and paraoxonase-1 (PON1) activity.	[1]
2.	*Curcuma longa* (Zingiberaceae)	Alzheimer's and Parkinson's disease	Improvement in motor performance and gross behavioral activity reversed GFAP and iNOS protein expressions, prevented the depletion of dopamine and tyrosine hydroxylase immunoreactivity, and reduced proinflammatory cytokines and total nitrite generation in the striatum.	[2, 3,4]
3.	*Tinospora cordifolia* (Menispermaceae)	Parkinson's disease	Reduced oxidative stress, increased dopamine levels and complex I activity, and restored 6-OHDA-induced behavioral changes in locomotor activity.	[6, 7, 8]
4.	*Apium graveolens* (Apiaceae)	Parkinson's disease	Attenuation of oxidative stress reduced monoamine oxidase activity, and protected dopaminergic neurons.	[9]
5.	Osmotin (*Nicotiana tabacum*)	Alzheimer's disease	Attenuation of Aβ accumulation and BACE-1 expression ameliorated memory impairment, prevented Aβ-induced neurotoxicity of neuronal HT22 cells and primary cultures of hippocampal neurons, and reversed synaptic deficits.	[12]
6.	β-Caryophyllene	Parkinson's disease	Attenuation of proinflammatory cytokines (IL-1b, IL-6, and TNF-α) in midbrain tissues and inflammatory mediators (COX-2 and iNOS expressions) in the cytoplasmic fraction of striatal tissue lysates, prevented the dopaminergic neuronal loss in the substantia nigra and striatal dopamine fibers, restored antioxidant enzymes and glutathione depletion and inhibited lipid peroxidation.	[3, 9]
7.	*Tribulus terrestris* (Zhygophyllaceae)	Parkinson's disease	Attenuation of inflammatory markers (iNOS and COX-2 mRNA expression), suppression of oxidative stress by increasing GSH and superoxide dismutase and catalase activities, ameliorated motor dysfunction, downregulation of CD11b mRNA expression (microglia marker), and improved striatal dopamine level.	[6, 13]
8.	Methanolic extract of *Lactuca capensis*	Alzheimer's disease	Attenuation of hippocampal apoptosis by lowering the enrichment factor of apoptosis, ameliorated cognitive impairment and memory deficits, decreased the lipid peroxidation and protein oxidation level, and acetylcholinesterase activity, and restored the level of GSH and activities of antioxidant enzymes (superoxide dismutase and glutathione peroxidase).	[14]
9.	*Nigella sativa* (Ranunculaceae)	Lateral and Multiple Sclerosis	Enhancement of remyelination in the cerebellum, decreased transforming growth factor beta-1 (TGF-β1) expression and suppressed inflammation.	[15,16]
10.	Safflower yellow	Alzheimer's disease	Downregulation of M1 microglial markers (iNOS and CD86) and upregulation of M2 microglial markers (arginase-1, CD2066, and YM-1), decreased inflammatory markers (iNOS, IL-1β, IL6, and TNF-α levels), reduced neuronal cell loss in hippocampus and cortex, and inhibited the activation of glial cells.	[17-19]
11.	Isogarcinol (*Garcinia mangostana*)	Lateral and Multiple Sclerosis	Alleviation of inflammation and demyelination in the brain and spinal cord, reduced number of cells differentiation by inhibiting Janus kinase (JAK)/ signal transducers and activators of transcription (STAT) signaling pathways, and reduced the activation of $CD4^+$ and $CD11b^+$ cell populations.	[20, 21, 25, 26]
12.	Rosmarinic acid	Parkinson's disease	Upregulation of the ratio of Bcl-2/Bax gene expression in the substantia nigra, increased the number of tyrosine hydroxylase, decreased the iron level in the substantia nigra, and restored striatal dopamine level.	[26, 27]
13.	White grape (Vitis vinifera)	Lateral and Multiple Sclerosis	Reduced TNF-α, iNOS, and PARP expression and nitro-tyrosine level, and inhibited the apoptosis (caspase-3 and Bcl-2 expressions).	[21, 22]
14.	Huperzine A (*Huperzia serrata*)	Alzheimer's disease	Improvement in memory, cognitive, and behavior functions, protects neurons from cytotoxicity and apoptosis, reduced Aβ-induced neuronal cell death, inhibited glutamate toxicity, and decreased oxidative damage.	[21, 26]
15.	Walnut extract	Lateral and Multiple Sclerosis	Downregulation of iNOS and Iba-1 expressions, and upregulation of calmodulin expressions.	[23a,b]

compounds have the potential to be neuroprotective and therapeutic agents as well as valuable resources for drug discovery against various neurodegenerative diseases. The therapeutic potential of natural products and their bioactive compounds to exert a neuroprotective effect on the pathologies of neurodegenerative disorders is described in Table 1 [9].

3. CLINICAL TRIALS ON VARIOUS NATURAL PRODUCTS FOR THEIR NEURO REGENERATIVE EFFECTS/NEUROPROTECTIVE EFFECTS

Several possible interventions aimed at various targets are being developed and tested in ongoing clinical trials, including anti-amyloid and anti-tau interventions, cognitive

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Table 2. Clinical studies on Natural products/phytochemicals used in the treatment of neurodegenerative disorders.

S. No.	Phytochemicals	Mechanism of Action	NCT Number	Status
1.	Ginkgo biloba	Antioxidant and anti-amyloid aggregation	NCT03090516	Recruiting
2.	Guanfacine	Alpha-2A-adrenoceptor agonist, a potent 5-HT2B receptor agonist	NCT03116126	Recruiting
3.	Coconut oil	Reduction in ADP-ribosylation factor 1 protein expression	NCT01883648	Terminated

enhancement, anti-neuroinflammation and neuroprotection interventions, neurotransmitter modification, and interventions to relieve behavioral psychological indications. The status of clinical studies on various natural products and their mechanism of action in the treatment of neurodegenerative disorders are described in Table **2**.

4. NEUROPROTECTIVE EFFECTS OF VARIOUS NATURAL PRODUCTS

A no. of natural products can elicit neuroprotective effects for the prevention of neurodegeneration. The use of natural products is widely supported by literature-based facts as they elicit various activities by different mechanisms and due to their multiple mechanisms of action, they do not cause resistance to the therapy and also the side effects of the natural products are minimal. Fig. (**2**) depicts the mechanism of action and the neuroprotective effects of various natural products [4].

The various natural (herbal compounds) eliciting a no. of mechanisms to act as neuroprotective are mentioned below:

- Resveratrol
- Quercetin
- Genistein
- Luteolin
- Apigenin
- Hesperidin
- Cyanobacteria
- Marine Macroalgae
- Other natural compounds

4.1. Resveratrol

Resveratrol (RSV) belongs to the class of phenolic stilbene compounds which is found in most of the flavonoid compounds of red wine, grapes, and nuts. A number of researchers had worked on RSV and they reported RSV to possess various activities like anticancer, antioxidant, neuroprotective, cardiovascular, antidiabetic, and many other effects. RSV works by scavenging reactive oxygen species (ROS) from the blood and thereby increasing the glutathione (GTH).it was found that the nanocapsule loaded RSV has more brain targeting efficacy as compared to the free RSV. The bio absorbance of RSV is good in the gastrointestinal tract but due to their rapid metabolism and clearance from the body, RSV lacks bio specificity. RSV was proven to reduce the inflammatory response by suppressing nitric oxide, Tumor necrosis factor and interleukins (IL-1B and IL-6) of astrocytes. Also, the removal of nuclear factor kappa B (NF-KB) also decreases the production of TNF and ILs. One study also revealed the fact that RSV significantly decreases the profile of mood states (POMS) and other fatigue and related properties but has no impact on memory and performance [10-13].

4.2. Quercetin

Quercetin (QCT) is a very well-known flavonoid compound that occurs in many herbs such as apples, red and black berries, pasta, tomatoes, and grapes. It is the most common flavonoid found in edible plants and is well known for its antioxidant properties. It had been reported that QCT enhances memory, learning properties, and consciousness in patients with neurological disorders such as Alzheimer's disease. Pharmacologically, QCT posses antiviral, anticancer, antioxidant, anti-inflammatory, and anti-amyloid effects. It was reported to induce the removal of end products of plasma daily with a half-life of 11-28 hours which makes it available to the body daily by the production inside the body. The higher amount of QCT can cross blood-brain barrier (BBB) when entrapped in liposomal preparations as compared to the free QCT which can further enhance the chances of higher neurotoxicity. In scopolamine induce loss impairment of memory in zebrafish, QCT was found to have memory-boosting effects with improved chlorogenic neurotransmission, found in one study. Moreover, there was a further need to have the toxicity data of the drug to determine its safety: toxicity data of the drug. It was observed the beta-mediated apoptosis was significantly reduced at the lower doses of the drug while the cytotoxicity was induced at the higher doses of the same. The ability of QCT to cross the BBB and the residence period & quantity of its metabolites in the brain are the most important factors in determining the dose of the QCT. Furthermore, the coadministration of alpha-tocopherol along with QCT was found to promote the crossing of BBB by QCT [2, 14-18].

4.3. Genistein

Genistein is abundantly found in soy isoflavone and is the type of bioflavonoid that exhibit multiple activities like antioxidant, anti-inflammatory, cardiovascular, anticancer, and proapoptotic properties like natural estrogen, protein tyrosine kinase inhibition activity and many other intercellular and intracellular activities. The evidences are there to support the fact that genistein inhibits various destructive conditions like atherosclerosis, estrogen deficiency and polycystic ovarian diseases, and hormone imbalanced diseases like hypothyroidism. Recently, the neuroprotective activity of genistein was evaluated by researchers. It was evaluated

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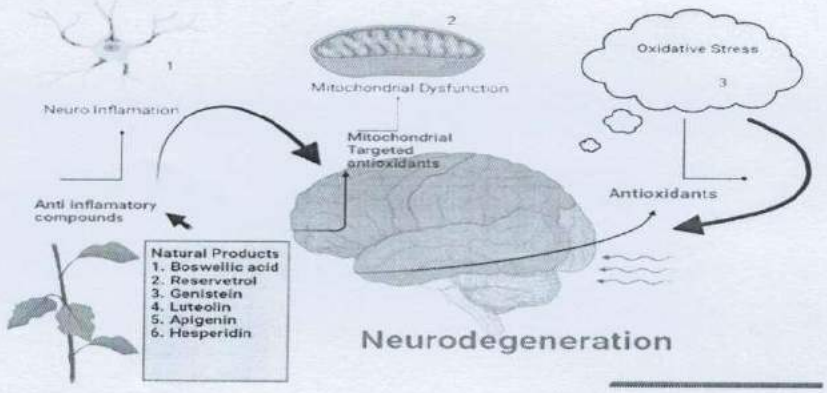

Fig. (2). Mechanism of Neurodegeneration and the role of natural products as neuroprotective. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

that genistein was effective in treating the neurotoxicity induced by a beta peptide in the neuronal cells of the wistar rats. It was also confirmed that genistein has the ability to cross the BBB and due to its antioxidant properties, it acts as a neuroprotective. Also, it can cross BBB, it was proven to be neuroprotective for a longer period of time [19-24].

4.4. Luteolin

Luteolin (Lu), being an isoflavone occurs in yellow color and crystalline appearance. It is commonly found in the families like Bryophyta, and Pteridophyte. In foods, it occurs in oregano, carrots, onion, olives, thyme, and peppermint, *etc*. it possesses various activities like antioxidant, neuroprotective, anti-inflammatory, anticancer, and anti-microbial properties, *etc*., and the antioxidant activity is associated with free radical scavenger activity of this plant. Due to this, Lu is able to remove the reactive oxygen species from our body and act as an antioxidant. This antioxidant action is one of the very important activities possessed by natural plants. The antioxidant action can serve as one of the important pathways in possessing the anticancer and neuroprotective action by the herb [25-27]. One study revealed the fact that Lu had directly inhibited the zinc-induced hypo phosphorylation not just because of its anti-oxidant activity but also through the regulatory mechanism of the tyrosine phosphokinase system. It was also reported that the BBB crossing activity of the Lu greatly enhanced when transported along with the Vitamin E Tocopherol [28-32].

4.5. Apigenin

Apigenin (Ap) is a subcategory of flavones and flavonoids. Very few evidences are there to support the fact that the Ap in the normal; diet can promote *in vivo* metabolic adverse reactions. Ap posses very important functions such as antioxidant, anti-inflammatory, anti-cancer, anti-proliferator, and anti-microbial properties. It also inhibits the strong metabolizing enzyme CYP450 and is the metabolizer of many prescription-based drugs. Moreover, it was also revealed by the researchers that, Ap is water soluble and intestinal-permeable flavonoid. The different transporters present in the intestine can absorb as well as transport the Ap in a good manner and the duodenum is the main site for absorption. Apart from this, it also posses various activities like antioxidants, anticancer, neuroprotective, enzymes inhibitor, anti-genotoxic, and anti-proliferator, *etc*. [33-37].

4.6. Hesperidin

Hesperidin (Hes)occurs in flavone glycosides such as oranges, lemons, sweet oranges, and grapes. Hes is a great memory booster as the administration of Hes for 16 weeks in transgenic mice enhanced the memory by enhancing the recognition index. Hes also impaired the learning and memory impairment caused by $AlCl_3$ and thereby functions as an anti-acetylcholinesterase. It possesses neuroprotective activities which can be well correlated from the signals of upregulation of beta cell lymphoma and downregulation of associated protein Bax. It also was reported that Hes possess neuroprotective activity against various neurological disorders like cerebral ischemia. It mainly passes through BBB easily and inhibits the release of glutamate [38-42].

4.7. Cyanobacteria

Cyanobacteria are aform of bacteria that are closely related to bacterial forms, they are prokaryotic, prosynthetic, and self-producing species. They are commonly known as blue-green algae and the members of the Oscillatoria family. Researchers have shown a keen interest in blue-green algae owing to their vast pharmacological activities. *Spirulina plantansis* is a well-known member of blue-green algae which is well known for its nutritional values. It possesses anti-inflammatory and antioxidant properties that protect it against pathogens. Also, research has shown that polysaccharides derived from spirulina possess an antioxidant effect on the dopaminergic neurons rather than the inhibition of mon-

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oamine oxidase (MAO). Another research also revealed the fact that it protects the memory damage caused by scopolamine in mice. Due to their antioxidant effect, they impart neuroprotective effects. It can cross BBB as suggested by the presence of c-phycocyanin which is a product of spirulina, in the hippocampus. Also, it has shown cytoprotective activity against neurodegeneration *via* an antioxidant mechanism [38, 39, 43-45].

4.8. Marine Macroalgae

Marine macroalgae (MM) is a plant-like substance which is typically found in coastal areas and well-known by the name seaweed. The common three groups are there viz. green, brown, and red algae. The main components of MM are phenolic compounds, phenols, proteins, pigments and a variety of other compounds are also present. Research revealed the fact that the compounds have good health impacts. One study revealed the high radical scavenging activity of the carotenoids. To add further to this, many studies suggested that they enhance cell viability and decrease oxidative stress, and have a healthy mitochondrial potential. These statements suggest the neuroprotective impact of the algae due to their antioxidant potential. However, researchers focused on their commercial use and tend to formulate the drug delivery system of the formulations particularly the nano drug delivery systems for the bacterial algae. However, the use of marine algae as a commercial drug delivery system is limited because of its inability to cross BBB [45-49].

4.9. *Astragalus Membranaceus*

Astragalus membranaceus (AM) is a terpenoid drug commonly used for recovery after a stroke in China. Clinical trials were performed to evaluate the clinical potential of the drug and the drug was found to be very effective in recovering patients after stroke [47, 50-53].

4.10. Other Natural Products as Neuro Protectives

A number of other compounds have been reported in the literature which possesses neuroprotective activities. Several compounds which treat neurodegeneration to a great extent are reported in Table **3**. The main mechanism includes their anti-oxidant potential, mitochondrial stabilization impact, anti-proliferative, free radical scavenging activity, and anti-cancer potential.

5. NATURAL COMPOUNDS TO MITIGATE EXCITOTOXICITY AND INFLAMMATION IN NEURODEGENERATION

Oxidative stress is responsible for the injury of vascular endothelium. It leads to tissue damage and occlusion of the artery followed by reperfusion. The specific reason behind this is the imbalance between the production and scavenging of the reactive oxygen species (ROS). Moreover, ischemic stroke happens as a result of the increased concentration of glutamate release which increases the concentration of Ca^{2+} ions. Furthermore, the elevated calcium ions result in the induction of enzymes such as proteases, phospholipase, and oxidase which further leads to the damage of DNA. Enhanced concentration of calcium ions also increases the concentration of lipid peroxidase (LPO) which further produces more free radicals and enhancement of oxidative stress also occurs in response to that. In lieu of this, some phytochemicals such as *Mucuna pruriensplant* or commonly known as velvet bean or Kapikacchu which contains a huge amount of natural antioxidants had proven to be beneficial in the reduction of oxidative stress and ROS. This is an Indian traditional herb that was previously used for the treatment of arthritis and depression and has shown its potential as a neurological substance due to its antioxidant activity [54-59].

6. ROLE OF NANOTECHNOLOGY-BASED DRUG DELIVERY FOR NEUROPROTECTIVE NATURAL PRODUCTS

The various phytoconstituents are responsible for a vast number of activities including their anticancer, antioxidant, and neuroprotective actions. However, their actions are restricted due to their solubility and bioavailability-related problems. Raw forms of natural products, if administered in the conventional forms, they suffered from the problem of bioavailability. Therefore, the lacks the scaleup from lab scale to commercialization. Nanotechnology has come up with the idea to overcome this problem. Nanotechnology-based drug delivery systems like nanosponges, nanoemul-

Table 3. Various plants and their products for their neuroprotective potential.

Sr. No.	Name of the Plant/Plant Part	Pharmacological Action	References
1	Extract of Yacon leaves	Prevents the memory defects	[18]
2	Aqueous extract of safflower	Improvement of short-term memory loss	[19]
3	*Lactucacapensis thunb.* leaves methanolic extract	Decrease the lipid peroxidation and protein oxidation	[20]
4	Haldi Powder	Improved quality of life	[2, 25]
5	Coconut oil	Improved cognitive functions	[21]
6	Osmotin	Enhanced random alterations	[26]
7	Brown rice (Germinated)	Reduced ROS activity	[27]

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sions, nanogels, nano micelles, and nanoparticles can improve the solubility and specificity of the natural bioactive. Also, the active targeting concept has brought more specificity to the phytoconstituents. The compounds specifically attack their site of action and prevent the side effects [56, 60-64].

7. LIMITATIONS, CHALLENGES, AND FUTURE PROJECTIONS

The process of natural neuroprotection against various neurodegenerative diseases is widely accepted nowadays by using various natural herbs whole or using their products. Despite a number of favorable incidents depicting the use of natural herbs for the treatment of neurodegeneration, a successful transformation of these herbs from research to commercialization are still lacking as preclinical evidence are there but clinical testing of the same is still lacking. Natural products and their byproducts face many challenges from the point of view of their solubility, biostability, physical and chemical stability, metabolism, BBB crossing, and therapeutic efficacy. A number of natural compounds like resveratrol, turmeric, and apigenin possess numerous neuroprotective potentials but face the problem in respect of stability, solubility, and bioavailability. The BBB prevents the crossing of the compounds and hereby hinders their movement from blood to the brain. However, nanotechnology and nanocarriers can play an important role in their bioavailability enhancement. Natural products when entrapped in nanocarriers can increase the bioavailability and stability of the products. Various types of nanocarriers like nanogels, nanoparticles, emulgel, nanosuspension, self-micro emulsifying drug delivery systems, nanostructured lipid carriers, and Nano micelles can be prepared for the delivery of phytoconstituents. A number of studies have been published in the literature in which nanocarriers have been used for the entrapment of phytoconstituents and they significantly enhanced their stabilization and solubilization [65-68].

CONCLUSION

The therapeutic potential of phytoconstituents to act as neuroprotective has been supported by a vast number of literature. Natural compounds are well known for their bioactivities such as reactive oxygen scavenging activity, antioxidant action, antiproliferative action, antimicrobial, anticancer, and neuroprotective actions. Various compounds such as luteolin, hesperidin, resveratrol, genistein, and many other compounds are there which are much more effective against neurodegeneration. However, their actions are limited due to their solubility, stability, and therapeutic efficacy-related problems. It was envisaged from the published data that the therapeutic action of the natural compounds can be enhanced by incorporating the phytoconstituents in the nanocarriers such as nanoparticles, nanogels, and nanostructured lipid carriers. They can enhance the stability and specificity of the bioactive compounds to a larger extent. The use of nanotechnology can also provide targeting which enhances their specificity to the respective site of action.

LIST OF ABBREVIATIONS

ALS	=	Amyotrophic Lateral Sclerosis
AM	=	*Astragalus membranaceus*
BBB	=	Blood-brain Barrier
LPO	=	Lipid Peroxidase
MAO	=	Monoamine Oxidase
MM	=	Marine Macroalgae
NDs	=	Neurodegenerative Disorders
OS	=	Oxidative Stress
ROS	=	Reactive Oxygen Species

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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REFERENCES

[1] Abdel-Salam, O.M.; Sleem, A.A.; Youness, E.R.; Yassen, N.N.; Shaffie, N.; El-Toumy, S.A. Capsicum protects against rotenone-induced toxicity in mice brain via reduced oxidative stress and 5-lipoxygenase activation. *J. Pharm. Pharmacogn. Res.*, **2018**, *2*(3), 60-77.

[2] Butterfield, D.A. Perspectives on oxidative stress in Alzheimer's disease and predictions of future research emphases. *J. Alzheimers Dis.*, **2018**, *64*(s1), S469-S479. http://dx.doi.org/10.3233/JAD-179912 PMID: 29504538

[3] (a) Jiang, T.; Sun, Q.; Chen, S. Oxidative stress: A major pathogenesis and potential therapeutic target of antioxidative agents in Parkinson's disease and Alzheimer's disease. *Prog Neurobiol.* **2016**, *147*, 1-19. http://dx.doi.org/10.1016/j.pneurobio.2016.07.005
(b) Paül, Valerià. "Hopes for the countryside's future. An analysis of two endogenous development experiences in south-eastern galicia. *J. Urban Region. Analysis.*, **2023**, 5(2), 169-192.

[4] da Costa, I.M.; de Moura Freire, M.A.; de Paiva Cavalcanti, J.R.L.; de Araújo, D.P.; Norrara, B.; Moreira Rosa, I.M.M.; de Azevedo, E.P.; do Rego, A.C.M.; Filho, I.A.; Guzen, F.P. Supplementation with Curcuma longa reverses neurotoxic and behavioral damage in models of Alzheimer's disease: a systematic review. *Curr. Neuropharmacol.*, **2019**, *17*(5), 406-421. http://dx.doi.org/10.2174/1570159X15666180117112610 PMID: 29338678

[5] Reddy, P.H.; Manczak, M.; Yin, X.; Grady, M.C.; Mitchell, A.; Kandimalla, R.; Kuruva, C.S. Protective effects of a natural product, curcumin, against amyloid β induced mitochondrial and synaptic toxicities in Alzheimer's disease. *J. Investig. Med.*, **2016**, *64*(8), 1220-1234. http://dx.doi.org/10.1136/jim-2016-000240 PMID: 27521081

[6] Mohd Sairazi, N. S.; Sirajudeen, K. Natural products and their bioactive compounds: neuroprotective potentials against neuro-

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degenerative diseases. *Evid. Based Complementary Altern. Med.*, **2020**, *2020*, 6565396.
http://dx.doi.org/10.1155/2020/6565396

[7] Rahman, M.H.; Bajgai, J.; Fadriquela, A.; Sharma, S.; Trinh, T.T.; Akter, R.; Jeong, Y.J.; Goh, S.H.; Kim, C.S.; Lee, K.J. Therapeutic potential of natural products in treating neurodegenerative disorders and their future prospects and challenges. *Molecules*, **2021**, *26*(17), 5327.
http://dx.doi.org/10.3390/molecules26175327 PMID: 34500759

[8] Ludovici, V.; Barthelmes, J.; Nägele, M.P.; Enseleit, F.; Ferri, C.; Flammer, A.J.; Ruschitzka, F.; Sudano, I. Cocoa, blood pressure, and vascular function. *Front. Nutr.*, **2017**, *4*, 36.
http://dx.doi.org/10.3389/fnut.2017.00036 PMID: 28824916

[9] Maher, P. Protective effects of fisetin and other berry flavonoids in Parkinson's disease. *Food Funct.*, **2017**, *8*(9), 3033-3042.
http://dx.doi.org/10.1039/C7FO00809K PMID: 28714503

[10] Nakajima, A.; Ohizumi, Y. Potential benefits of nobiletin, a citrus flavonoid, against Alzheimer's disease and Parkinson's disease. *Int. J. Mol. Sci.*, **2019**, *20*(14), 3380.
http://dx.doi.org/10.3390/ijms20143380 PMID: 31295812

[11] Ullah, A.; Munir, S.; Badshah, S.L.; Khan, N.; Ghani, L.; Poulson, B.G.; Emwas, A.H.; Jaremko, M. Important flavonoids and their role as a therapeutic agent. *Molecules*, **2020**, *25*(22), 5243.
http://dx.doi.org/10.3390/molecules25225243 PMID: 33187049

[12] Li, F.; Gong, Q.; Dong, H.; Shi, J. Resveratrol, a neuroprotective supplement for Alzheimer's disease. *Curr. Pharm. Des.*, **2012**, *18*(1), 27-33.
http://dx.doi.org/10.2174/138161212798919075 PMID: 22211686

[13] Albani, D.; Polito, L.; Signorini, A.; Forloni, G. Neuroprotective properties of resveratrol in different neurodegenerative disorders. *Biofactors*, **2010**, *36*(5), 370-376.
http://dx.doi.org/10.1002/biof.118 PMID: 20848560

[14] López-Miranda, V.; Soto-Montenegro, M.L.; Vera, G.; Herradón, E.; Desco, M.; Abalo, R. Resveratrol: a neuroprotective polyphenol in the Mediterranean diet. *Rev. Neurol.*, **2012**, *54*(6), 349-356.
PMID: 22403148

[15] Dajas, F. Life or death: Neuroprotective and anticancer effects of quercetin. *J. Ethnopharmacol.*, **2012**, *143*(2), 383-396.
http://dx.doi.org/10.1016/j.jep.2012.07.005 PMID: 22820241

[16] Boyina, H.K.; Geethakhrishnan, S.L.; Panuganti, S.; Gangarapu, K.; Devarakonda, K.P.; Bakshi, V.; Guggilla, S.R. *In silico* and *in vivo* studies on quercetin as potential anti-Parkinson agent. *GeNeDis 2018. Genetics and Neurodegeneration*, **2020**, 1-11.
http://dx.doi.org/10.1007/978-3-030-32633-3_1 PMID: 32468451

[17] Aciıkara, O.B.; Karatoprak, G.Ş.; Yücel, Ç.; Akkol, E.K.; Sobarzo-Sánchez, E.; Khayatkashani, M.; Kamal, M.A.; Kashani, H.R.K. A critical analysis of quercetin as the attractive target for the treatment of parkinson's disease. *CNS Neurol Disord Drug Targets*, **2022**, *21*(9), 795-817.

[18] Tavares Luiz, M.; Delello Di Filippo, L.; Carolina Alves, R.; Sousa Araújo, V.H.; Lobato Duarte, J.; Maldonado Marchetti, J.; Chorilli, M. The use of TPGS in drug delivery systems to overcome biological barriers. *Eur. Polym. J.*, **2021**, *142*, 110129.
http://dx.doi.org/10.1016/j.eurpolymj.2020.110129

[19] Trieu, V.N.; Uckun, F.M. Genistein is neuroprotective in murine models of familial amyotrophic lateral sclerosis and stroke. *Biochem. Biophys. Res. Commun.*, **1999**, *258*(3), 685-688.
http://dx.doi.org/10.1006/bbrc.1999.0577 PMID: 10329446

[20] (a) Aras, A.B.; Guven, M.; Akman, T.; Alacam, H.; Kalkan, Y.; Silan, C.; Cosar, M. Genistein exerts neuroprotective effect on focal cerebral ischemia injury in rats. *Inflammation*, **2015**, *38*(3), 1311-1321.
(b) Duan, X.; Li, Y.; Xu, F.; Ding, H. Study on the neuroprotective effects of Genistein on Alzheimer's disease. *Brain Behav.*, **2021**, *11*(5), e02100.
PMID: 33704934

[21] Sun, X.Y.; Wei, Y.P.; Xiong, Y.; Wang, X.C.; Xie, A.J.; Wang, X.L.; Yang, Y.; Wang, Q.; Lu, Y.M.; Liu, R.; Wang, J.Z. Synaptic released zinc promotes tau hyperphosphorylation by inhibition of protein phosphatase 2A (PP2A). *J. Biol. Chem.*, **2012**, *287*(14), 11174-11182.
http://dx.doi.org/10.1074/jbc.M111.309070 PMID: 22334661

[22] Hajialyani, M.; Hosein Farzaei, M.; Echeverria, J.; Nabavi, S.; Uriarte, E.; Sobarzo-Sánchez, E. Hesperidin as a neuroprotective agent: a review of animal and clinical evidence. *Molecules*, **2019**, *24*(3), 648.
http://dx.doi.org/10.3390/molecules24030648 PMID: 30759833

[23] Kim, J.; Wie, M.B.; Ahn, M.; Tanaka, A.; Matsuda, H.; Shin, T. Benefits of hesperidin in central nervous system disorders: a review. *Anat. Cell Biol.*, **2019**, *52*(4), 369-377.
http://dx.doi.org/10.5115/acb.19.119 PMID: 31949974

[24] Kuzu, M.; Kandemir, F.M.; Yıldırım, S.; Çağlayan, C.; Küçükler, S. Attenuation of sodium arsenite-induced cardiotoxicity and neurotoxicity with the antioxidant, anti-inflammatory, and antiapoptotic effects of hesperidin. *Environ. Sci. Pollut. Res. Int.*, **2021**, *28*(9), 10818-10831.
http://dx.doi.org/10.1007/s11356-020-11327-5 PMID: 33099738

[25] Kempuraj, D.; Thangavel, R.; Kempuraj, D.D.; Ahmed, M.E.; Selvakumar, G.P.; Raikwar, S.P.; Zaheer, S.A.; Iyer, S.S.; Govindarajan, R.; Chandrasekaran, P.N.; Zaheer, A. Neuroprotective effects of flavone luteolin in neuroinflammation and neurotrauma. *Biofactors*, **2021**, *47*(2), 190-197.
http://dx.doi.org/10.1002/biof.1687 PMID: 33098588

[26] (a) Nabavi, S.F.; Khan, H.; D'onofrio, G.; Šamec, D.; Shirooie, S.; Dehpour, A.R.; Argüelles, S.; Habtemariam, S.; Sobarzo-Sanchez, E. Apigenin as neuroprotective agent: Of mice and men. *Pharmacological Research*, **2018**, *128*, 359-365.
(b) Zhao, L.; Wang, J.-L.; Liu, R.; Li, X.-X.; Li, J.-F.; Zhang, L. Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer's disease mouse model. *Molecules*, **2013**, *18*(8), 9949-9965.
PMID: 23966081

[27] Wang, D.; Liu, L.; Zhu, X.; Wu, W.; Wang, Y. Hesperidin alleviates cognitive impairment, mitochondrial dysfunction and oxidative stress in a mouse model of Alzheimer's disease. *Cell. Mol. Neurobiol.*, **2014**, *34*(8), 1209-1221.
http://dx.doi.org/10.1007/s10571-014-0098-x PMID: 25135708

[28] Liu, Q.; Huang, Y.; Zhang, R.; Cai, T.; Cai, Y. Medical application of Spirulina platensis derived C-phycocyanin. *Evid. Based Complementary Altern. Med.*, **2016**, *2016*

[29] Chen, J.-C.; Liu, K.S.; Yang, T.-J.; Hwang, J.-H.; Chan, Y.-C.; Lee, I.-T. Spirulina and C-phycocyanin reduce cytotoxicity and inflammation-related genes expression of microglial cells. *Nutr. Neurosci.*, **2012**, *15*(6), 252-256.
PMID: 22687570

[30] Kim, S.-K.; Pangestuti, R. Prospects and potential applications of seaweeds as neuroprotective agents. *Marine Nutraceuticals: Prospects and Perspectives, CRC Press: Florida, US*, **2013**, *17*.

[31] Barbalace, M.C.; Malaguti, M.; Giusti, L.; Lucacchini, A.; Hrelia, S.; Angeloni, C. Anti-inflammatory activities of marine algae in neurodegenerative diseases. *Int. J. Mol. Sci.*, **2019**, *20*(12), 3061.
http://dx.doi.org/10.3390/ijms20123061 PMID: 31234555

[32] Hannan, M.; Dash, R.; Haque, M.; Mohibbullah, M.; Sohag, A.A.M.; Rahman, M.; Uddin, M.J.; Alam, M.; Moon, I.S. Neuroprotective potentials of marine algae and their bioactive metabolites: Pharmacological insights and therapeutic advances. *Marine drugs*, **2020**, *18*(7), 347.

[33] PereirL, L.; Valado, A. The seaweed diet in prevention and treatment of the neurodegenerative diseases. *Mar. Drugs*, **2021**, *19*(3), 128.
PMID: 33652930

[34] Chen, C.-C.; Lee, H.-C.; Chang, J.-H.; Chen, S.-S.; Li, T.-C.; Tsai, C.-H.; Cho, D.-Y.; Hsieh, C.-L. Chinese herb astragalus membranaceus enhances recovery of hemorrhagic stroke: double-blind, placebo-controlled, randomized study. *Evid. Based Complementary Altern. Med.*, **2012**, *2012*

[35] Costa, I.M.; Lima, F.O.V.; Fernandes, L.C.B.; Norrara, B.; Neta, F.I.; Alves, R.D.; Cavalcanti, J.R.L.P.; Lucena, E.E.S.; Cavalcante, J.S.; Rego, A.C.M.; Filho, I.A.; Queiroz, D.B.; Freire, M.A.M.; Guzen, F.P. Astragaloside IV supplementation promotes a neuroprotective effect in experimental models of neurological disorders: a systematic review. *Curr. Neuropharmacol.*, **2019**, *17*(7), 648-665.
http://dx.doi.org/10.2174/1570159X16666180911123341 PMID: 30207235

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[36] Yang, J.; Li, J.; Lu, J.; Zhang, Y.; Zhu, Z.; Wan, H. Synergistic protective effect of astragaloside IV–tetramethylpyrazine against cerebral ischemic-reperfusion injury induced by transient focal ischemia. *J. Ethnopharmacol.*, **2012**, *140*(1), 64-72. http://dx.doi.org/10.1016/j.jep.2011.12.023 PMID: 22207211

[37] Martinez-Oliveira, P.; de Oliveira, M.F.; Alves, N.; Coelho, R.P.; Pilar, B.C.; Güllich, A.A.; Ströher, D.J.; Boligon, A.; da Costa Escobar Piccoli, J.; Mello-Carpes, P.B.; Manfredini, V. Yacon leaf extract supplementation demonstrates neuroprotective effect against memory deficit related to β-amyloid-induced neurotoxicity. *J. Funct. Foods*, **2018**, *48*, 665-675. http://dx.doi.org/10.1016/j.jff.2018.08.004

[38] Zhang, L.; Zhou, Z.; Zhai, W.; Pang, J.; Mo, Y.; Yang, G.; Qu, Z.; Hu, Y. Safflower yellow attenuates learning and memory deficits in amyloid β-induced Alzheimer's disease rats by inhibiting neuroglia cell activation and inflammatory signaling pathways. *Metab. Brain Dis.*, **2019**, *34*(3), 927-939. http://dx.doi.org/10.1007/s11011-019-00398-0 PMID: 30830599

[39] Sharma, S.; Sharma, S.; Chourasia, R.; Pandey, A.; Rai, A.K.; Sahoo, D. Alzheimer's disease: ethanobotanical studies. In: *Naturally Occurring Chemicals Against Alzheimer's Disease*; Elsevier: Amsterdam, **2021**; pp. 11-28. http://dx.doi.org/10.1016/B978-0-12-819212-2.00044-X

[40] Rahim, N.S.; Lim, S.M.; Mani, V.; Abdul Majeed, A.B.; Ramasamy, K. Enhanced memory in Wistar rats by virgin coconut oil is associated with increased antioxidative, cholinergic activities and reduced oxidative stress. *Pharm. Biol.*, **2017**, *55*(1), 825-832. http://dx.doi.org/10.1080/13880209.2017.1280688 PMID: 28118770

[41] Ali, T.; Yoon, G.H.; Shah, S.A.; Lee, H.Y.; Kim, M.O. Osmotin attenuates amyloid beta-induced memory impairment, tau phosphorylation and neurodegeneration in the mouse hippocampus. *Sci. Rep.*, **2015**, *5*(1), 11708. http://dx.doi.org/10.1038/srep11708 PMID: 26118757

[42] Mamiya, T.; Asanuma, T.; Kise, M.; Ito, Y.; Mizukuchi, A.; Aoto, H.; Ukai, M. Effects of pre-germinated brown rice on β-amyloid protein-induced learning and memory deficits in mice. *Biol. Pharm. Bull.*, **2004**, *27*(7), 1041-1045. http://dx.doi.org/10.1248/bpb.27.1041 PMID: 15256737

[43] Postu, P.A.; Noumedem, J.A.K.; Cioanca, O.; Hancianu, M.; Mihasan, M.; Ciorpac, M.; Gorgan, D.L.; Petre, B.A.; Hritcu, L. *Lactuca capensis* reverses memory deficits in Aβ1-42-induced an animal model of Alzheimer's disease. *J. Cell. Mol. Med.*, **2018**, *22*(1), 111-122. http://dx.doi.org/10.1111/jcmm.13299 PMID: 28816008

[44] Zhang, R.; Lu, H.; Tian, S.; Yin, J.; Chen, Q.; Ma, L.; Cui, S.; Niu, Y. Protective effects of pre-germinated brown rice diet on low levels of Pb-induced learning and memory deficits in developing rat. *Chem. Biol. Interact.*, **2010**, *184*(3), 484-491. http://dx.doi.org/10.1016/j.cbi.2010.01.043 PMID: 20138853

[45] Neta, F.; Da Costa, I.; Lima, F.; Fernandes, L.; Cavalcanti, J.; Freire, M.; Lucena, E.D.S.; Do Rêgo, A.M.; De Azevedo, E.; Guzen, F. Effects of *Mucuna pruriens* (L.) supplementation on experimental models of Parkinson's disease: A systematic review. *Pharmacogn. Rev.*, **2018**, *12*(23).

[46] Nayak, V.S.; Kumar, N.; D'Souza, A.S.; Nayak, S.S.; Cheruku, S.P.; Pai, K.S.R. The effects of Mucuna pruriens extract on histopathological and biochemical features in the rat model of ischemia. *Neuroreport*, **2017**, *28*(18), 1195-1201. http://dx.doi.org/10.1097/WNR.0000000000000888 PMID: 28953092

[47] Singh, J.; Mittal, P.; Vasant Bonde, G.; Ajmal, G.; Mishra, B. Design, optimization, characterization and in-vivo evaluation of Quercetin enveloped Soluplus®/P407 micelles in diabetes treatment. *Artif Cells, Nanomed Biotechnol.*, **2018**, *46*(sup3), S546-S555.

[48] Vardhan, H.; Mittal, P.; Adena, S.K.R.; Mishra, B. Long-circulating polyhydroxybutyrate-co-hydroxyvalerate nanoparticles for tumor targeted docetaxel delivery: Formulation, optimization and *in vitro* characterization. *Eur. J. Pharm. Sci.*, **2017**, *99*, 85-94. http://dx.doi.org/10.1016/j.ejps.2016.12.007 PMID: 28002762

[49] Vardhan, H.; Mittal, P.; Adena, S.K.R.; Upadhyay, M.; Mishra, B. Development of long-circulating docetaxel loaded poly (3-hydroxybutyrate-co-3-hydroxyvalerate) nanoparticles: Optimization, pharmacokinetic, cytotoxicity and *in vivo* assessments. *Int. J. Biol. Macromol.*, **2017**, *103*, 791-801. http://dx.doi.org/10.1016/j.ijbiomac.2017.05.125 PMID: 28536023

[50] Vardhan, H.; Mittal, P.; Adena, S.K.R.; Upadhyay, M.; Yadav, S.K.; Mishra, B. Process optimization and *in vivo* performance of docetaxel loaded PHBV-TPGS therapeutic vesicles: A synergistic approach. *Int. J. Biol. Macromol.*, **2018**, *108*, 729-743. http://dx.doi.org/10.1016/j.ijbiomac.2017.10.172 PMID: 29111267

[51] (a) Vardhan, H.; Pooja, M.; Sandeep, K.R.A.; Brahmeshwar, M. Long-circulating polyhydroxybutyrate-co-hydroxyvalerate nanoparticles for tumor targeted docetaxel delivery: Formulation, optimization and *in vitro* characterization. *Eur. J Pharmaceut Sci.*, **2017**, *99*, 85-94.
(b) Wilson, K.; Saharan, A.; Mittal, P.; Gautam, R.K.; Saini, V. Formulation, development and evaluation of topical intradermal drug delivery system for anti-acne product. *Indian Drugs*, **2021**.

[52] Ajmal, G.; Bonde, G.V.; Mittal, P.; Khan, G.; Pandey, V.K.; Bakade, B.V.; Mishra, B. Biomimetic PCL-gelatin based nanofibers loaded with ciprofloxacin hydrochloride and quercetin: A potential antibacterial and anti-oxidant dressing material for accelerated healing of a full thickness wound. *Int. J. Pharm.*, **2019**, *567*, 118480. http://dx.doi.org/10.1016/j.ijpharm.2019.118480 PMID: 31255776

[53] Ajmal, G.; Bonde, G.V.; Thokala, S.; Mittal, P.; Khan, G.; Singh, J.; Pandey, V.K.; Mishra, B. Ciprofloxacin HCl and quercetin functionalized electrospun nanofiber membrane: fabrication and its evaluation in full thickness wound healing. *Artif. Cells Nanomed. Biotechnol.*, **2019**, *47*(1), 228-240. http://dx.doi.org/10.1080/21691401.2018.1548475 PMID: 30688107

[54] Bairagi, U.; Mittal, P.; Singh, J.; Mishra, B. Preparation, characterization, and *in vivo* evaluation of nano formulations of ferulic acid in diabetic wound healing. *Drug Dev. Ind. Pharm.*, **2018**, *44*(11), 1783-1796. http://dx.doi.org/10.1080/03639045.2018.1496448 PMID: 29973105

[55] Bharti, K.; Mittal, P.; Mishra, B. Formulation and characterization of fast dissolving oral films containing buspirone hydrochloride nanoparticles using design of experiment. *J. Drug Deliv. Sci. Technol.*, **2019**, *49*, 420-432. http://dx.doi.org/10.1016/j.jddst.2018.12.013

[56] Bonde, G. V.; Ajmal, G.; Yadav, S. K.; Mittal, P.; Mishra, B. Lapatinib-loaded nanocolloidal polymeric micelles for the efficient treatment of breast cancer. **2020**, *10*(9), 023-029.

[57] Bonde, G.V.; Ajmal, G.; Yadav, S.K.; Mittal, P.; Singh, J.; Bakde, B.V.; Mishra, B. Assessing the viability of Soluplus® self-assembled nanocolloids for sustained delivery of highly hydrophobic lapatinib (anticancer agent): Optimisation and *in-vitro* characterisation. *Colloids Surf. B Biointerfaces*, **2020**, *185*, 110611. http://dx.doi.org/10.1016/j.colsurfb.2019.110611 PMID: 31704609

[58] Bonde, G.V.; Upadhyay, M.; Ajmal, G.; Mittal, P.; Hardikar, S.R.; Mishra, B. Polyethylene glycol block polymeric micelle: A promising delivery vehicle for lymphatic targeting. **2018**.

[59] Santos, J.R.; Gois, A.M.; Mendonça, D.M.F.; Freire, M.A.M. Nutritional status, oxidative stress and dementia: The role of selenium in Alzheimer's disease. *Front. Aging Neurosci.*, **2014**, *6*, 206. http://dx.doi.org/10.3389/fnagi.2014.00206 PMID: 25221506

[60] Bonde, G.V.; Yadav, S.K.; Chauhan, S.; Mittal, P.; Ajmal, G.; Thokala, S.; Mishra, B. Lapatinib nano-delivery systems: a promising future for breast cancer treatment. *Expert Opin. Drug Deliv.*, **2018**, *15*(5), 495-507. http://dx.doi.org/10.1080/17425247.2018.1449832 PMID: 29521126

[61] Mittal, P. Microneedles based drug delivery systems. Rx Pharmatutor-Pharmacy Infopedia. *Development and Evaluation of Paclitaxel and Genistein loaded Nanoformulations for Improved and Safe Treatment of Ovarian Cancer. IIT (BHU) varanasi*, **2018**.

[62] Mittal, P.; Kapoor, R.; Mishra, B. Dendrimers: Role in novel drug delivery. In: *Nanopharmaceutical Advanced Delivery Systems*; Wiley, New Jersey, **2021**; pp. 79-97.

[63] Mittal, P.; Kapoor, R.; Saharan, A.; Gautam, R.K. Targeting Molecular and Cellular Mechanisms in Respiratory Syncytial Virus

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(RSV) Infection. In: *Targeting Cellular Signalling Pathways in Lung Diseases*; Springer: Singapore, **2021**; pp. 501-516. http://dx.doi.org/10.1007/978-981-33-6827-9_23

[64] Mittal, P.; Seth, N.; Rana, A. Self-microemulsifying drug delivery system (SMEDDS): An alternative approach for hydrophobic drugs. *Int. J Nat Prod Sci.*, **2012**, *1*, 80.

[65] Mittal, P.; Vardhan, H.; Ajmal, G.; Bonde, G.V.; Kapoor, R.; Mittal, A.; Mishra, B. Formulation, optimization, hemocompatibility and pharmacokinetic evaluation of PLGA nanoparticles containing paclitaxel. *Drug Dev. Ind. Pharm.*, **2019**, *45*(3), 365-378. http://dx.doi.org/10.1080/03639045.2018.1542706 PMID: 30394795

[66] Mittal, P.; Vrdhan, H.; Ajmal, G.; Bonde, G.; Kapoor, R.; Mishra, B. Formulation and characterization of genistein-loaded nanostructured lipid carriers: pharmacokinetic, biodistribution and *in vitro* cytotoxicity studies. *Curr. Drug Deliv.*, **2019**, *16*(3), 215-225.

[67] Mittal Pooja, C. A.; Aggarwal, Jyoti Potential assessment of Transcutol P and Lauroglycol FCC as Co-Surfactants for formulation of self microemulsifying drug delivery systems (Smedds). *Int. J. Pharma Sci.*, **2012**, *4*(1), 1742-1745.

[68] Mittal Pooja, R.A.; bala, Rajni; Nimrata, Seth. Lipid based Self microemulsifying drug delivery systems (SMEDDS) for lipophilic drugs-an acquainted review. *IRJP*, **2011**, *2*(12), 75-80.

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Dietary Supplementation with Resveratrol Attenuates Serum Melatonin Level, Pro-Inflammatory Response and Metabolic Disorder in Rats Fed High-Fructose High-Lipid Diet under Round-the-Clock Lighting

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Abstract: This study aims to investigate the effect of resveratrol on systemic inflammatory response and metabolic disorder in rats fed a high-fructose high-lipid diet (HFHLD) and exposed to round-the-clock lighting (RCL). 21 adult male Wistar rats were randomly divided into 3 groups: control (group 1, $n = 7$); HFHLD for 8 weeks + round-the-clock lighting (RCL) (group 2, $n = 7$); HFHLD + RCL + Resveratrol (in a daily dose of 5 mg/kg intragastrically (group 3, $n = 7$). Results show that the combined effect of HFHLD and RCL reduces the serum melatonin ($p < 0.001$) and accelerates pro-inflammatory activities, oxidative stress, and metabolic disorder. There is a significant increase in the serum tumour necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) (both $p < 0.001$), blood malondialdehyde—thiobarbituric acid adducts ($MDA\text{-}TBA_2$) ($p < 0.001$), serum glucose ($p < 0.01$), insulin concentration, and the homeostatic model assessment insulin resistance (HOMA-IR) index (both $p < 0.001$), serum with very low-density lipoprotein (VLDL), and triacylglycerol (TAG) (both $p < 0.001$). At the same time, the decrease in the serum high-density lipoprotein (HDL) level ($p < 0.001$) is observed in the HFHLD + RCL group compared to the control. In the HFHLD + RCL + Resveratrol group, hypomelatonaemia ($p < 0.001$), pro-inflammatory actions, oxidative stress, and metabolic disorder were mitigated. Resveratrol can cause a significant rise in the serum melatonin and reduce serum TNF-α and CRP levels (both $p < 0.001$), blood $MDA\text{-}TBA_2$ ($p < 0.001$), serum glucose (both $p < 0.01$), insulin concentration, and HOMA-IR (both $p < 0.001$), serum VLDL and TAG (both $p < 0.001$) compared to the group 2, while serum HDL level increases ($p < 0.01$). Resveratrol attenuates pro-inflammatory responses and prevents considerable metabolic disorder in rats fed HFHLD under RCL.

Keywords: resveratrol; high-fructose high-lipid diet; light-dark cycle; melatonin; pro-inflammatory activity; oxidative stress; metabolic disorder; rats

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1. Introduction

Substantial changes in the daily light-dark cycle can result in the disorganisation of the circadian system, including melatonin rhythm alterations. The relevance of this issue is conditioned by the changes reshaping the sleep/work cycle in industrialised countries and the growing tendency to a night work regime with the rise in the exploitation of visual display units, smartphones, and light-emitting diode lighting that cause a shift in the light spectrum toward artificial lighting sources [1].

In the long term, light-dependent disorganisation of the circadian system appears to be very detrimental to health. A number of epidemiological studies have shown that

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under these conditions there is a significant increase in rates of several diseases, including metabolic syndrome, type 2 diabetes, obesity, cardiovascular diseases, mood disorders, cancer, and age-related risks [2–4]. Voluminous experimental and clinical studies evidence the role of hypomelatonaemia in the mechanisms contributing to the development of carbohydrate and lipid metabolic disorders, systemic inflammation, endothelial dysfunction, and nitro-oxidative stress [5].

In our opinion, these disorders can vary considerably under the combined impact of light-dependent disorganisation of the circadian system and diet. Recent reports demonstrate the impact of diets on serum melatonin and its circadian fluctuation. For instance, the capability of certain concentrations of caffeine and alcohol, as well as deficiency of some nutrients (folate, magnesium, and zinc), to reduce the nocturnal production of melatonin, but this effect is minor compared with the light–dark cycle [6].

Our more recent studies on rats have proven that the melatonin concentration in the blood serum can considerably decrease under combined exposure to round-the-clock lighting (RCL) and a high-fructose high-lipid diet (HFHLD) for 60 days [7]. The administration of melatonin under this condition partly lessens the manifestation of carbohydrate and lipid metabolism disorders and signs of nitro-oxidative stress in the skeletal muscles and the liver of rats, though a homeostatic model assessment insulin resistance (HOMA-IR) index under this condition demonstrates no significant changes [8]. It is evident that under these conditions the normalisation of the melatonin level is not sufficient to correct metabolic disorder, which can be achieved by suppressing the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) or by inducing the nuclear-factor-E2-related factor-2 (Nrf2), antagonistic to NF-κB [9,10]. The best safety profile among agents that can inhibit NF-κB and/or activate Nrf2 is found in plant polyphenols, particularly in bioflavonoids, epigallocatechin-3-gallate [11,12], and quercetin [13,14].

Resveratrol (3,4′,5-trihydroxy-trans-stilbene), a natural phytoalexin, is also able to concurrently suppress NF-κB and activate Nrf2–antioxidant response element (ARE) signalling pathway [15,16], alleviate NF-κB-dependent pro-inflammatory hypercytokinaemia and endothelium dysfunction (in higher degree compared to quercetin) [17], and, in combination with melatonin, enhances antioxidant activity and decreases gene expression of the insulin-regulated glucose transporter GLUT4, sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in heart tissue in a diabetic aged rat model [18].

Given that the role of transcriptional factors NF-κB and Nrf2 is associated with modifying the mammalian circadian clock [19,20], it seems important to focus on the capability of resveratrol to modulate serum melatonin levels under the co-effect of RCL and HFHLD. Another reason to carry out this study is to investigate the effect produced by resveratrol on the systemic inflammatory response and carbohydrate and lipid metabolism under the combined action of the circadian system disorganisation and "western diet", considering the fact that efficacy of specific NF-κB inhibitor ammonium pyrrolidine diothiocarbamate or Nrf2 inducer dimethyl fumarate is limited because of their toxicity [9,10].

Therefore, this study aims to investigate the effect of resveratrol on serum melatonin levels, systemic inflammatory response, and metabolic disorder in rats fed a HFHLD and exposed to RCL.

2. Materials and Methods

2.1. Chemicals

Crystalline D-Fructose (≥99.5%, Ph. Eur.) was purchased from ADM, Turkey, while resveratrol (3,4′,5-Trihydroxy-trans-stilbene,5-[(1E)-2-(4-Hydroxyphenyl)ethenyl]-1,3- benzenediol, ≥99%)was obtained from Merck Life Science, Poland. Diagnostic kits for determining insulin, tumour necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) in the blood serum were purchased from MyBioSource.com, USA. Analytical kits for measuring glucose and assessing the lipid profile in the blood serum were procured from Filisit-Diagnostics, Ukraine.

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2.2. Experimental Subjects

The 21 adult male Wistar rats (weight: 235 ± 20 g) used for this study were bred in an experimental biological clinic (vivarium) at the Petro Mohyla Black Sea National University, Mykolayiv, Ukraine. The animals were kept under standard environmental conditions (air temperature: +22 ± 2 °C, air humidity: 30–60%). The animals had free access to water and rodent pellets. Prior to the commencement of the study, bioethical approval was obtained from the Commission on Bioethics of Petro Mohyla Black Sea National University, Mykolayiv, Ukraine.

2.3. Experimental Design

The rats were randomly divided into 3 groups: control (group 1, $n = 7$); High-Fructose High-Lipid Diet (HFHLD) + round-the-clock lighting (RCL) (group 2, $n = 7$); HFHLD + RCL + Resveratrol (group 3, $n = 7$). Group 1 was fed standard chow (Table 1) and kept on a 12/12 h light/dark cycle. Group 2 waskept on a HFHLD for 8 weeks and exposed to RCL. Group 3 received resveratrol in a daily dose of 5 mg/kg [21] intragastrically. Resveratrol was administered together with carbohydrates (20% aqueous solution of fructose) whichincreased the solubility and bioavailability of stilbenoids [22]. Rats fromthe first two groups, instead of receiving resveratrol, were given 1 mL of a 20% solution of fructose intragastrically as a "placebo".

Table 1. Components and contents in standard and high-lipid diet.

Standard Rat Chow		High-Lipid Diet	
Nutrients	g/kg Total	Nutrients	g/kg Total
Protein	160	Protein	94
Fat	70	Fat	313
Carbohydrate	480	Carbohydrate	466
Fibre	68	Fibre	14
Sodium	2.7	Sodium	4
Vitaminand mineral mix	36	Vitaminand mineral mix	40
		Ingredients:	
		Refined wheat flour	450
		Skimmed milk powder	200
		Table margarine 82% fat	200
		Starch	100
		Peroxidised sunflower oil	40
		Sodium chloride	10
Total calorie	2720 kcal/kg	Total calorie	4477 kcal/kg

The rats were kept on a HFHLD for 2 months: the animals received a 20% aqueous solution of fructose for drinking and ahigh-lipid diet, whose components and content are given in Table 1. Fromthe 30th day of the experiment, the rats were exposed to RCL with an intensity of 1500 lx over the next 30 days, as previously reported [23].

After the experiment, the rats were sacrificed under thiopental anaesthesia in the morning (8.00–10.00), minimizing the effect of daily fluctuations in pineal melatonin secretion. The animals were given thiopental sodium (50 mg/kg, intraperitoneally, manufacturer: Kyivmedpreparat, Arterium Corporation, Kyiv, Ukraine). After that, they were dissected, and blood was taken via cardiac puncture sample bottles containing lithium heparin (30 IU per 1 mL of blood) (article LG3902, obtained from Sky Medica, Kyiv, Ukraine). Then heparinised blood was centrifuged (3000× g, 15 min) at room temperature. Each sample's separated top layer of serum was used for the analysis.

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2.4. Biochemical and Enzyme-Linked Immunosorbent Assays

To assess the serum melatonin levels, TNF-α, CRP, and insulin we used highly sensitive and specific ELISA kits for rat samples. Optical density readings were taken with awavelength of 450 nm (Stat Fax 2100 Microplate Reader, Awareness Technology, Inc., Palm City, FL, USA).

To measure secondary products of lipid peroxidation (LPO) in the blood—malondialdehyde (MDA)—thiobarbituric acid (TBA) adducts ($MDA\text{-}TBA_2$) with maximum light absorption of 532 nm, we used a spectrophotometer ULAB 101 (China). A rise in their concentration after 1.5 h incubation in a pro-oxidant ascorbate-iron buffer (pH = 7.4; 1 litre of the buffer contained 1.9 g tris-(2-hydroxy-methyl)-aminomethane hydrochloride, 50 mL 0.1 N HCl, 1.4 g ascorbic acid, and 32 mg $FeSO_4 \times 7H_2O$) and was used to measure the general antioxidant blood potential (the ability of the blood to resist LPO under the aggressive pro-oxidant environment) [24].

Serum glucose, total cholesterol (CH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triacylglycerol (TAG) concentrations were measured using enzymatic methods employing photometric equipment for measuring the optical density of materials that can measure the optical density of solutions at a wavelength of 490–600 nm (spectrophotometer ULAB 101, China) and standard laboratory reagent kits.

Insulin resistance was assessed by the homeostatic model assessment insulin resistance (HOMA-IR) index using the equation: HOMA-IR = fasting glucose (mmol/L) × fasting insulin (μU/mL)/22.5 [25].

2.5. Statistical Analysis

The findings were statistically analysed using the Microsoft Office Excel software package with the Real Statistics 2019 extension. We exploited the Shapiro-Wilk test to verify the normality of variances. The arithmetic mean and standard error of mean (SEM) were computed. The results are presented as mean ± SEM. Assuming that all samples had a normal distribution, we used the parametric analysis of variance (ANOVA), which was followed by a pairwise comparison of groups using the Student's t-test for independent samples and Tukey's honestly significant difference analysis. Multiple comparisons were avoided by employing the Dunn—Šidák correction. $p < 0.05$ was used to determine whether the differences between the arithmetic means were significant.

3. Results

3.1. Effects of Resveratrol on the Melatonin Level in the Serum of the Rats Fed a High-Fructose High-Lipid Diet under Round-the-Clock Lighting

Under the combined effect of RCL and HFHLD, the serum melatonin concentration significantly decreased by 4.5 times, achieving 7.1 ± 0.7 pg/mL ($p < 0.001$) (Figure 1). The dietary supplementation with resveratrol under RCL and HFHLD resulted in the growth of the melatonin level, which was 1.9 times higher than the respective values in group 2 ($p < 0.001$).

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Figure 1. Serum Melatonin, $n = 7$. Values were expressed as Mean ± SEM. Control, HFHLD (high-fructose high-lipid diet) + RCL (round-the-clock lighting), and HFHLD + RCL + Resveratrol; ** $p < 0.001$ is significant compared to group 1; ## $p < 0.001$ is significant compared to group 2.

3.2. Effects of Resveratrol on the Systemic Inflammatory Response Indices in the Serum of Rats Fed a High-Fructose High-Lipid Diet under Round-the-Clock Lighting

The animals exposed to RCL and kept on HFHLD demonstrated considerable deterioration of systemic inflammatory response indices, such as serum concentrations of the TNF-α, a pro-inflammatory cytokine, CRP, and an acute phase reactant (Table 2).

Table 2. Effects of resveratrol on the systemic inflammatory response indices in the serum of rats fed a high-fructose high-lipid diet (HFHLD) under round-the-clock lighting (RCL).

Groups/Parameters	TNF-α (pg/mL)	CRP (ng/mL)
1. Group 1 (Control), $n = 7$	34.0 ± 2.0	4.1 ± 0.1
2. Group 2 (HFHLD + RCL), $n = 7$	109.8 ± 6.0 **	12.8 ± 0.3 **
3. Group 3 (HFHLD + RCL + Resveratrol), $n = 7$	43.6 ± 4.9 ##	5.3 ± 0.3 *,##

Note: The table represents the mean ± SEM; * $p < 0.01$, and ** $p < 0.001$ is significant compared to group 1; ## $p < 0.001$ is significant compared to group 2; TNF-α—Tumour necrosis factor-alpha; CRP—C-reactive protein.

The serum TNF-α and CRP levels in rats with RCL and HFHLD were 3.2-fold and 3.1-fold higher, respectively, than in the control animals (both $p < 0.001$).

The administration of resveratrol during the RCL and HFHLD combined exposure led to a statistically significant reduction in the TNF-α and CRP concentration by 2.5 and 2.4 times, respectively, compared to the results in group 2 (both $p < 0.001$).

3.3. Effects of Resveratrol on Lipid Peroxidation in the Blood of Rats Fed a High-Fructose High-Lipid Diet under Round-the-Clock Lighting

Simultaneous action of the RCL and HFHLD was accompanied by the changes in LPO and general antioxidant blood potential in the rats' blood (Table 3). When compared to the corresponding values in the control group, the MDA-TBA_2 concentration under RCL and HFHLD nearly doubled: it grew 2.1-fold before the incubation and 1.9-fold after the incubation (both $p < 0.001$). The MDA-TBA_2 increment throughout incubation in the pro-oxidant buffer solution was 1.8 times higher than the results in the control rats ($p < 0.01$), indicating a significant decline in general antioxidant blood potential.

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Table 3. Effects of resveratrol on lipid peroxidation (LPO) in the blood of rats fed a high-fructose high-lipid diet (HFHLD) under round-the-clock lighting (RCL).

Groups/Parameters	MDA-TBA_2 (μmol/L)		
	Before Incubation	After Incubation	Increment over Incubation Time
1. Group 1 (Control), $n = 7$	11.2 ± 0.9	25.4 ± 2.0	14.2 ± 2.4
2. Group 2 (HFHLD + RCL), $n = 7$	23.0 ± 0.6 **	48.3 ± 2.1 **	25.3 ± 1.9 *
3. Group 3 (HFHLD + RCL + Resveratrol), $n = 7$	12.6 ± 0.9 ##	27.7 ± 1.9 ##	15.1 ± 2.2 #

Note: The table represents the mean ± SEM; * $p < 0.01$, and ** $p < 0.001$ is significant compared to group 1; # $p < 0.01$, and ## $p < 0.001$ is significant compared to group 2; MDA-TBA_2—Malondialdehyde—thiobarbituric acid adducts.

Resveratrol administered under RCL and HFHLD lowered the MDA-TBA_2 level in the blood by 1.8 times (before incubation) and by 1.7 times (after incubation) in comparison to the group 2 values (both $p < 0.001$). The MDA-TBA_2 increment over incubation in a pro-oxidant buffer solution was 1.7 times less than the respective values in group 2 ($p < 0.01$), which evidences the growth in general antioxidant blood potential under the dietary supplementation of resveratrol.

3.4. Effects of Resveratrol on Carbohydrate Metabolism in the Serum of Rats Fed a High-Fructose High-Lipid Diet under Round-the-Clock Lighting

The animals exposed to RCL and kept on HFHLD demonstrated considerable deterioration of carbohydrate metabolism (Table 4). There was a 1.4-fold difference between the serum glucose levels and the corresponding values in group 1 ($p < 0.01$). The serum insulin concentration and HOMA-IR were 3.7 times higher compared to the control group (both $p < 0.001$).

Table 4. Effects of resveratrol on carbohydrate metabolism in the serum of rats fed a high-fructose high-lipid diet (HFHLD) under round-the-clock lighting (RCL).

Groups/Parameters	Glucose (mmol/L)	Insulin (μU/mL)	HOMA-IR
1. Group 1 (Control), $n = 7$	4.94 ± 0.24	1.5 ± 0.2	0.32 ± 0.06
2. Group 2 (HFHLD + RCL), $n = 7$	6.89 ± 0.25 *	5.5 ± 0.2 **	1.17 ± 0.04 **
3. Group 3 (HFHLD + RCL + Resveratrol), $n = 7$	4.45 ± 0.18 #	2.1 ± 0.1 ##	0.42 ± 0.03 ##

Note: The table represents the mean ± SEM; * $p < 0.01$, and ** $p < 0.001$ is significant compared to group 1; # $p < 0.01$, and ## $p < 0.001$ is significant compared to group 2; HOMA-IR—Homeostasis Model Assessment of Insulin Resistance.

Resveratrol administered under RCL and HFHLD combination reduced the glucose level, which was 1.5 times inferior to the result in group 2 ($p < 0.01$). In comparison to the results in group 2, the serum insulin level and HOMA-IR fell 2.6 and 2.8 times, respectively (both $p < 0.001$).

3.5. Effects of Resveratrol on Lipid Profile in the Serum of Rats Fed a High-Fructose High-Lipid Diet under Round-the-Clock Lighting

The animals, when exposed to RCL and receiving HFHLD, demonstrated a considerable 2.7-fold fall in serum HDL compared to the respective value in the control rats ($p < 0.001$) (Table 5). On the other hand, VLDL and TAG levels considerably rose and were 3.3 and 3.2 times higher than in group 1, respectively (both $p < 0.001$).

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Table 5. Effects of resveratrol on lipid profile in the serum of rats fed a high-fructose high-lipid diet (HFHLD) under round-the-clock lighting (RCL).

Groups/Parameters	Total CH (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	TAG (mmol/L)
1. Group 1 (Control), $n = 7$	2.39 ± 0.29	0.63 ± 0.04	1.47 ± 0.29	0.29 ± 0.02	0.65 ± 0.05
2. Group 2 (HFHLD + RCL), $n = 7$	2.62 ± 0.30	0.23 ± 0.02 **	1.43 ± 0.29	0.96 ± 0.04 **	2.10 ± 0.10 **
3. Group 3 (HFHLD + RCL + Resveratrol), $n = 7$	2.28 ± 0.22	0.48 ± 0.02 *,#	1.29 ± 0.22	0.51 ± 0.03 **,##	1.11 ± 0.06 **,##

Note: The table represents the mean ± SEM; * $p < 0.01$, and ** $p < 0.001$ is significant compared to group 1; # $p < 0.01$, and ## $p < 0.001$ is significant compared to group 2. CH—Cholesterol; HDL—High-density lipoprotein; LDL—Low-density lipoprotein; VLDL—Very low-density lipoprotein; TAGs—Triacylglycerol.

The HDL concentration was increased by dietary resveratrol supplementation under RCL and HFHLD combination and was 2.1 times higher than the results in group 2 ($p < 0.01$). When compared to the results in group 2, the levels of VLDL and TAG decreasedby1.9 times for each (both $p < 0.001$).

4. Discussion

Earlier, we demonstrated that the development of hypomelatoninaemia naturally occurs in rats due to long-term RCL exposure and does not manifest when the rats are kept on HFHLD. However, when these factors act together, the melatonin level is significantly reduced compared to the effects resulting from the separate use of the above-mentioned factors [7]. This confirms the ability of alimentary factors under certain conditions to influence melatonin secretion, which was discovered by other researchers [3]. Dietary components such as glucose, sodium, ethanol, and caffeine have been shown to affect circadian rhythms and melatonin production by altering the expression of circadian oscillator proteins [3]. We can presume that other carbohydrates (besides glucose) and lipids are also capable of reducing pineal melatonin production through similar mechanisms.

It is noteworthy that the dietary supplementation with resveratrol over RCL and HFHLD significantly elevated the serum melatonin level compared with the findings of group 2. Earlier studies on the diabetic-aged rat model demonstrated that the effect of resveratrol and melatonin administered separately and their combination could increase SIRT1 gene expression in heart tissue [18] that can lead to the reduction of the activity of several pro-inflammatory and pro-oxidant transcription factors, such as NF-κB, STAT3, FOXO, and p53 [26]. The NF-κB suppression restricts the transcription of the crucial enzyme in pineal melatonin synthesis—arylalkylamine N-acetyltransferase [27]. This points out that resveratrol can be considered as both an inducer of melatonin synthesis and a compound that has a synergic impact along with this hormone on the immune system and metabolism due to their effects on the same transcription factors.

Some studies have revealed the association between systemic inflammatory response and diets rich in fructose [28] and fats [29]. For instance, fructose intake increases the pro-inflammatory cytokines, intestinal permeability, and lipid accumulation in the liver, and increases pro-inflammatory cytokines [28]. Elevated free fatty acids and cytokines can activate the inhibitory kappa B kinase (IKK) complex and NF-κB both through activating Toll-like receptors and through inducing cellular stresses (oxidative or/and endoplasmic reticulum) [29]. High fructose consumption can activate NF-κB via sphingosine kinase 1/sphingosine-1-phosphate induction [30]. Excessive lighting in the dark phase leads to the perturbation of clock genes (CLOCK, PER1, PER2) in humans and rodents by increasing immune activation and producing pro-inflammatory cytokines, even in the absence of immune challenge [31,32]. Some proteins of the circadian oscillator, and, in particular, CLOCK, are known to be involved in the NF-κB activation [33].

In fact, according to our findings, the rats exposed to RCL and kept on HFHLD demonstrated an increase in TNF-α and CRP, which are markers of the systemic inflammatory response. The obtained results on the statistically significant reduction in the serum TNF-α

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and CRP under RCL and HFHLD under the resveratrol administration are supported by the data from randomised controlled trials according to which dietary supplementation with resveratrol dramatically decreased TNF-α and CRP levels [34]. Resveratrol is supported as an adjuvant to pharmacologic therapy of metabolic disorders, significantly improving inflammatory markers.

Previously we have found out that the simultaneous effect of RCL and HFHLD leads to more marked metabolic disorders, such as LPO, and a decrease in the general antioxidant blood potential in the blood of rats, hyperinsulinaemia, hypo-α-lipoproteinaemia, hypertriacylglycerolaemia, and increased visceral fat mass, in comparison with the animals kept on the HFHLD only [7]. This study demonstrated that resveratrol administered under RCL and HFHLD reduces the MDA-TBA_2 level and its increment over 1.5-h incubation in pro-oxidant ascorbate-iron buffer that points out the LPO restrain and growth in general antioxidant blood potential. It has been suggested that the strong antioxidant properties of resveratrol allow it to reduce oxidative stress and inhibit free radicals, especially those produced by LPO. The findings of this study are consistent with other published results, which demonstrated that rats fed with various levels of resveratrol had decreased serum LPO products. For instance, 4-month supplementation of 0.04% and 0.06% resveratrol significantly lowered MDA concentration in the serum of rats fed high fructose diet (63%) [35]. A 10-week resveratrol diet supplementation (at a daily dose of 30 mg/kg·bw) significantly decreased hepatic MDA, improved its superoxide dismutase and catalase, and reduced glutathione values in rats fed HFHLD; additionally, serum total antioxidant capacity was insignificantly affected compared to the HFHLD group [36]. Resveratrol is known as an antioxidant exerting a dual effect: it can increase the activity of antioxidant enzymes (manganese-containing superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, and glutathione reductase) and can act as a scavenger of superoxide, hydroxyl and other free radicals that prevents LPO in cell membranes and DNA lesions [37]. It is known that the expression of the genes encoding the aforementioned antioxidant enzymes is associated with the activation of the Nrf2–ARE by resveratrol [38].

Under RCL, the antioxidant effect of resveratrol can also be associated with its capability, discovered in this study, to increase serum melatonin levels. This hormone, known for its direct antiradical properties [39], promotes the expression of a number of antioxidant enzymes (superoxide dismutase, glutathione peroxidase) [40].

The dietary supplementation with resveratrol under RCL and HFHLD produces sufficient impact on carbohydrate and lipid metabolism under the experimental conditions by lowering serum glucose and insulin, HOMA-IR, as well as serum VLDL and TAG, and increasing HDL level compared with the findings in group 2.

Our results correspond with data on the effect of resveratrol on carbohydrate and lipid metabolism, demonstrated under long-term resveratrol administration. Thus, resveratrol considerably improves the lipid profile (significantly declines the VLDL and TAGs concentrations, normalizes CH and LDL levels, and increases the HDL level), insulin sensitivity, and hepatic mRNA expression of peroxisome proliferator-activated receptor alpha (PPARα), and diminishes hepatic NF-κB and morphological characteristics of liver steatosis [36]. The role of disorganisation of the circadian system in the mechanisms of metabolic disorders is confirmed by the data on the capability of resveratrol to abolish 11-week high-fat diet-induced circadian desynchrony and ameliorate the impaired lipid profiles, the plasma leptin rhythmities in mice that, as we suggest, can be associated with its impact on the expression of clock genes (BMAL1, CLOCK, and PER2) and clock-controlled lipid metabolism-related genes (SIRT1, PPARα, SREBP-1c, ACC1, and FAS) [41].

Obviously, the mitigation of pro-inflammatory responses and metabolic disorder under the combined pathogenic impact of a high-calorie carbohydrate-lipid diet and light-dependent disruption of the biological clocks results from the effect caused by resveratrol on key signalling pathways involved in the regulation of immune response, inflammation, oxidative stress, carbohydrate and lipid metabolism, and circadian oscillator. This multidirectional action of resveratrol favourable distinguishes it from other polyphenols.

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We are aware of some limitations in this study. The rat model is not a complete simulation of the pathological processes in patients who experience the pathogenic effects of the western lifestyle, particularly a high-calorie carbohydrate-lipid diet, and light-dependent disruption of the biological clocks. Therefore, additional clinical data are required to support the results so far. Since this study investigated the effects of resveratrol under the combined action of two factors (HFHLD and RCL), which can cause more pronounced inflammatory and metabolic disorders, as shown in our previous paper [7], the assessment of effects produced by resveratrol under separate action of these factors requires special experimental design, including additional control groups. Moreover, the measurement of serum melatonin in the morning does not reveal the regularities of its circadian rhythmicity in rats during the experiment. Determining the MDA-TBA_2 increment over the blood incubation in pro-oxidant buffer solution evaluated only the general antioxidant blood potential regardless of the contribution from enzymatic and low-molecular antioxidant compounds that requires further in-depth investigation.

5. Conclusions

The administration of resveratrol considerably restrains the fall of serum melatonin, reduces serum TNF-α and CRP levels, blood MDA-TBA_2, serum glucose and insulin concentrations, HOMA-IR, serum VLDL, and TAC levels, increases serum HDL, and thereby prevents pro-inflammatory activities, LPO and metabolic disorder, as well as improving general antioxidant blood potential in rats fed a high-fructose high-lipid diet under round-the-clock lighting.

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References

1. Blume, C.; Garbazza, C.; Spitschan, M. Effects of light on human circadian rhythms, sleep and mood. *Somnologie* **2019**, *23*, 147–156. [PubMed]
2. Touitou, Y.; Reinberg, A.; Touitou, D. Association between light at night, melatonin secretion, sleep deprivation, and the internal clock: Health impacts and mechanisms of circadian disruption. *Life Sci.* **2017**, *173*, 94–106.
3. Arango, C.; Buitelaar, J.K.; Fegert, J.M.; Olivier, V.; Pénélaud, P.-F.; Marx, U.; Chimits, D.; Falissard, B.; Barylnik, J.; Birdeanu, L.; et al. Safety and Efficacy of Agomelatine in Children and Adolescents with Major Depressive Disorder Receiving Psychosocial Counselling: A Double-Blind, Randomised, Controlled, Phase 3 Trial in Nine Countries. *Lancet Psychiatry* **2022**, *9*, 113–124. [PubMed]
4. Shkodina, A.D.; Tan, S.C.; Hasan, M.M.; Abdelgawad, M.; Chopra, H.; Bilal, M.; Boiko, D.I.; Tarianyk, K.A.; Alexiou, A. Roles of Clock Genes in the Pathogenesis of Parkinson's Disease. *Ageing Res. Rev.* **2022**, *74*, 101554. [PubMed]
5. Imenshahidi, M.; Karimi, G.; Hosseinzadeh, H. Effects of melatonin on cardiovascular risk factors and metabolic syndrome: A comprehensive review. *NaunynSchmiedebergs Arch. Pharmacol.* **2020**, *393*, 521–536.
6. Peuhkuri, K.; Sihvola, N.; Korpela, R. Dietary factors and fluctuating levels of melatonin. *Food Nutr. Res.* **2012**, *56*. [CrossRef]

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7. Belikova, O.I.; Cherno, V.S.; Frenkel, Y.D.; Kostenko, V.O. Influence of chronic hypomelatoninemia on carbohydrate and lipid metabolism of rats kept on "Western pattern diet". *Fiziol. Zh.* **2018**, *64*, 52–60. [CrossRef]
8. Belikova, E.I.; Frenkel, Y.D.; Cherno, V.S. Influence of exogenous melatonin on free radical processes in rats exposed to light around the clock under modeling of insulin resistance syndrome. *Med. Probl. Hyg. Radiat. Environ. Med.* **2017**, *7*, 35–51.
9. Belikova, O.I.; Frenkel, Y.D.; Cherno, V.S.; Kostenko, V.O. Influence of nuclear factor κB inhibitor on biochemical markers of insulin resistance syndrome under hypopinealism and high-calorie carbohydrate-lipid diet. *World Med. Biol.* **2017**, *3*, 80–82. [CrossRef]
10. Frenkel, Y.D.; Cherno, V.S.; Kostenko, V.O. Nrf2 induction alleviates metabolic disorder and systemic inflammatory response in rats under a round-the-clock lighting and high-carbohydrate-lipid diet. *Rom. J. Diabetes Nutr. Metab. Dis.* **2022**, *29*, 194–201.
11. Yelins'ka, A.M.; Shvaykovs'ka, O.O.; Kostenko, V.O. Epigallocatechin-3-gallate prevents disruption of connective tissue in periodontium and salivary glands of rats during systemic inflammation. *Wiad. Lek.* **2018**, *71*, 869–873.
12. Kozaeva, R.; Klymenko, M.O.; Katrushov, O.V.; Kostenko, V.O. Bioflavonoids as agents for correcting nitro-oxidative stress and salivary gland functions in rats exposed to alcohol during modeled lipopolysaccharide-induced systemic inflammatory response. *Wiad. Lek.* **2022**, *75*, 685–690. [CrossRef] [PubMed]
13. Yelins'ka, A.M.; Liashenko, L.I.; Kostenko, V.O. Quercetin potentiates antiradical properties of epigallocatechin-3-gallate in periodontium of rats under systemic and local administration of lipopolisaccharide of Salmonella typhi. *Wiad. Lek.* **2019**, *72*, 1499–1503. [CrossRef] [PubMed]
14. Yavtushenko, I.V.; Nazarenko, S.M.; Katrushov, O.V.; Kostenko, V.O. Quercetin limits the progression of oxidative and nitrosative stress in the rats' tissues after experimental traumatic brain injury. *Wiad. Lek.* **2020**, *73*, 2127–2132. [CrossRef] [PubMed]
15. Zhu, X.; Liu, Q.; Wang, M.; Liang, M.; Yang, X.; Xu, X.; Zou, H.; Qiu, J. Activation of Sirt1 by resveratrol inhibits TNF-α induced inflammation in fibroblasts. *PLoS ONE* **2011**, *6*, e27081. [CrossRef] [PubMed]
16. Farkhondeh, T.; Folgado, S.L.; Pourbagher-Shahri, A.M.; Ashrafizadeh, M.; Samarghandian, S. The therapeutic effect of resveratrol: Focusing on the Nrf2 signalling pathway. *Biomed. Pharmacother.* **2020**, *127*, 110234. [CrossRef]
17. Chekalina, N.I.; Kazakov, Y.M.; Mamontova, T.V.; Vesnina, L.E.; Kaidashev, I.P. Resveratrol more effectively than quercetin reduces endothelium degeneration and level of necrosis factor α in patients with coronary artery disease. *Wiad. Lek.* **2016**, *69*, 475–479. [PubMed]
18. Akgun-Unal, N.; Ozyildirim, S.; Unal, O.; Gulbahce-Mutlu, E.; Mogulkoc, R.; Baltaci, A.K. The effects of resveratrol and melatonin on biochemical and molecular parameters in diabetic old female rat hearts. *Exp. Gerontol.* **2023**, *172*, 112043. [CrossRef] [PubMed]
19. Shen, Y.; Endale, M.; Wang, W.; Morris, A.R.; Francey, L.J.; Harold, R.L.; Hammers, D.W.; Huo, Z.; Partch, C.L.; Hogenesch, J.B.; et al. NF-κB modifies the mammalian circadian clock through interaction with the core clock protein BMAL1. *PLoS Genet.* **2021**, *17*, e1009933. [CrossRef] [PubMed]
20. Wible, R.S.; Ramanathan, C.; Sutter, C.H.; Olesen, K.M.; Kensler, T.W.; Liu, A.C.; Sutter, T.R. NRF2 regulates core and stabilizing circadian clock loops, coupling redox and timekeeping in Mus musculus. *eLife* **2018**, *7*, e31656. [CrossRef]
21. Mozafari, M.; Nekooeian, A.A.; Panjeshahin, M.R.; Zare, H.R. The effects of resveratrol in rats with simultaneous type 2 diabetes and renal hypertension: A study of antihypertensive mechanisms. *Iran J. Med. Sci.* **2015**, *40*, 152–160. [PubMed]
22. Vesely, O.; Baldovska, S.; Kolesarova, A. Enhancing Bioavailability of Nutraceutically Used Resveratrol and Other Stilbenoids. *Nutrients* **2021**, *13*, 3095. [CrossRef] [PubMed]
23. Frenkel, Y.D.; Zyuzin, V.O.; Cherno, V.S.; Kostenko, V.O. Effect of epigallocatechin-3-gallate and quercetin on the production of reactive oxygen and nitrogen species in liver of rats exposed to round-the-clock light and kept on carbohydrate-lipid diet. *Fiziol. Zh.* **2022**, *68*, 20–27. [CrossRef]
24. Akimov, O.Y.; Kostenko, V.O. *Oxidative-Nitrosative Stress and Methods of Its Research*; Mahnoliya: Lviv, Ukraine; p. 152p.
25. Chao, P.C.; Li, Y.; Chang, C.H.; Shieh, J.P.; Cheng, J.T.; Cheng, K.C. Investigation of insulin resistance in the popularly used four rat models of type-2 diabetes. *Biomed. Pharmacother.* **2018**, *101*, 155–161. [CrossRef]
26. Poulose, N.; Raju, R. Sirtuin regulation in aging and injury. *Biochim. Biophys. Acta* **2015**, *1852*, 2442–2455. [CrossRef]
27. Markus, R.P.; Cecon, E.; Pires-Lapa, M.A. Immune-pineal axis: Nuclear factor κB (NF-kB) mediates the shift in the melatonin source from pinealocytes to immune competent cells. *Int. J. Mol. Sci.* **2013**, *14*, 10979–10997. [CrossRef]
28. Wang, Y.; Qi, W.; Song, G.; Pang, S.; Peng, Z.; Li, Y.; Wang, P. High-Fructose Diet Increases Inflammatory Cytokines and Alters Gut Microbiota Composition in Rats. *Mediators Inflamm.* **2020**, *2020*, 6672636. [CrossRef]
29. Duan, Y.; Zeng, L.; Zheng, C.; Song, B.; Li, F.; Kong, X.; Xu, K. Inflammatory Links Between High Fat Diets and Diseases. *Front. Immunol.* **2018**, *9*, 2649. [CrossRef]
30. Wang, X.; Zhang, D.M.; Gu, T.T.; Ding, X.Q.; Fan, C.Y.; Zhu, Q.; Shi, Y.W.; Hong, Y.; Kong, L.D. Morin reduces hepatic inflammation-associated lipid accumulation in high fructose-fed rats via inhibiting sphingosine kinase 1/sphingosine 1-phosphate signalling pathway. *Biochem. Pharmacol.* **2013**, *86*, 1791–1804. [CrossRef]
31. Vieira, E.; Mirizio, G.G.; Barin, G.R.; de Andrade, R.V.; Nimer, N.F.S.; La Sala, L. Clock Genes, Inflammation and the Immune System-Implications for Diabetes, Obesity and Neurodegenerative Diseases. *Int. J. Mol. Sci.* **2020**, *21*, 9743. [CrossRef]
32. Ojetola, A.A.; Asiwe, J.N.; Adeyemi, W.J.; Ogundipe, D.J.; Fasanmade, A.A. Dietary Supplementation with D-Ribose-L-Cysteine Prevents Hepatic Stress and Pro-Inflammatory Responses in Male Wistar Rats Fed a High-Fructose High-Fat Diet. *Pathophysiology* **2022**, *29*, 621–639. [CrossRef] [PubMed]

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33. Boiko, D.I.; Shkodina, A.D.; Hasan, M.M.; Bardhan, M.; Kazmi, S.K.; Chopra, H.; Bhutra, P.; Baig, A.A.; Skrypnikov, A.M. Melatonergic Receptors (Mt1/Mt2) as a Potential Additional Target of Novel Drugs for Depression. *Neurochem. Res.* **2022**, *47*, 2909–2924. [CrossRef] [PubMed]
34. Koushki, M.; Dashatan, N.A.; Meshkani, R. Effect of Resveratrol Supplementation on Inflammatory Markers: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *Clin. Ther.* **2018**, *40*, 1180–1192.e5. [CrossRef] [PubMed]
35. Kopec, A.; Piatkowska, E.; Leszczynska, T.; Koronowicz, A. Effect of long term administration of resveratrol on lipid concentration in selected organs and liver's histology in rats fed high fructose diet. *J. Funct. Foods* **2013**, *5*, 299–305. [CrossRef]
36. Reda, D.; Elshopakey, G.E.; Mahgoub, H.A.; Risha, E.F.; Khan, A.A.; Rajab, B.S.; El-Boshy, M.E.; Abdelhamid, F.M. Effects of Resveratrol Against Induced Metabolic Syndrome in Rats: Role of Oxidative Stress, Inflammation, and Insulin Resistance. *Evid. Based Complement. Alternat. Med.* **2022**, *2022*, 3362005. [CrossRef] [PubMed]
37. Kuršvietienė, L.; Stanevičienė, I.; Mongirdienė, A.; Bernatonienė, J. Multiplicity of effects and health benefits of resveratrol. *Medicina* **2016**, *52*, 148–155. [CrossRef] [PubMed]
38. Javkhedkar, A.A.; Quiroz, Y.; Rodriguez-Iturbe, B.; Vaziri, N.D.; Lokhandwala, M.F.; Banday, A.A. Resveratrol restored Nrf2 function, reduced renal inflammation, and mitigated hypertension in spontaneously hypertensive rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2015**, *308*, R840–R846. [CrossRef]
39. Kuznetsova, T.Y.; Solovyova, N.V.; Solovyov, V.V.; Kostenko, V.O. Antioxidant activity of melatonin and glutathione interacting with hydroxyl and superoxide anion radicals. *Ukr. Biochem. J.* **2017**, *89*, 22–30. [CrossRef]
40. Abadi, S.H.M.H.; Shirazi, A.; Alizadeh, A.M.; Changizi, V.; Najafi, M.; Khalighfard, S.; Nosrati, H. The Effect of Melatonin on Superoxide Dismutase and Glutathione Peroxidase Activity, and Malondialdehyde Levels in the Targeted and the Non-targetedLung and Heart Tissues after Irradiation in Xenograft Mice Colon Cancer. *Curr. Mol. Pharmacol.* **2018**, *11*, 326–335. [CrossRef]
41. Sun, L.; Wang, Y.; Song, Y.; Cheng, X.R.; Xia, S.; Rahman, M.R.; Shi, Y.; Le, G. Resveratrol restores the circadian rhythmic disorder of lipid metabolism induced by high-fat diet in mice. *Biochem. Biophys. Res. Commun.* **2015**, *458*, 86–91. [CrossRef]

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REVIEW ARTICLE

3D Printing: A Promising Revolutionary Technology in Pharmaceutical Drug Development and Health Care

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ABSTRACT

The three-dimensional (3D) printing technique is an emerging paradigm shift in the field of drug manufacturing. It also proved to be a promising technology with respect to the pharmaceutical, clinical medicine, and regulatory sciences. It is a modern additive manufacturing technology in which digital information is used to produce a physical model. Unlike other subtractive and formative manufacturing processes which involve removing sections of material by machining or by cutting it away, in 3D printing technology objects are prepared from 3D model data in the process of joining materials layer by layer. In drug therapy, 3D printing has abundant opportunities for rapid preparation of multifunctional customized drug delivery systems with improved drug release features, flexible and personalized dosage forms, implants matching specific patient anatomical needs as well as cell-based materials for regenerative drug therapy and prosthesis. The 3D printing methods have gained vast importance in the field of pharmaceutical and medical applications. It is an interdisciplinary approach with the aim of exploring newer drug-delivery systems. 3D printing could also become a part of the drug production line in the pharmaceutical industry which tends to move towards personalized medicine along with mass manufacture. Presently 3D printing technology is broadly investigated in the field of drug delivery after the approval of the first 3D printed tablet containing an antiepileptic drug, levetiracetam under the trade name of Spritam® by Aprecia Pharmaceuticals in 2015. The present review recapitulates the novel applications of 3D printing technology in the field of pharmaceutical drug development and health care. It also reviews the working principle of various techniques of 3D printing along with their advantages and disadvantages.

Keywords
3D printing technology; Personalized Therapy; 3D printed dosage forms; Customized drug delivery systems; Patient compliance

Abbreviations: 3D printing – Three-Dimensional Printing; CJP- Colour Jet Printing; DLP- Digital Light Processing; DLS- Digital Light Synthesis; DMLS- Direct Metal Laser Sintering; DOD- Drop on Drop; DOS- Drop on Solid; FDM- Fused Deposition Modeling; FFF- Fused Filament Fabrication; LCM- Lithography-Based Ceramic Manufacturing; MJM- Multi Jet Modeling; NPJ- Nanoparticle Jetting; PAM- Pressure Assisted Microsyringe method; PJP- Polyjet Printing; SLA/STL- Stereolithography; SLM- Selective Laser Melting; SLS- Selective Laser Sintering

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Introduction

Three-dimensional or 3D printing is a modern manufacturing technology in which digital information is used to produce a physical model. It is an additive manufacturing process in which three-dimensional solid objects are generated from a digital file with the aim of achieving actually customized and personalized drug delivery approach. Unlike other subtractive and formative manufacturing processes which involve removing sections of material by machining or by cutting it away, in 3D printing technology objects are prepared from 3D model data in the process of joining materials layer by layer. In drug therapy, 3D printing has the abundant opportunity of rapid preparation of multifunctional customised drug delivery systems with improved drug release features, flexible and personalized dosage forms, implants matching specific patient anatomical needs as well as cell-based materials for regenerative drug therapy, and prosthesis[1]. Being a technology of relating precise manufacturing of individually developed dosage forms, tissue engineering, and disease modeling 3D printing technology has become one of the most revolutionary and powerful means. The 3D printing methods have gained vast importance in the field of pharmaceutical and medical applications. It is an interdisciplinary approach with the aim of exploring newer drug-delivery systems. 3D printing could also become a part of the drug production line in the pharmaceutical industry which tends to move towards personalised medicine along with mass manufacture.

The 3D printing (3DP) process was first patented in 1986; though, it has been utilized in the fields of medicines, bio-fabrication, and pharmaceutical printing only since the last decade. International Standard Organization (ISO) defined the 3D printing (3DP) process as the "fabrication of objects through the deposition of a material using a print head, nozzle, or another printer technology"[2]. Fabrication of highly complex drug delivery devices, uncomplicated modifications of a product at a designed level, the opportunity of manufacturing even nano-sized systems, great reproducibility, and fast and cost-effectiveness are the most important benefits of 3DP or additive manufacturing. FDA has already approved 3D-printed medical devices and implants earlier the recent years which have been used successfully. A 3D- printed tracheal splint was approved for use in 2012. In February 2013, the FDA approved a personalised 3D- printed plate used in a surgical skull repair for a specific patient[3]. Presently 3D printing technology is broadly investigated in the field of drug delivery after the approval of the first 3D printed tablet containing an antiepileptic drug, levetiracetam under the trade name of Spritam® by Aprecia Pharmaceuticals in 2015[4]. In pharmaceutical fields, a wide variety of 3DP techniques are used such as inkjet-based 3DP[5-7] extrusion-based

Table 1 Various Type of 3d Printing Techniques [1,49,50,51,52,53]

Printing technique		Building material	Binding agent
Inkjet Printing	Drop on Solid (DOS)	Powders	Liquid binding agent
	Drop on Drop (DOD)	Solution/ suspensions	• UV polymerization • Cooling/ heating • Solvent evaporation
	Multi Jet Modeling(MJM)		
	Polyjet Printing (PJP)		
	Nanoparticle Jetting (NPJ)	Photopolymer	UV polymerization
	Colour Jet Printing (CJP)	Powders	Liquid binding agent
	Tridimensional Inkjet Printing	Powders	Liquid binding agent
Beam Based Printing	Stereolithography (STL/SLA)	Photopolymer	UV light
	Digital Light Synthesis (DLS)	Photopolymer	Digital light
	Digital Light Processing (DLP)	Photopolymer	Digital light
	Lithography Based Ceramic Manufacturing (LCM)	Photopolymer with suspended ceramic particles	Laser beam
	Selective Laser Sintering (SLS)	Polymeric powder	Laser beam
	Selective Laser Melting (SLM)	Polymeric powder	Laser beam
	Direct Metal Laser Sintering (DMLS)	Metal powder	Laser beam
Extruder Nozzle Based Printing	Fused Deposition Modeling (FDM)	Melted solid/ thermoplastic material in semisolid consistency	Temperature change • Cooling/ heating
	Pressure Assisted Microsyringe method(PAM)		
	Fuse Filament Fabrication(FFF)		

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fused deposition modeling (FDM)[8-25], selective lasersintering (SLS)[25] stereolithography (SLA)[28-30]digital light processing (DLP)[31-33], pressure-assisted microsyringe (PAM)[34-35] printing, semi-solid extrusion (SSE)[36] direct powder extrusion (DPE)[36], and many more techniques Table 1. These technologies along with their advantages make it possible for young researchers to expand existing pharmaceutical applications and investigate newer possibilities. These printing technologies encompass the use of diverse types of materials, and various resolutions and speeds to produce personalised dose print lets (3D printed tablets)[10,16,29,30], Implants, Physiological channels[31], sustained release tablets[32], modified release tablets[18,26,33], bilayer tablets[17,21], hydrogels[27] gold nanoparticles[5], Oral coated 3D printed tablets[6] multicompartment polyfills[13,29,30], solid self-micro emulsifying drug delivery systems (S-SMEDDS)[34], orodispersible printlets[25], gastric floating system[15], pressure-controlled drug delivery system[14], Chewable printlets[19], Osmotic pump with dual release[20], fast dissolving printlets [37] and many more.

Steps involved on 3D Printing

3D Printing is additive manufacturing that involves many steps starting from the generation of a digital file using computer-aided design, CAD to the physical three-dimensional solid resultant parts. However different 3D printing techniques will involve variations to a certain degree, broadly eight steps are needed to complete fabrication using any of the 3D printing techniques [38].

The first step is a computer-aided design (CAD) which involves solid modeling software or reverse engineering equipment such as laser and optical scanning, to create a 3D solid or surface representation. The second step is to convert a 3D solid representation into STL file employing a computer-aided design system. STL file illustrates the external closed surfaces of the original CAD model and forms the basis for the calculation of the layers. The third step is transferring STL File to the printing machine and further manipulating it for its correct size, position, and orientation for building. In the fourth step, the machine setup is done in terms of build parameters like material constraints, energy source, layer thickness, timings, etc. Building of physical model is the fifth step. Removal of prepared print let from the machine, post-processing step such as cleaning if required and finally application of finished 3D printlets are the further steps. Figure 1.

Principle, major benefits, and limitations of 3d printing techniques (table 2)

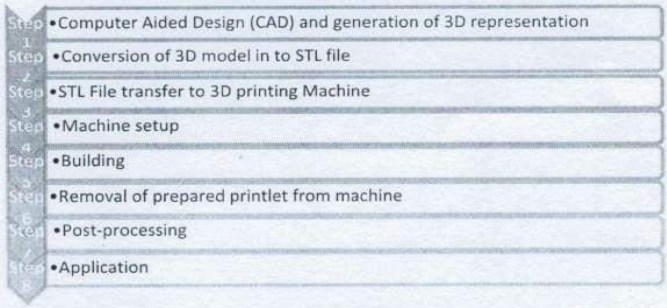

Fig. 1. Basic steps involved in 3D Printing

Table 2 Evaluation of Various Types 3 D Printing Techniques

Printing technique	Principle	Benefits	Limitations
1. Inkjet Printing[6,7,54,55,56, 57, 58, 59]	In this technique an ink which comprises of different combinations of drug, liquid binder and other excepients is sprayed accurately in small drops in varying sizes layer by layer into a substrate (which canbe a powder or a solution or suspension) where it solidifies into a solid dosage form.	• High resolution • The possibility of printing more than one material during the same printing process • Processing at room temperature • Highly porous matrix can be printed	• Drying step is needed • Long printing times • Limited geometries of the shapes can be printed (in particular, cavities are difficult to incorporate).

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Printing technique	Principle	Benefits	Limitations
2. Beam Based Printing[25-33, 60]	It is based on the principle of photopolymerization, the ultraviolet, digitalor laser light beam isaided by baffles to crossthe surface of the liquid resin, to accurately represent the previously designed 3D model.	• Versatile as drugs can be mixed with the photopolymer prior to printing, and become trapped in the solidified matrices • Able to manufacture submicron and micron-sized layers • The technique is more suited to fabrication of dosage forms encapsulating thermally labile drugs • Higher resolution compared to other 3D printing technique.	• Requirement of prior production of filaments. • Requires curing after printing • A restricted number of photopolymer availability • Residual analysis wouldbe necessary for pharmaceutical applications • Long printing time if high resolution is selected Because only a single laser is used
3. Extruder Nozzle Based Printing[36-37]	A thermoplastic polymer is melted and extruded through a mobile heated nozzle layer by layer in a repeated manner along x-y-z stage, followed by solidification by means of temperature change to generate a previously defined shape by computer aided design.	• Higher resolution and uniformity compared with powder-bed printing • Good mechanical strength with insignificant friability • better dosing accuracy and high drug loading • Commonly available • Lowest cost setup • Post-printing drying/solidification is not needed • Tablets with multi-release profiles can be prepared	• Limited choice of thermoplastic materials with satisfactory melt viscosity properties for extrusion. • Inability to load temperature sensitive substances during extrusion due to the high processing temperature. • Controlling the material flow-rate through the nozzle quiet challenging

Advantages of 3d printing over conventional solid dosage form

Conventional solid dosage form poses a certain limitation, such as

- Limited dose flexibility
- Formulation of tablet requires adequate flow characteristics
- Poorly soluble, low-potency or low-density materials may result in poor dose uniformity
- lengthy and time
- These limitations can be superseded by using 3D Printing technology, which has many advantages[11] Figure 2 such as
- Improved product complexity
- High dose flexibility
- Suitable for formulation development
- Cheap and easy to manufacture
- Personalization suitable for paediatric and geriatric patients.
- Speed up First-in-human (FIH) trials
- On-demand manufacturing with the possibility foron-site preparation
- Automated equipment available for production
- Only short-term stability data required
- Taste masking and blinding possible
- Thermal methods could serve as an enabling strategy

Role of 3D printing technology in personalized treatmentand its applications (Figure 3, Table 3)

Conventionally, mass manufacturing of pharmaceuticals is done in only a small number of distinct dose strengths, which is based on the optimum dose required for an appropriate effect in the majority of the population. However, it is obvious that one dose might not fit all patients. Their requirements can vary depending upon many factors. Thus conventional dosage forms cannot be administered to all patients because of the variability found among individuals of different races with variable metabolism.3-D printing has remarkable potential to be utilized in the pharmaceutical industry with the objective of designing personalized dosage forms. Advances in computer and 3D printing technology, as

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well as pharmacogenomics research, make it possible to modify treatments according to the needs of an individual patient. Drugs will be selected and dosed based on genetic profiles, patient-specific allergens can be replaced with safer ingredients, and all of the medications the patient needs will be combined into a single pill. 3-D printing had permitted pharmacists to adjust medication in terms of dosage, shape, size, and dose combinations in particular to the needs of the patient according to their age, race or gender[39-40]. This technique also provides opportunities for producing new formulations of drugs, which could be particularly helpful for patients with multiple conditions. For instance, a pharmacist could make one pill with multiple active ingredients. Reducing the number of pills a patient has to take from ten to one may significantly improve medication compliance. MakerBot Replicator is the innovator of a technology that allows the 3D printing of pills into specific geometrical shapes and sizes which affects the pharmacokinetics. Release kinetics as more rapid or sustained is an important driver of medication production for the consumer. The surface area-to-volume ratio is an important property and determinant in the kinetics of drug release. Release from a pyramid and standard cylinder is not found equal, the pyramid is ableto deliver the drug more appropriately based on kinetic studies. The inference is that the one-size-fits-all approach is not optimal for patients who require continuous adjustments in their dosage. Researchers at the UCL School of Pharmacy, University College London have developed a technique using 3D printing referred to as "hot melt extrusion" to produce many odd-shaped medications which are quite difficult to manufacture using standard production techniques. The true advantage of hot melt extrusion is that such odd-shaped medications demonstrate improved kinetics for drug release. Since some patients require medications that are faster acting, while others need medications to be released more gradually over a longer period of time, 3Dprinting offers one the ability to customize medications for individual patient needs by developing medication inspecific shapes releasing drugs at satisfactory release rate[41]. It is reported that 3D printing permits the manufacture of tablets in such shapes which are otherwise unfeasible by conventional powder compaction methods. A number of paracetamol-loaded geometric shapes such as cubes, discs, pyramids, spheres, and torus constant surface area/volume ratio were prepared, and it was reported that the rate of dissolution is directly proportional to the SA/V ratio[42]. One of the best examples of personalised manufacturing is Fabrx, London which was established in 2014. It provides a novel and flexible platform for the production of personalised medicines, revolutionizing medical treatment for patients aroundthe world[43].

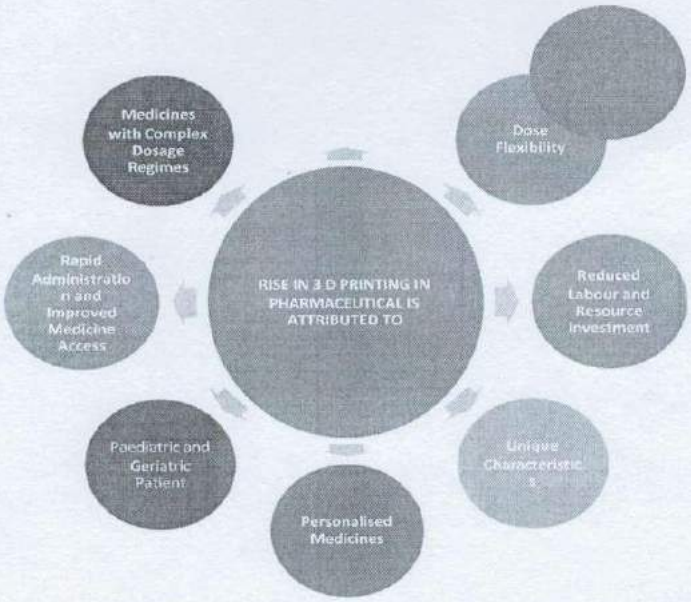

Fig. 2. Rise in 3 D printing in pharmaceutical development

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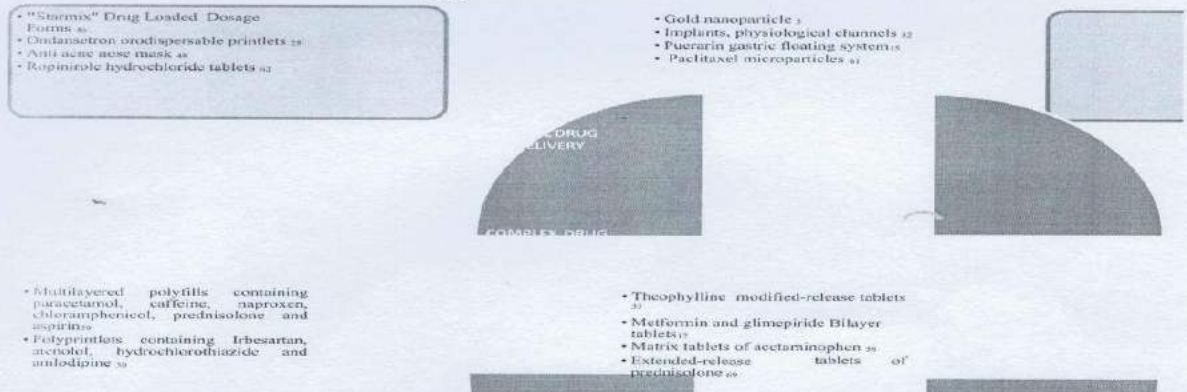

Fig. 3. Recent pharmaceutical applications of 3D printing technology Table 3
Applications of 3d Printing Technology in Designing of Drug Delivery Systems

Sno.	Technique	Drug	Excipient	Application	Outcome	Referance
1	Inkjet printing	Gold	Arabinose	Gold nanoperticle	• Stable • Biodegradable • Biocompatible	Begines *et al.* 2019
2	Inkjet printing Drop on solid deposition printing	Warfarin sodium	D-sucrose, pregelatinized starch, povidone K30, microcrystalline cellulose, and silicon dioxide, 38% (v/v) ethanol as liquid binder	Mouth-disintegrating tablets	• Formulation of mouth-disintegrating tablets with excellent dose accuracy • Good mechanical strength • Faster disintegration and dissolution	Tian *et al.* 2018
3	Piezoelectric inkjet printing	Paclitaxel	Poly (lactic-co-glycolic acid) (PLGA)	Microparticles	• Homogeneous shape and size • Geometry dependent drug release rate in the order of honeycomb>grid, ring>circle shapes	Lee *et al.* 2012
4	Piezoelectric inkjet printing	Ropinirole hydrochloride	Poly (ethylene glycol diacrylate) (PEGDA) hydrogel matrix	3D-printed solid dispersion tablets	• Personalized therapy	Clark *et al.* 2017
5	Digital light processing (DLP)	Diclofenac sodium, Ibuprofen	Poly (ethylene glycol) diacrylate, PEG, Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide, tartrazine	Implants, Physiological channels	• Good morphology • Excellent integrity • Satisfactory perfusion behavior	Yang et al 2020

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Sno.	Technique	Drug	Excipient	Application	Outcome	Reference
6	Digital light processing (DLP)	Paracetamol	Polyethylene glycol diacrylate and diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide	Sustained release tablets	• Improved drug release rate • Tensile strength of printlets.	Krkobabic *et al.* 2019[55]
7	Digital light processing (DLP)	Theophylline	Poly (ethylene glycol) diacrylate PEGDA	Modified-release tablets.	• Tablets with distinct layers and smooth outer surface • well-defined shapes with different release profiles.	Kadry *et al.* 2019[56]
8	Selective laser sintering (SLS) 3D printing	Ondansetron	Drug-cyclodextrin complexes and mannitol	Orodispersable printlets with characteristics similar to a commercial ODT	• Orodispersible printlets with characteristics similar to a commercial ODT which can be personalised	Allahham *et al.* 2020[45]
9	Selective laser sintering (SLS) 3D printing	Paracetamol and ibuprofen	Ethyl cellulose, Kollicoat Instant Release	3D Printlets	• Dual release profile was obtained • Instant release for one drug and sustained release for another drug	Awad *et al.* 2019[46]
10	Direct powder extrusion	Itraconazole	Hydroxyl propylcellulose	3D printed tablets	• Unable formulation of tablets from amorphous solid dispersion	Goyanes *et al.* 2019[14]
11	Stereolithographic (SLA) 3D printing	Ibuprofen	Polyethylene glycol diacrylate	Ibuprofen-loaded hydrogels	• Faster drug release due to higher water content of hydrogels	Martinez et al 2017[47]
12	Stereolithographic (SLA) 3D printing	Paracetamol and aspirin	Poly (ethylene glycol) diacrylate, poly(caprolactone) triol, diphenyl(2,4,6-trimethylbenzoyl), phosphine oxide	Personalised tablets	• Better drug loading and modulated drug release • Patient specific batcheswere formulated. • 28 drug dosage forms were formulated in one single print cycle	Healy *et al.* 2019[48]
13	Stereolithographic (SLA) 3D printing	Paracetamol, caffeine, naproxen, chlorampheni col, prednisolone and aspirin	Polyethylene glycol diacrylate and diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide	Multilayered polyfills	• Excellent physical properties and the different material inclusions enabled distinct drug release profiles of the six actives within dissolution test	Martinez et al 2018[49]
14	Stereolithographic (SLA) 3D printing	Irbesartan, atenolol, hydrochlorothiazide and amlodipine	Polyethylene glycol diacrylate and diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide	Polyprintlets	• Reduced pill burden and improve patient compliance	Xu *et al.* 2020[50]
15	Fused-deposition 3-dimensional printing	Aminosalicylate (4-ASA and 5-ASA)	Polyvinyl alcohol filaments were loaded with the drugs in an ethanolic drug solution	Modified-release tablets	• Mechanically strong tablets • Significant thermal degradation of the active 4-ASA (50%) occurred indicating that the method may not be appropriate for thermolabile drugs	Goyanes *et al.* 2015[51]
16	Fused-deposition 3-dimensional printing	Metformin and glimepiride	Eudragit® RL sustained release layer and polyvinyl alcohol	Bilayer tablets	• Desirable drug release for both drugs was obtained	Gioumeouxouzis *et al.* 2017[52]

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Sno.	Technique	Drug	Excipient	Application	Outcome	Reference
17	3D Extrusion-Based Printing	Puerarin	Hydroxypropyl cellulose Hydroxypropyl methyl cellulose Microcrystalline cellulose Lactose	Gastric floating system	• Appropriate gastric residence time • Controlled release	Li et al 2019[19]
18	Filaments co-extrusion (modified FDM)	Aripiprazole	Kollicoat® IR (BASF®, Germany)	3D-printed tablets	• Better dissolution of aripiprazole • Prolonged release.	Jamroz *et al.* 2018[20]
19	Hot-melt extrusion (FDM)	Isoniazid	Hydroxypropyl-methylcellulose Hydroxypropyl-cellulose, Polyethylene Oxide, Eudragit® RS PO, RL PO and L 100 Triethyl citrate Buffering agents, potassium dihydrogen phosphate and sodium hydroxide pellets, Polylactic acid	3D-printed oral dosage forms	• Facilitating adjustable dose and drug release properties.	Oblom *et al.* 2019[21]
20	3D extrusion based printing process	Aspirin, hydrochlorothiazidefor immediate release pravastatin, atenolol, and ramipril for sustained release.	Polyvinylpyrrolidine, lactose D-mannitol Sodium starch glycolate, Hydroxypropyl methylcelloluse	Multi-active 5 in 1 solid dosage form Polypill	• Planned immediate and sustained release profilesfrom a single dosage unit • Avoidance of chances of incompatibility	Shaban *et al.* 2015[22]
21	Fused-deposition 3-dimensional printing	Acetaminophen	Eudragit® RS, mannitol, Fumed silica	Capsule for pressure-controlled drug delivery system	• Development of novel drug delivery system	Krause *et al.* 2019[23]
22	Fused-deposition 3-dimensional printing	Isoleucine	E sucrose, pectin, maltodextrin, water, flavourings and colourants	Chewable printlets	• Oral tailored-dose therapies in a hospital setting.	Goyanes *et al.* 2019[24]
23	Fused-deposition 3-dimensional printing	Captopril, nifedipine and glipizide	Polyethylene glycol 6000, hydroxypropyl methylcelloluse, sodium chloride tromethamine, Dmannitol 99, Croscarmellose sodium, microcrystalline cellulose	Osmotic pump with the drug captopril and sustained release compartments with the drugs nifedipine and glipizide	• Well-defined and separate controlled release profiles for three different drugs	Khaled *et al.* 2015[25]
24	Fused-deposition 3-dimensional printing	Guaifenesin	Hydroxypropyl methylcelloluse,	Bilayer tablets	• High drug loading of up to600 mg • Immidiate release and sustained release was obtained	Khaled *et al.* 2014[21]

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specialized software, allowing the selection of the required dose by the pharmacist according to the prescription given by the clinician. The incorporation of a modern fingerprint access control alongside a data matrix reader ensures manufacturing reliability, as only qualified personnel will have access to the technology's outstanding features. Moreover, the system is fitted with advanced in-line quality control procedures alongside camera monitoring of the printing process to track the progress and detect any faults during manufacture. A choice between three different printing nozzles allows theuser to adapt the system to their manufacturing needs. The M3DIMAKER™ has vast applications in product drug development including the manufacture of small batches for pre-clinical and clinical studies as well as clinical practice for personalised medicines. Depending on the medicine being made, the preparation of one month's medication can be carried out in very less time, revolutionizing the drug manufacture timeline.

Conclusion

Three-dimensional printing is a layer-by-layer, automated modern manufacturing technology in which digital information is used to produce a physical model with the aim of achieving essentially a customised and personalised drug delivery approach. The three- dimensional printing approach plays an integral role in developing various complex drug delivery systems. In recent years, many studies reported enhanced safety, bioavailability, effectiveness, and acceptability of 3-D printed pharmaceutical drug products. This approach has proven to be a possible and functional means of drug therapy for patients in need of specific medicine for individualized therapy. 3D printing technology rised with an exceptionally speedy development rate after the approval of the first 3D printed tablet containing an antiepileptic drug, levetiracetam under the trade name of Spritam® manufactured by Aprecia Pharmaceuticals. Studies on solid oral dosage forms, topical dosage forms, and Implants have proven the tremendous scope of 3D printing technology in the field of pharmaceutical drug development and health care. This promising technology offers many advantages over conventional manufacturing techniques such as improved product complexity, high dose flexibility, personalization suitable for pediatrics and geriatric patients speed up first-in-human (FIH) trials, on-demand and quick manufacturing with the possibility for on-site preparation. Furthermore, this technology also provides new prospects for thedevelopment of multifunctional novel drug delivery systems for individualised therapy. A number of active pharmaceutical ingredients can be incorporated into a single pill using 3-D printing technology which further leads to improved patient compliance leading to adherence to the drug therapy. By proper understanding of the working principle of various techniques of 3D printing along with their advantages and disadvantages, it is possible to achieve undeniable benefits for further growth of pharmaceutical drug delivery systems and satisfactory outcomes at the industrial level.

References

1. Jamroz W, Szafraniec J, Kurek M, Jachowicz R. 3D Printing in Pharmaceutical and Medical Applications Recent Achievements and Challenges. Pharm Res 35. 2018 .p. 176.
2. https://www.iso.org/obp/ui/#iso:std:iso-astm:52900:ed-1:v1:en. ISO/ASTM 52900:2015en Additive manufacturing – General principles – Terminology. 2018.
3. https://www.pharmacytimes.com/contributor/beth-bolt-rph/2014/12/3d-drug-printing-the-next-generation-of-pharmacy 3D Drug Printing: TheNext Generation of Pharmacy
4. https://www.kqed.org/futureofyou/22005/why-3d-printing-has tremendous-potential-for-big-pharma. Why 3D Printing has 'Tremendous Potential' for Big Pharma
5. Begines B et al. Design of highly stabilized nanocomposite inks based on biodegradable polymermatrix and gold nanoparticles for Inkjet Printing. Scientific Reports.2019.
6. Kejing S, Deck K, Nokhodchi A. Mohammed M. Drop-On-Powder 3D Printing of Tablets with an Anti-Cancer Drug.5-Fluorouracil. Pharmaceutics. 2019 11.p.150.
7. Tian P, Yang F, Yuan X, Min-Mei L, Li-Ping Y, Wei L, Qi-Feng L, Zhu-Fen L, Si-Yu H & Yan- Zhong COral disintegrating patient-tailored tablets of warfarin sodium produced by 3D printing. Drug Development and IndustrialPharmacy. 2018.
8. Goyanes A, Scarpa M, Kamlow M, Gaisford S, Basit, AW, Orlu M. Patient acceptability of 3D printed medicines. Int. J. Pharm.530. 2017 p. 71– 78.
9. Kollamaram G, Croker DM, Walker GM, Goyanes A, Basit AW, Gaisford S. Low temperature fused deposition modeling fdm 3D printing of thermolabile drugs. Int. J. Pharm. 545, 2018.p.144–152.
10. Oblom H, Zhang J, Pimparade M, Speer I, Preis M, Repka, M, Sandler N. 3D-printed isoniazid tablets for the treatment and prevention of tuberculosis—personalized dosing and drug release. AAPS Pharmscitech, 2019 .p. 20-52.
11. Alhnan MA, Okwuosa TC, Sadia M, Wan KW, W Ahmed, B Arafat. Emergence of 3D printed dosage forms: Opportunities and challenges. Pharm. Res. 33. 2016. p.1817–1832.
12. Madla CM, Trenfield SJ, Goyanes A, Gaisford S, Basit AW. 3D printing technologies, implementation and regulation: An overview. Eds.; Springer International Publishing: Cham. Switzerland. 2018. p. 21–40.

INDORE INSTITUTE OF PHARMACY IQAC INDORE

Principal
Indore Institute of Pharmacy.
INDORE (M.P.)

13. Shaban A, Jonathan Burley C, Morgan Alexander R, Jing Y, Clive R J. 3D printing of five-in-one dose combination polypill with defined immediate and sustained release profiles. Journal of Controlled Release 217. 2015. p. 308–314.
14. Krause J, Bogdahn M, Schneider F. Design and characterization of a novel 3D printed pressure-controlled drug delivery system. European Journal of Pharmaceutical Sciences. 2019.
15. Pingfei L, Zhang S, Sun W, Mengsuo C, Wen H, LiQ, Weisan P, and Xinggang Y. Flexibility of 3D Extruded Printing for a Novel Controlled-Release Puerarin Gastric Floating Tablet: Design of Internal Structure. AAPS PharmSciTech 2019.p. 20:236.
16. Jamroz W, Kurek M, Czech A, Szafraniec J, Gawlak K, Jachowicz R. 3D printing of tablets containing amorphous aripiprazole by filaments co-extrusion. Eur J Pharm Biopharm. 2018. Oct.131. p. 44-47
17. Christos I. Gioumouxouzis, Baklavaridis A, Orestis LK, Catherine K. Markopoulou, Nikolaos B, Dimitrios T, Dimitrios G.Fatouros.A 3D printedbilayer oral solid dosage form combining metformin for prolonged and glimepiride for immediate drug delivery. European Journal of Pharmaceutical Sciences, Volume 120, 30 July,2018.p.40-52
18. Goyanes A, Buanz AB, Hatton GB, Gaisford S, Basit AW. 3D printing of modified-release aminosalicylate 4-ASA and 5-ASA tablets: Eur J Pharm Biopharm. Jan. 89. 2015. P.157-62.
19. Goyanes A, Madla CM, Umerji A, Duran Pineiro G, Giraldez Montero JM, Lamas Diaz MJ, Gonzalez Barcia M, Taherali F, Sanchez Pintos P, Couce ML. Automated therapy preparation of isoleucine formulations using 3D printing for the treatment of MSUD: First single-centre, prospective, crossover study in patients. Int. J. Pharm. 2019. 567-118497.
20. Khaled S, Burley JC, Alexander MR, Yang J, Roberts CJ. 3D printing of tablets containing multiple drugs with defined release profiles. Int. J. Pharm. 494.2015.p. 643–650.
21. Khaled S, Burley J, Alexander M, Roberts CJ. Desktop 3D printing of controlled release pharmaceutical bilayer tablets. Int. J. Pharm. 461. 2014. p.105–111.
22. Linares V, Casas M, Caraballo I. Printfills: 3D printed systems combining fused deposition modelling and injection volume filling. Application to colon-specific drug delivery. Eur. J. Pharm. Biopharm. 134. 2019.p. 138–143.
23. Araujo MRP, Barreto LLS, Gratieri T, Gelfuso GM, Filho CM. The digital pharmacies era: How 3D printing technology using fused deposition modeling can become a reality. Pharmaceutics. 2019.p.11, 128.
24. Sadia M, So Snicka A, Arafat B, Isreb A, Ahmed W, Kelarakis A, Alhnan MA Adaptation of pharmaceutical excipients to FDM 3D printing for the fabrication of patient-tailored immediate release tablets. Int. J. Pharm. 513. 2016. p. 659–668.
25. Nour A, Fina F, Marcuta C, Kraschew L, Wolfgang M, Gaisford Simon, W. Basit A, and Goyanes A, e Laser Sintering S. 3D Printing ofOrally Disintegrating Printlets Containing Ondansetron. Pharmaceutics. 2020.p. 12- 110
26. Jie W, Alvaro G, Simon G, Abdul BW. Stereolithographic SLA 3D printing of oral modified-release dosage forms. International Journal of Pharmaceutics. Volume 503. Issues 1–2. 2016. p. 207-212.
27. Martinez PR, Goyanes A, Basit AW, Gaisford S. Fabrication of drug-loaded hydrogels with stereolithographic 3D printing. Int J Pharm. 5321. 2017.p. 313-317.
28. Healy AV, Fuenmayor E, Doran P, Geever LM, Higginbotham CL, Lyons JG. Additive manufacturing of personalized pharmaceutical dosage forms via stereolithography. Pharmaceutics.11. 2019.p. 645.
29. Robles Martinez P, Xu X, Trenfield, SJ, Awad A, Goyanes A, Telford R, Basit AW, Gaisford S. 3D printing of a multi-layered polypill containing six drugs using a novel stereolithographic method. Pharmaceutics, 11; 2019. 274.
30. Xu X, Martinez RP, Madla CM, Goyanes A, Joubert F, Basit AW, Gaisford S .Stereolithography SLA 3D printing of an antihypertensive polyprintlet: Case study of an unexpected photopolymer-drug reaction. Addit. Manuf. 2020.p. 101071.
31. Yan Y, Yanjun Z, Xiao L, Qingliang Y, Gengshen Y. Printability of External and Internal Structures Based on Digital Light Processing 3D Printing Technique. Pharmaceutics. 12.2020 .p. 207
32. Krkobabic M, Medarevic D, Cvijic S, Grujic B, Ibric S. Hydrophilic excipients in digital light processing DLP printing of sustained release tablets: Impact on internal structure and drug dissolution rate. Int. J. Pharm. 572. 2019. p. 118790
33. Kadry H, Wadnap S, Xu C, Ahsan F. Digital light processing DLP 3D-printing technology and photoreactive polymers in fabrication of modified-release tablets. Eur. J. Pharm. Sci. 135.2019.p. 60–67.
34. Vithani K, Goyanes A, Jannin V, Basit AW, Gaisford S, Boyd BJ. A proof of concept for 3D printing of solid lipid-based formulations of poorly water-soluble drugs to control formulation dispersion kinetics. Pharm. Res. 36. 2019.p. 102.
35. Kapilkumar V, Alvaro G, Vincent J, Abdul W B, Simon G, BJ Ben An Overview of 3D Printing Technologies for Soft Materials and Potential Opportunities for Lipid-based Drug Delivery Systems. Pharm Res 36.2019.p. 4

INDORE INSTITUTE OF PHARMACY IQAC INDORE

Principal
Indore Institute of Pharmacy,
INDORE (M.P.)

36. Alvaro G, Nour A, Sarah TJ, Edmont S, Simon G, Abdul BW. Direct powder extrusion 3D printing: Fabrication of drug products using a novel single-step process. International Journal ofPharmaceutics. 2019. P. 567 -118471
37. Conceicao J, Vaamonde F, Goyanes A, Adeoye O, Concheiro A, Cabral MH, Sousa Lobo JM, Alvarez L. Hydroxypropyl-cyclodextrin-based fast dissolving carbamazepine print let's prepared by semisolid extrusion 3D printing. Carbohydr. Polym. 2019.p. 221, 55–6
38. Ian G, David R, Brent S. Additive Manufacturing Technologies 3D Printing: Rapid Prototyping and Direct Digital Manufacturing Second Edition. Springer New York Heidelberg Dordrecht London. 2015.
39. Zeeshan F, Madheswaran T , Pandey M , Gorain B. Three-Dimensional 3-D Printing Technology Exploited for the Fabrication of Drug Delivery Systems: Curr Pharm Des. 2442. 2018.p. 5019-5028.
40. Chandekar A, Mishra DK., Sharma S, Saraogi GK,Gupta U, Gupta G. 3D Printing Technology: A New Milestone in the Development of Pharmaceuticals. Current Pharmaceutical Design, volume 25, issue 9.2019 p.937-945
41. https://www.forbes.com/sites/robertglatter/2015/06/01/why-shape-matters-the-rise-of-3d-printing-in-pharma/#c8f5c1f24c09
42. Martinez PR, Goyanes A, Basit AW, Gaisford S. Influence of Geometry on the Drug Release Profiles of Stereolithographic SLA 3D- Printed Tablets. AAPS PharmSciTech. Nov. 198: 2018.p. 3355-3361.
43. https://www.fabrx.co.uk/2020/04/06/fabrxs-pharmaceutical-3d-printer-for-personalised-medicines-m3dimaker-is-now-available https://www.fabrx.co.uk/about/
44. Zema L, Melocchi A, Maroni A, Gazzaniga A. Three-dimensional printing of medicinal products and the challenge of personalized therapy. J. Pharm. Sci. 106. 2017. P. 1697–1705.
45. Trenfield SJ, Xian Tan H, Awad A, Buanz A, Gaisford S, Basit Goyanes AW. Track-and-trace: Novel anti-counterfeit measures for 3D printed personalized drug products using smart material inks. Int. J. Pharm. 2019.p. 567- 118443.
46. Scoutaris N. 3D printed Starmix drug loaded dosage forms for paediatric applications. Pharm. 2018. p. 35- 34.
47. Elliott I. The practice and clinical implications of tablet splitting in international health. Trop. Med. Int. Health. 19.2014. P. 754–760.
48. Goyanes A, Del-Amornrat U, Wang J, Basit AW, Gaisford S. 3D scanning and 3D printing as innovative technologies for fabricating personalized topical drug delivery systems. J Control Release. 234.2016. p. 41-8.
49. Kotta S, Nair A, Alsabeelah N. 3D Printing Technology in Drug Delivery: Recent Progress and Application. Current Pharmaceutical Design. 24.2018.p. 5039-5048
50. Anna A, Augusto P, Bernardo I. The Role of 3D Printing in Medical Applications: A State of the Art. Hindawi Journal of Healthcare Engineering, Article ID 5340616. 2019.p.10 pages
51. Afsana, Jain V, Haider N, Jain K. 3D Printing in Personalized Drug Delivery. Current Pharmaceutical Design. Vol. 24 42.2018. p.5039-63.
52. Trenfield SJ, Awad A, Goyanes A, Gaisford S, Basit AW. 3D printing pharmaceuticals: Drug development to frontline care. Trends Pharmacol. Sci. 39. 2018.p. 440–451.
53. James N, Rapti D, Christine Moore MV, Mansoor Khan A, Khairuzzaman Akm. A new chapter in pharmaceutical manufacturing: 3D-printed drug products. Advanced Drug Delivery Reviews. 108. 2017. p. 39–50.
54. Huang W, Zheng Q, Sun W, Xu H, Yang X. Levofloxacin implants with predefined microstructure fabricated by three dimensional printing techniques. Int J Pharm.339 1-2. 2007. P.33–8.
55. Wu W, Zheng Q, Guo X, Sun J, Liu Y. A programmed release multi-drug implant fabricated by three-dimensional printing technology for bone tuberculosis therapy. Biomed Mater. 46. 2009.p. 065005
56. Daly R, Harrington TS, Martin GD, Hutchings IM. Inkjet printing for pharmaceutics–a review of research and manufacturing. Int J Pharm. 4942. 2015. p. 554–67.
57. Kyobula M, Adedeji A, Alexander MR, Saleh E, Wildman R, Ashcroft I. 3D inkjet printing of tablets exploiting bespoke complex geometries for controlled and tuneable drug release. J Control Release. 261. 2017.p. 207–15.
58. Yu DG, Yang XL, Huang WD, Liu J, Wang YG, Xu H. Tablets with material gradients fabricated bythree-dimensional printing. J Pharm Sci. 969. 2007. p.2446–56.
59. Rowe C, Katstra W, Palazzolo R, Giritlioglu B, Teung P, Cima M Multi mechanism oral dosage forms fabricated by three dimensional printing™. J Control Release. 661: 2000.p.11–7.
60. Chockalingam K, Jawahar N, Chandrasekhar U. Influence of layer thickness on mechanical properties in stereolithography. Rapid Prototyp J. 122.2006. p.106–13.
61. Lee BK, Yun YH, Choi JS, Choi YC, Kim JD, Cho YW. Fabrication of drug-loaded polymer microparticles with arbitrary geometries using a piezoelectric inkjet printing system. Int J Pharm. 4272. 2012.p. 305-10.
62. Clark EA, Alexander MR, Irvine DJ, Roberts CJ, Wallace MJ.3D printing of tablets using inkjet with UV photo initiation Int J of Pharm. 5291-2.2017.p.523-30.

INDORE INSTITUTE OF PHARMACY IQAC INDORE

Principal
Indore Institute of Pharmacy,
INDORE (M.P.)

63. Awad A , Fina F, Trenfield, Sarah J , Patel P , Goyanes A, Gaisford S, and Basit AW. 3D Printed Pellets Miniprintlets: A Novel, Multi- Drug, Controlled Release Platform Technology. Pharmaceutics114.2019.p.148
64. Gioumouxouzis CI, Katsamenis OL, Bouropoulos N, Fatouros DG. 3D printed oral solid dosage forms containing hydrochlorothiazide for controlled drug delivery. J. Drug Deliv. Sci. Tec. 40. 2017. p. 164–171.
65. Yi HG, Choi YJ, Kang KS. A 3D-printed local drug delivery patch for pancreatic cancer growth suppression. J Control Release. 238. 2016.p.231- 41
66. Genina N, Boetker JP, Colombo S, Harmankaya N, Rantanen J, Bohr A. Anti-tuberculosis drug combination for controlled oral delivery using 3D printed compartmental dosage forms: From drug product design to in vivo testing. J. Control. Release. 268.2017. P. 40–48.
67. Beck RCR, Chaves PS, Goyanes A, Vukosavljevic B, Buanz A, Windbergs M, Basit AW, Gaisford S.3D printed tablets loaded with polymeric nanocapsules: An innovative approach to produce customized drug delivery systems. Int. J. Pharm.2017.p.528, 268–279.
68. Sadia M, Arafat B, Ahmed, Forbes W, Alhnan MA. Channelled tablets: An innovative approach to accelerating drug release from 3D printed tablets. J. Control. Release. 269. 2018. p. 355–363.
69. Skowyra J, Pietrzak K, Alhnan MA. Fabrication of extended release patient-tailored prednisolone tablets via fused deposition modelling FDM 3D printing. Eur J Pharm Sci. 68. 2015.p. 11-7.

INDORE INSTITUTE OF PHARMACY IQAC INDORE

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polymer, HPMC K100M as floating polymer, and calcium chloride as a cross-linking agent were obtained experimentally, and the results were found to fit the design model. The quantitative effect of these factors at different levels on the responses could be predicted using polynomial equations, and high linearity was observed between predicted and actual values of response variables. The results of the present study showed that the responses i.e, particle size, entrapment efficiency, and in vitro release are significantly affected by the concentration of polymer and a cross-linking agent. The formulation OF1 was found to be the optimum formulation predicted by the point prediction of the design expert software. The in vitro drug release was found to be controlled for more than 12 h and followed the Higuchi model. The validations of RSM for three dependent variables were 100.09%, 99.68%, and 97.02%. Therefore, it can be concluded that a floating microsphere for repaglinide was developed and optimized using a three-factor, three-level Box – Behnken design.

Keywords: Box-Behnken design, Sodium alginate, Floating microspheres, Solvent evaporation.

Introduction

The oral route is the most simple, flexible, and convenient for the patient among the various drug administration routes[1]. Drugs with a narrow absorption window should be kept at the absorption site for a longer period to achieve controlled drug release. Sedimentation, floatation, expansion, mucoadhesion, and a modified shape system have all been developed to keep the formulation in the gastrointestinal tract. Drugs with a short half-life are quickly eliminated from the bloodstream, requiring frequent dosing; as a result, controlled-release formulations of such drugs have been developed to improve bioavailability[2].

In a meglitinide class of oral anti-hyperglycemic agents, repaglinide is having short half-life (1 hour) due to which it requires repeated dosing. The persistent dosing of the drug it gives rise to side effects on the gastrointestinal tract[3]. The gastroretentive system increases the effectiveness of dosage forms by releasing the medication in a controlled way and thus keeping its concentration for a longer duration of time[4]. Thus, repaglinide is a worthy candidate for formulating a floating drug delivery system as it possesses a short duration of action, early clearance, and enzymatic stability in the upper part of GIT[5].

A novel microsphere-based drug delivery system had increased the interest of researchers from the pharmaceutical field, as it controlled the release of drugs by diffusion or mass transfer behavior[6]. Floating drug delivery is useful for drugs, which are poorly soluble at an alkaline pH, short half-life, and have a narrow window of absorption[7,8]. Floating drug delivery systems remain buoyant in the stomach for an extended period of time and thereby enhance the bioavailability of the drug. Floating microsphere has more advantage that they pass uniformly through the gastrointestinal tract to avoid variabilities in gastric emptying and give sustained release, thereby reducing inter-subject variability in absorption and local irritation[9,10].

Using process optimization software, we developed a gastroretentive floating multiunit system of antidiabetic, narrow absorption window drug Repaglinide to increase bioavailability and patient compliance. To design and select the best formulation, a three-factor, three-level Box – Behnken design was used[11]. The ionotropic gelation method was used to prepare the formulations, involving independent variables Sodium alginate (mg), HPMC K100 (mg), and $CaCl_2$ (%w/v) and dependent variables Entrapment efficiency (%), In-vitro release of drug (%), and Swelling index (%).

Materials and Methods

Repaglinide was procured from Torrent Pharmaceuticals, Ahmedabad, (Gujrat), Sodium alginate, and HPMC K100M from Loba Chemie Pvt ltd. Mumbai and Colorcon Asia Pvt ltd. and calcium chloride from Arva synthesis Pvt ltd. (Hyderabad). All the other reagents used were of analytical grade.

Preliminary trials: Based on an earlier observation reported elsewhere, microsphere formulations were created by varying the concentration of polymers and cross-linking agents in different ratios. Lower sodium alginate concentrations resulted in a less viscous gel and smaller microsphere particle size, whereas higher calcium chloride concentrations increased drug entrapment efficiency[12,13].

Formulation of floating microsphere: Ionotropic gelation was used to make floating microspheres. With stirring, an accurately weighed amount of drug and sodium alginate were dispersed uniformly in ethanol. HPMC

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K100M in various concentrations was mixed in appropriate proportions with this dispersion, and stirring was continued. To the above solution, the required amount of sodium bicarbonate was added. Next, added the resultant dispersion drop by drop to calcium chloride solution using a 22G syringe needle with continuous stirring on a magnetic stirrer[14,15]. To improve mechanical strength, the formed microspheres were suspended in the solution for 1 hour before being collected, then washed with distilled water, and air-dried. The formulations were created using a Box – Behnken experimental design, and statistical screening was used to find the best formulation. The experiment's seventeen runs were

evaluated for particle size, surface morphology, swelling index, drug entrapment efficiency, and in vitro drug release.

Experimental design: Response surface methodology was used in this study for optimizing floating microspheres of repaglinide and investigating the correlation between responses and factors. DESIGN EXPERT (Version 13 Stat-Ease Inc., Minneapolis, USA) was used for statistical experimental design. The factor interaction between the variables was assessed using response surface graphs. Concentrations of sodium alginate (A), HPMC K100M (B), and calcium chloride (C) were investigated as independent variables, while Entrapment efficiency (%) and In vitro drug release and Swelling index (%) were the dependent variables with their three factorial levels low (-1), medium (0) and high (+1) for theses are given in Table 1.

In the case that involves 3-4 variables, the preferred design is Box-Behnken as it comprises of lesser runs as compared with that of central composite design. The developed design consists of 17 runs, for which the nonlinear computer-generated quadratic model can be expressed as follows:

$$R = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

where R is a response, b_0 is intercept, b_1–b_{33} are regression coefficients, and A, B, and C are independent variables while the interaction terms are (AB, AC, and BC) and (A^2, B^2, and C^2) are the quadratic one, respectively. Linear and second-order polynomials were fitted to the experimental data to obtain the regression equation[16]. Table 2. Depicts their observed and predicted responses.

Table 1 Level of independent and dependent variables used in experiments.

		Levels		
		Low	Medium	High
Factors	Process Parameter	(-1)	(0)	(+1)
Independent Variables				
A	Sodium alginate (mg)	400	800	1200
B	HPMC K100M (mg)	100	200	300
C	Calcium chloride (%w/v)	1	3	5
Dependent Variables			Constrains	
R1	Entrapment efficiency (%)		Maximize	
R2	In vitro drug release (%)		Maximize (sustained release)	
R3	Swelling index (%)		Maximize	

Each formulation contains 2 mg of repaglinide, sodium bicarbonate (5%),

Table 2 Observed responses in Box-Behnken design for repaglinide floating microspheres.

Batch	Repaglinide floating microspheres								
	Independent variables			Dependent variables (Actual Responses)			Dependent variables (Predicted Responses)		
	A (mg)	B (mg)	C (%w/v)	R1 (%)	R2 (%)	R3 (%)	R1 (%)	R2 (%)	R3 (%)
F1	400	200	1	63.4	76.02	68.2	63.34	76.08	63.66
F2	800	300	5	78.2	76.9	62.2	77	76.70	53.60
F3	800	200	3	81.32	79.4	60.01	80.73	79.53	54.34
F4	800	200	3	80.52	77.6	52.2	80.73	79.53	54.34
F5	800	100	1	70.4	77.2	40.6	71.6	77.41	49.20
F6	1200	200	5	72.98	72.62	80.4	73.04	72.56	84.94
F7	800	300	1	78.4	77.8	62.2	76.8	78.19	65.28
F8	800	200	3	82.02	80.02	48.4	80.73	79.53	54.34

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Batch	Repaglinide floating microspheres								
	Independent variables			Dependent variables (Actual Responses)			Dependent variables (Predicted Responses)		
	A (mg)	B (mg)	C (%w/v)	R1 (%)	R2 (%)	R3 (%)	R1 (%)	R2 (%)	R3 (%)
F9	400	200	5	73.4	84.8	38.1	72.95	79.53	54.34
F10	1200	200	1	72.8	76.2	79.2	73.25	75.55	72.06
F11	800	200	3	81.8	80.42	58.6	80.73	79.53	54.34
F12	1200	300	3	67.8	71.42	53.2	68.95	71.68	57.26
F13	800	100	5	79.2	85.68	58.4	80.8	85.29	55.33
F14	800	200	3	78	80.2	52.5	80.73	79.53	54.34
F15	400	300	3	62.3	80.42	34.4	63.96	79.97	35.86
F16	400	100	3	64.4	82.56	30.1	63.25	82.3	26.04
F17	1200	100	3	69.92	76.72	54.2	68.26	77.17	52.74

Surface morphology and particle size characterization

The prepared microspheres were morphologically examined for shape, size, surface morphology, and topological properties using a scanning electron microscope (JEOL, Model JSM -840, USA Inc.) after gold sputtering at a pressure of 5.13E to 4 pascals and 5 kV at 0°C were maintained to get the photographs. Particle sizes of microspheres were determined by optical microscopy[17]. An optical microscope was fitted with an eyepiece micrometer which was then calibrated with a stage micrometer. About 100 microspheres were randomly selected from each formulation and then the mean diameter was calculated[18].

Drug entrapment efficiency

To calculate the entrapment efficiency, an accurately weighed quantity of microspheres (50 mg) were taken along with a suitable solvent (50 ml) in a volumetric flask and kept for 24 hours. It was then filtered, suitably diluted, and then analyzed by UV spectrophotometry at 241 nm[19].

% Entrapment Efficiency = (Drug loading/Theoretical drug loading) × 100

Swelling index

An accurately weighed amount of microspheres (100 mg) were immersed in 25 ml of 0.1 M HCl (pH 1.2). Microspheres were removed from the medium after fixed intervals of 5 h. They were immediately wiped with paper and weighed. Using the formula below, the weight change of the microspheres with regard to time has been calculated[20,21].

Swelling ratio (%) = Ws – Wi / Wi × 100

Where,

Wi initial weight of the microspheres

Ws weight of the microspheres in the swollen state

In vitro drug release

A six-basket USP XXIV dissolution apparatus type I (Model 8000, Labindia Analytical Instruments Pvt. Ltd., Mumbai, INDIA) was used to simulate the gastric environment with rotation at 100 rpm maintained at 37 ± 0.5 °C and 0.1N HCl (pH 1.2) as dissolution medium (900 ml). A series of aliquots were removed at defined intervals up to 12 h after dilution, and the measurements were performed by ultraviolet spectroscopy at a maximum wavelength of 241 nm[22] (Shimadzu UV-1800 UV-VIS spectrophotometer, Japan). For sink conditions to be maintained, the withdrawn volume was replaced with an equivalent volume of fresh medium. Each experiment was replicated three times[23,24].

Drug release kinetics and transport mechanism

Based on the drug release data, the kinetics of drug release were assessed by fitting it to zero-order, first-order, and Higuchi models. Additionally, data were analysed with the Korsmeyer-Peppas model to calculate the value of n, the release exponent that describes the mechanism by which the drug is released[25].

Statistical analysis

Results were calculated and expressed as a mean ± standard deviation of three determinations. The design expert 13 software was used for statistical analysis. The statistical analysis was carried out with the Response surface methodology (RSM) using the Design of experiments technique (DOE) (Box - Behnken Design). The process variables, and its effect on particle size, entrapment efficiency, and drug release was statistically analyzed using ANOVA.

Optimization

In design expert, to validate the polynomial equation ANOVA application was used[26]. A total of seventeen runs (F1 – F17) were evaluated in terms of statistically significant coefficients and R squared values. To validate the chosen experimental design and polynomial equations, one optimum

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Table 4a Quadratic model and coefficients for release of drug from formulations, entrapment efficiency and swelling index for repaglinide floating microspheres.

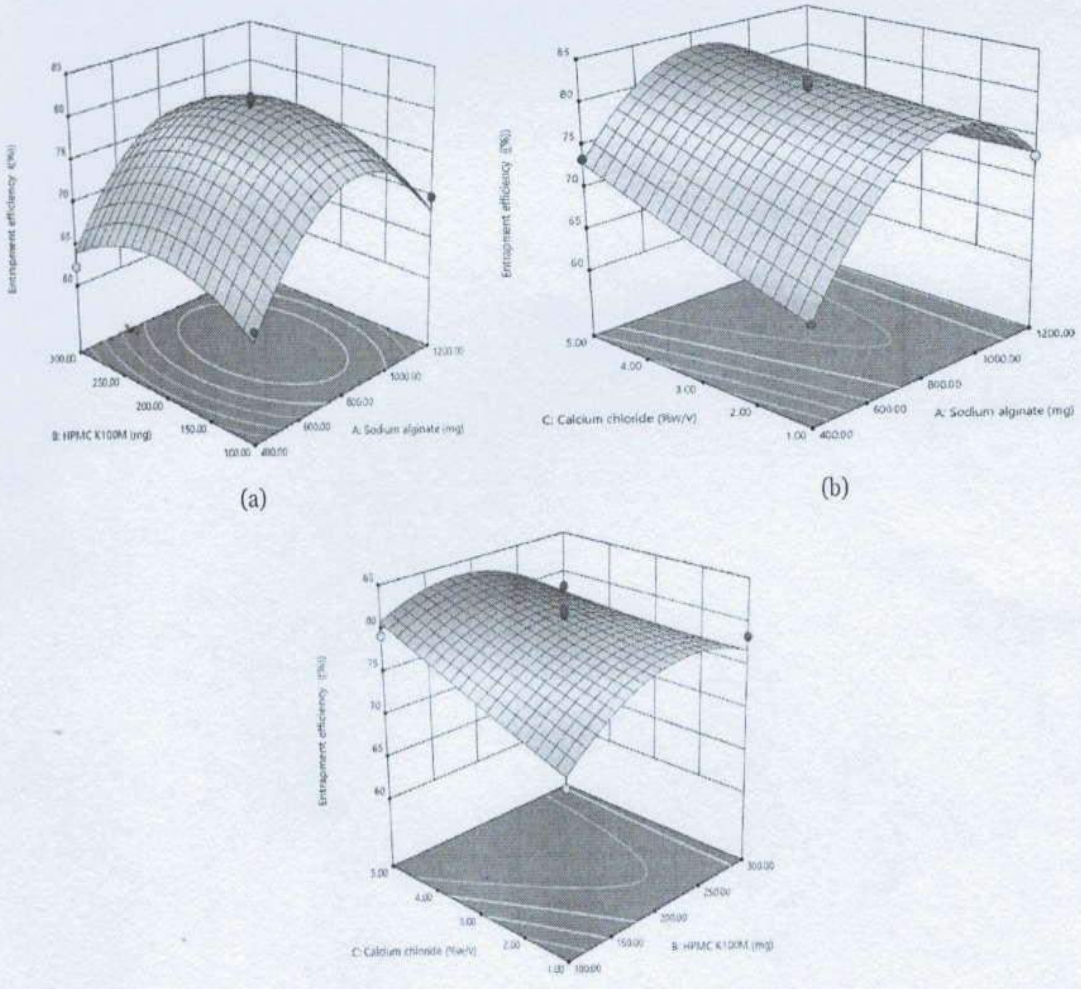
Terms	Entrapment efficiency (R1)			In vitro drug release (R2)			Swelling index (R3)		
	Coefficients	SE	Range*	Coefficients	SE	Range*	Coefficients	SE	Range*
Constants	80.73	0.88	78.65 to 82.82	79.528	0.45	78.47 to 80.59	54.342	3.55	45.95 to 62.73
Sodium alginate (A)	2.5	0.7	0.85 to 4.15	-3.355	0.35	-4.19 to -2.52	12.025	2.81	5.39 to 18.66
HPMC K100M (B)	0.35	0.7	-1.3 to 2	-1.9525	0.35	-2.79 to -1.12	3.5875	2.81	-3.05 to 10.22
CaCl2 (C)	2.35	0.7	0.7 to 4	1.5975	0.35	0.76 to 2.43	-1.3875	2.81	-8.02 to 5.25
A × B	-5.00E-03	0.99	-2.34 to 2.33	-0.79	0.5	-1.97 to 0.39	-1.325	3.97	-10.71 to 8.06
A × C	-2.46	0.99	-4.79 to -0.12	-3.09	0.5	-4.27 to -1.91	7.825	3.97	-1.56 to 17.21
B × C	-2.25	0.99	-4.58 to 0.082	-2.345	0.5	-3.53 to -1.16	-4.45	3.97	-13.83 to 4.93
A^2	-10.27	0.96	-12.54 to -7.99	-1.8665	0.49	-3.02 to -0.71	-0.371	3.87	-9.51 to 8.77
B^2	-4.36	0.96	-6.63 to -2.09	0.1185	0.49	-1.03 to 1.27	-10.996	3.87	-20.14 to -1.85
C^2	0.18	0.96	-2.09 to 2.45	-0.2515	0.49	-1.4 to 0.90	12.504	3.87	3.36 to 21.65

Table 4b ANOVA for response surface quadratic model

Results of the ANNOVA	Entrapment efficiency	In-vitro drug release	Swelling index
Regression			
Sum of squares	686.29	218.83	2716.23
Degree of freedom	9	9	9
Mean squares	76.25	24.31	301.80
F value	19.61	24.29	4.79
P value	0.0004	0.0002	0.0254
Residual			
Sum of squares	10.65	5.22	93.55
Degree of freedom	4	4	4
Mean squares	2.66	1.31	23.39
Lack of fittest			
Sum of squares	16.57	1.78	347.18

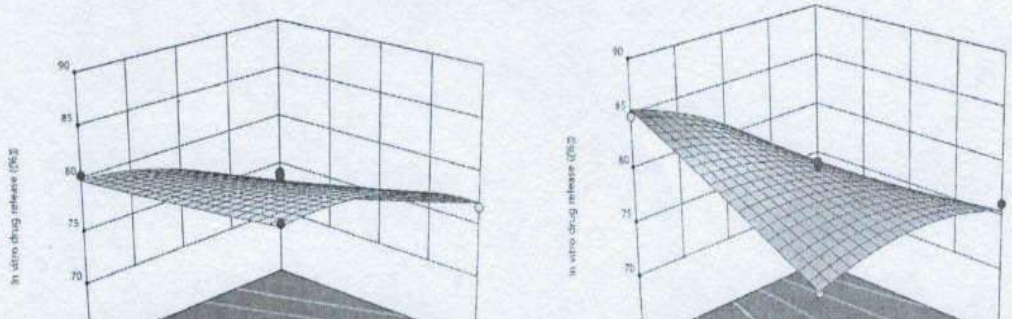
Response surface analysis

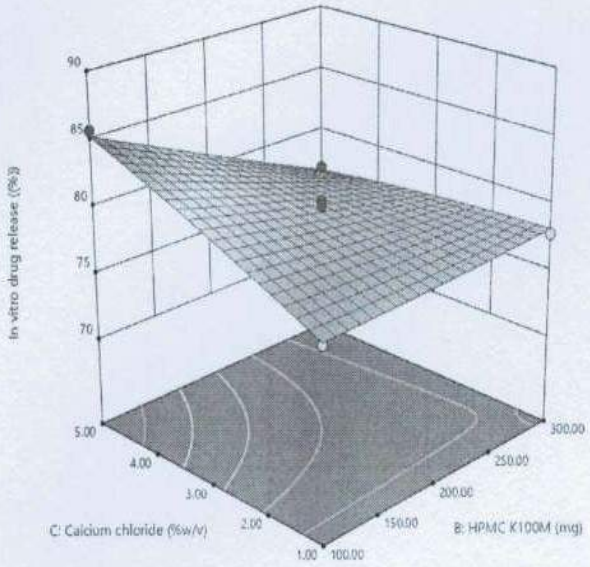
For all the responses Three-dimensional (3D) surface plots were generated which are depicted in Figures 2a - 2c, Figures 3a - 3c, and Figures 4a - 4c for responses R1, R2, and R3, respectively.



(a) (b) (c)

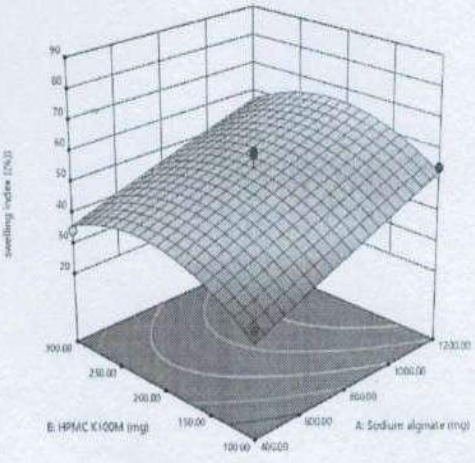
Fig. 2. The effect of independent variables on response R1 (Entrapment efficiency): (a) Effect of A and B; (b) Effect of B and C; and (c) Effect of A and C.



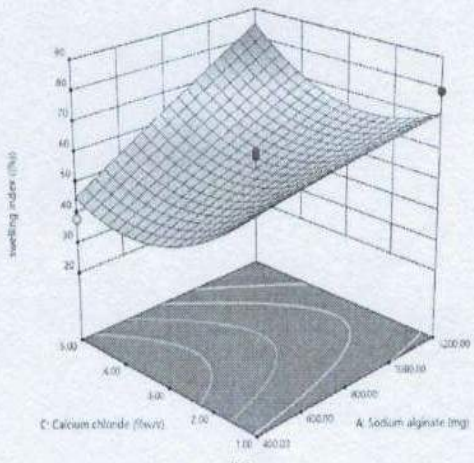


(c)

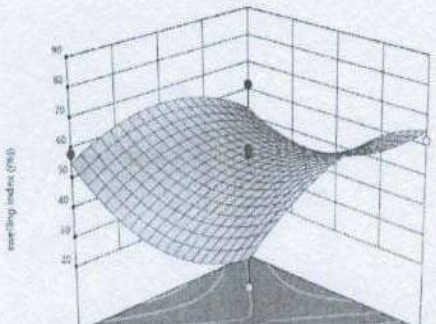
Fig. 3 The effect of independent variables on response R2 (In vitro drug release): (a) Effect of A and B; (b) Effect of A and C; and (c) Effect of B and C.



(a)



(b)



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Response R1: Effect on entrapment efficiency

$R1 = 80.73 + 2.50A + 0.35B + 2.35C - 5.00AB - 2.46AC - 2.25BC - 10.27A^2 - 4.36B^2 + 0.18C^2$

Based on model F-value (19.61), there is a 0.04% chance that such a "Model F-Value" could occur due to noise. Model terms A, B, C, AC, A^2 and B^2 were significant, indicated by values of "Prob > F" <0.0500(P-value = 0.0004). Figures 2a – 2c are the response surface plot showing the effect of different independent variables on the entrapment efficiency of microspheres.

Response R2: Effect on In vitro drug release

In vitro drug release:

$R2 = 79.53 - 3.35A - 1.95B + 1.60C - 0.79AB - 3.09AC - 2.34BC - 1.87A^2 + 0.12B^2 - 0.25C^2$

Where A is the concentration of sodium alginate, B is the concentration of HPMC K100M, and C is the concentration of calcium chloride. This means that the model's F-value of 24.29 indicates its significance. The Model F-value of 24.29 implies the model is significant. There is a 0.02% chance that a "Model F-value" this large could occur due to noise. Model terms A, B, C, AC, BC, A^2 were significantly indicated by the values of "Prob>F"<0.0500. Figures 3a - 3c are the response surface plot showing the effect of different independent variables on in vitro drug release.

Response R3: Effect on swelling index

The model proposes the following equation for swelling index

$R3 = 54.34 + 12.03A + 3.59B - 1.39C - 1.32AB + 7.83AC - 4.45BC - 0.37A^2 - 11.0B^2 + 12.50C^2$

Based on Model F value (4.79) that there is a 2.54% chance that such a "Model F- value" occurs due to noise. Model terms A, B^2 and C^2 were significant, indicated by value "Prob>F<0.0500" (P = 0.0254). Factor A and B, showed more pronounced effect on the swelling index. Figures 4a - 4c is the response surface plot showing the effect of different independent variables on in swelling index.

Selection of optimized formulation using point prediction method

The desirability approach has been employed for the selection of optimized formulation. Numeric optimization indicated the levels of independent variables namely sodium alginate, HPMC K100M, and calcium chloride (A, B, and C) used to prepare the optimized formulation with the maximum entrapment efficiency, in vitro drug release and swelling index (R1, R2, and R3) illustrated in Table 1. The optimized formulation was prepared in the triplicate manner through the optimal levels of the independent variables, suggested by the Design Expert®13. The predicted and actual values of dependent and independent variables were given in Table 5. All data are presented as mean ± SD, (n = 3).

In vitro drug release

The In vitro studies revealed that on increasing the polymer concentration the release of drug decreases (P < 0.05). Figure 5 depicts the release behaviour of OF-1.

Table 5 Composition of optimised formulation with predicted and observed values of responses

Batch code	Sodium alginate (mg)	HPMC K100M (mg)	Calcium chloride (%w/v)	Entrapment efficiency (%)	In vitro drug release (%)	Swelling index (%)	Desirability
	Predicted formulation						
RM 1	969.947	186.307	5.00	81.604	78.435	73.814	0.922
	Actual Optimised Formulation						
OF1	970.00	186.00	5.00	78.22 ± 2.20	73.39 ± 3.09	70.46 ± 2.05	------

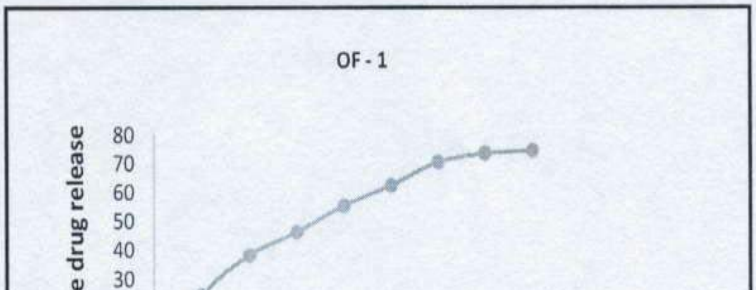

Table 6 Release kinetics and transport mechanism of optimized formulation

Model	Equation	Parameters	Optimised formulation (OF1)
Zero order	$m_o - m = kt$	r^2	0.768
First order	In m = kt	r^2	0.989
Higuchi's model	$m_o - m = kt1/2$	r^2	0.996
Korsmeyer-peppas	Log $(m_o - m)$ = log k + nlog t	r^2	0.982
Diffusion coefficient		N	0.662

Mechanism of release

The drug release data obtained from the in vitro studies were applied to different kinetic models of release kinetics. The drug transport mechanism was characterized by the Korsmeyer-Peppas equation (Table 6). Where m_o is the initial drug amount; m is the amount of drug remaining at a specific time; k is the rate constant; t is the time; r^2 is correlation coefficient; n is diffusion coefficient.

Discussion

In this study, we used Repaglinide encapsulate into sodium alginate microparticles and evaluate the resulting microparticles. Our findings suggest that the microparticles formed using the ionic gelation method and Box - Behnken Design® software for optimization could be useful for encapsulating a variety of therapeutic agents for a wide range of drug delivery systems, including oral, transdermal, and ocular.

Preliminary trials: Different proportions of polymers and cross-linking agents were used to create microspheres. Since a lower amount of polymers resulted in irregularly shaped microspheres, the sodium alginate and HPMCK100M contents were set at 400–1200 mg and 100–300 mg, respectively, with the medium level at 800 and 200 mg. As per literature, a concentration of calcium chloride of 1-5% w/v was selected to control the level of cross-linking between the bivalent cation Ca^{2+} and the acid group of alginate[27,28].

Formulation optimization

Using a three-factor, three-level set-up, seventeen formulations were prepared and evaluated. The evaluated responses were particle size, entrapment efficiency, and in-vitro drug release. Due to poor molecular packing and cross-linking, microspheres made with low concentrations of polymers (- level) were rough and irregular in shape, whereas microspheres made with medium and high concentrations of polymers (+ level) were larger and more spherical. With different polymeric concentrations, the size and morphology of the microspheres varied due to differences in the availability of reacting/binding sites for cross-linking cations. Smooth, spherical, and smaller microspheres were obtained as the cross-linking agent content was increased, and they were well-packed and discrete[29] (Figure 1).

Further, we investigated the influence of polymer concentration on drug entrapment efficiency which was more intricate. Both polymers affected drug entrapment efficiency but in a reciprocal manner. The effect of the concentration of sodium alginate was directly proportional to entrapment efficiency, while the effect of HPMC K100M was inversely proportional. The formulation with the lowest sodium alginate content and the highest HPMC K100M (F15) content had the lowest DEE of 62.30. Both formulations (F16) with low levels of sodium alginate and HPMC K100M showed 64.40 percent drug entrapment, which was in the middle of the range. The F8 formulation (82.02 percent) had the highest entrapment efficiency, with all factors at a medium level.

In the drug release study, minimum drug release was observed in the formulation containing the highest concentration of polymers (+1 level), and the maximum drug release was observed in formulation F13 (85.68%) containing sodium alginate and HPMC K100M at 0 and -1 levels, and cross-linking agent at +1 level, respectively. The formulations containing a similar amount of sodium alginate but with lower levels of HPMC K100M showed better drug release at 76.72%. Similarly, a high amount of drug release (84.8%) was obtained in formulation F9 containing -1 and 0 levels of sodium alginate and HPMC K100M, and a higher level of cross-linking agent. From this, it is clear that both sodium alginate and HPMC K100M controlled the release of the drug and were more or less compensating for each other as far as drug release was concerned.

Total of seventeen runs were generated by box - Behnken design for three factors at three levels to identify the optimum levels of different independent process parameters. The observed values for entrapment efficiency, drug release, and swelling index range from 62.3 to 82.02%, 85.68 to 71.42%, and 30.01 to 80.4%, respectively. The values of R- squared, Adj - squared, Pred R- squared, SD, and % CV are shown in Table 3. along with the regression equation. Moreover, the closeness between the adjusted and predicted R^2 values of all the responses for the regression equations showed the statistical significance and validity of these equations for the optimization of repaglinide microspheres. From the regression equations (Table 4a), it is clear that the entrapment efficiency mainly depends on the sodium

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alginate and HPMC K100M concentration, whereas the release of drug and swelling index was mainly affected by the HPMC K100M and $Cacl_2$.

Values of ANOVA for various responses are mentioned in Table 4b. and all statistically significant ($p < 0.05$) coefficients are included in the equations.

A positive value in an optimization design indicates a favorable optimization, and a negative value indicates an inverse relationship between the response and the factor. In this study, it is clear that all the three independent variables, including sodium alginate concentration (A), HPMC K100M (B), and $Cacl_2$ (C), interacted with each other to lead to three potential estimates, namely drug entrapment efficiency (R1), in-vitro drug release (R2), and swelling index (R3).

Response surface analysis

Response R1: Effect on entrapment efficiency

The amount of both polymer and the crosslinking agents showed a pronounced effect on drug release (Bhavesh Patel et al., 2021). The "lack of Fit F-value" of 2.07 implies lack of fit is not significant relative to the pure error. The "Pred R-Squared" of 0.6051 is not as close to the "Adj R-Squared" of 0.9128 as one might normally expect. "Adeq Precision" value of 11.602 shows the presence of adequate signal and the model can be used to navigate the design space. Figures 2a – 2c is the response surface plot showing the effect of different independent variables on the entrapment efficiency of microspheres. Formulations F3, F4, F8, F11, and F14 showed maximum entrapment efficiency, that is, 78- 82.02% (all the three factors at zero level), while F15 showed minimum entrapment efficiency of 62.3% (sodium alginate at -1 level, HPMC K100M +1 level, and calcium chloride at 0 levels). This shows that a lower concentration of sodium alginate has a negative effect on entrapment efficiency. However, stirring speed and stirring time have a negative effect on drug entrapment efficiency as an increase in stirring speed and exposure time, microsphere breaks down because of higher shear force and particle size decreased. Results in a decrease in entrapment efficiency (Vijay Bahadur et al., 2020).

Response R2: Effect on In vitro drug release

The amount of polymer showed a pronounced effect on drug release. The lack of fit was not significantly indicated by the "Lack of Fit F-value" of 0.46. There is a 72.78% chance that a "Lack of Fit F-value" (quite high) could occur due to noise. The "Pred R-squared" of 0.8374 is in reasonable agreement with the "Adj R-squared" of 0.9291. "Adeq Precision" at 17.994 indicates an adequate signal to use the model to navigate the design space.

Figures 3a - 3c are the response surface plot showing the effect of different independent variables on in vitro drug release. Formulations F9, F11, F13, F14, F15, and F16 showed maximum in vitro drug release, that is, 84.8%, 80.42%, 85.68%, 80.2%, 80.42% and 82.56%, respectively. This indicated that in vitro drug release was affected by the concentration of both the polymer and the cross-linking agent significantly.

Response R3: Effect on swelling index

An increase in the amount of polymer and crosslinking agent increased swelling of the microsphere. While calcium chloride decreases the swelling ratio[31]. The lack of fit was not significantly indicated by the "Lack of Fit F-value" of
4.95. High R^2 value for all three responses obtained from the linear correlation plots between the predicted and the experimental values. The R^2 values for responses R1, R2 and R3 were in the range 0.9598 - 0.9618, 0.9668 - 0.9690 and 0.8596 - 0.8604. Here the ratio of 11.602 (for R1), 17.944 (for R2), and 9.678 (for R3) indicates an adequate signal, hence this model could be used to navigate the response surface design space.

Optimization

Numerical optimization technique provides the optimum values of each response, that is, particle size (R1), drug entrapment efficiency (R2), and % in vitro drug release (R3) which is used to prepared the optimized formulation The optimum formulation was selected to achieve. The constraints for the response R1, R2, and R3 were found to be in the range $62.3 \leq R1 \geq 82.02$, $71.42 \leq R2 \geq 85.68$, $30.1 \leq R3 \geq 80.4$. The closeness in the predicted and actual values of these variables signifies the use of Design-Expert as a reliable optimization tool to formulate floating microspheres.

In vitro floating test

In vitro floating test, indicated that the optimized formulation showed the acceptable floating time. As HPMC is a cellulosic hydrocolloid polymer (S. Prajapati et al., 2011). Formulation OF1 exhibited fairly good floating time up to 12 hr.

In vitro drug release

The In vitro studies revealed that on increasing the polymer concentration the release of drug decreases ($P < 0.05$). It is caused because the viscosity of the polymer matrix increases thereby leading to the formation of large size microspheres which ultimately makes the diffusional path length of the drug increase. Also, therelease of drug and the swelling capacity decreases on increasing the amount of cross-linking agent due to the presence of smaller cavities, thereby causing a gradual decline in the effective surface area for diffusion of the drug. The cumulative amount of drug release from the optimized formulation, OF1, was found to be 82.72. Figure 5 depicts the release behavior of OF-1.

Mechanism of release

The drug release data obtained from the in vitro studies were applied to different kinetic models of release kinetics. The best fit was predicted by the Higuchi model (r^2, 0.996) than by zero-order (r^2, 0.768) and first-order (r^2, 0.989) models, which finally showed the release of repaglinide from the formulated mucoadhesive microspheres is diffusion controlled. To find out release kinetics, the experimental data were further applied to the Korsmeyer-Peppas equation. It was found that, optimized formulation OF-1 have release exponent (n) value was 0.662 ($0.5 < n < 1$), indicates non-fickian or

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diffusion-controlled drug release and it is controlled by swelling and relaxation of polymer.

Hydrophilic polymers were used which gets swell when coming in contact with an aqueous phase. This causes enhancement in swelled polymer porosity and decreased path length thereby increasing the release, whereas a decrease in concentration gradient delays the drug release. The optimized formulation has an optimal concentration, ensure constant release of drug toits desired site of action.

Conclusion

Repaglinide floating microspheres were successfully prepared and optimized by Box - Behnken design using sodium alginate as release retarding polymer, HPMC K100M as floating polymer, and calcium chloride as a cross-linking agent. The important formulation parameters such as particle size, entrapment efficiency, and in vitro drug release for different combinations of independent variables, were obtained experimentally, and the results were found to fit the design model.

The results for the present study showed that the responses i.e, particle size, entrapment efficiency, and in vitro release are significantly affected by the concentration of polymer and a cross-linking agent. The optimized formulation OF1 showed controlled release for more than 12 hr and followed the Higuchi model. The validations of RSM for three dependent variables were 100.09%, 99.68%, and 97.02%. Therefore, it can be concluded that developed floating microspheres provides a versatile platform for controlled delivery and improving the bioavailability of repaglinide using a Box - Behnken design.

Declaration of interest

The authors report no declarations of interest.

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Conflict of interests:

Declared none

References

1. A Gaur PK, Mishra S, Kumar A, Panda BP. Development and optimization of gastroretentive mucoadhesive microspheres of gabapentin by Box–Behnken design. Artif Cells Nanomedicine Biotechnol. 2014 Jun;42(3):167–77.
2. Waterman KC. A Critical Review of Gastric Retentive Controlled Drug Delivery. Pharm Dev Technol. 2007 Jan;12(1):1–10.
3. Hatorp V. Clinical Pharmacokinetics and Pharmacodynamics of Repaglinide: Clin Pharmacokinet. 2002;41(7):471–83.
4. Joshi G, Saini S, Arora S, Sharma A, Kumar V. Innovation in gastroretentive drug delivery systems: formulations and techniques for anti- diabetic drugs. Int J Adv Pharm. 2011;2(4):11.
5. M Sharma et al. In-vitro and in-vivo evaluation of repaglinide loaded floating microspheres prepared from different viscosity grades of HPMC polymer. Res Artic. 2015;23(6):675–82.
6. Ganesan P, Deepa John AJ, Sabapathy L, Duraikannu A. Review on Microsphere. Am J Drug Discov Dev. 2014 Jun 15;4(3):153–79.
7. K Vinchurkar et al. Features & Facts of Gastroretentive Drug Delivery System – A Review. Turk J Pharm Sci. 2021 Jun 18;1–21.
8. Yeole PG et al. Floating drug delivery systems: need and development. Indian JPharm Sci. 2004;3(67):265–72. 3
9. Soliman M, Elmowafy E, Almogerbi A, Mansour S. Dual buoyant/mucoadhesive macroporous polypropylene microparticles for gastric delivery of Repaglinide. Int J Drug Deliv. 2016;7(4):197–207.
10. Metkari VB, Kulkarni LV, Patil PS, Jadhav PA, Bamane GS, Kumbhar CM. Microspheres - A New Drug Delivery System: A Review. 2014;4(2):1128– 33.
11. Singh B et al. Developing micro-/nanoparticulate drug delivery systems using "design of experiments." Rev Artic. 2011;1(2):75–87.
12. Chowdary KPR, Rao YS. Design and in vitro and in vivo evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: A technical note. AAPS Pharm Sci Tech. 2003 Sep;4(3):87–92.
13. Prajapati SK, Tripathi P, Ubaidulla U, Anand V. Design and Development of Gliclazide Mucoadhesive Microcapsules: In Vitro and In Vivo Evaluation. AAPS PharmSciTech. 2008 Mar;9(1):224.
14. Chowdary KPR, Rao YS. Preparation-and- evaluation-of-mucoadhesive-microcapsules-of-indomethacin.pdf. Indian JPharm Sci. 2003;65(1):49–52.
15. Madhusudhan S, Panda AK, Parimalakrishnan S, Manavalan R, Manna PK. Design, in vitro and in vivo evaluation of glipizide Eudragit microparticles. J Microencapsul. 2010 Jan;27(4):281–91.
16. Abdallah MH. Box-behnken design for development and optimization of acetazolamide microspheres. Int J Pharm Sci Res. 2014;5(4):1228–39.

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REVIEW ARTICLE

Nanocarriers and Diabetes: New Vistas and the Way Ahead

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Abstract: World Health Organization has reported an estimated 1.5 million deaths directly due to diabetes in 2019. Center for Disease Control and Prevention, in its National Diabetes Statistics Report, 2020, says that 1 in 10 United States residents has diabetes. This rapid progression of diabetes is noteworthy despite significant advances in the field of antidiabetic medicine. The critical challenges in treatment are dyslipidemia, hyperinsulinemia, and hyperglycemia. The latest research has also linked diabetes to carcinogenesis. The diabetic condition accelerates cell growth, proliferation, migration, inflammation, angiogenesis, metastasis, and inhibition of apoptosis in cancer cells. In addition, diabetic complications of nephropathy, retinopathy, neuropathy, cardiomyopathy, peripheral arterial disease, coronary artery disease, and stroke increase morbidity. Amidst all these challenges, a ray of hope is the advent of nanocarriers. The nano size helps in the targeted and controlled delivery of drugs. In addition, nanocarrier formulation helps in the delivery of acid-labile and enzyme-labile molecules and plant-based macromolecules *via* the oral route. Its use in the form of dendrimers, ethosomes, niosomes, transfersomes, and polymeric nanoparticles is established. In addition, different polymers used to formulate nanocarriers are also established for targeting diabetes. Thus, this review aims to compile approaches involving the use of nanocarriers for the betterment of pharmacotherapy of diabetes and to provide a way ahead for researchers in the field.

Keywords: Hypoglycemic agents, liposomes, ethosomes, niosomes, transfersomes, dendrimers, cancer, diabetic complications.

1. INTRODUCTION

Over the past few decades, we have witnessed that the extensiveness of diabetes is spreading abundantly at an incredible rate, affecting various age groups. Globally, the number of cases of diabetes shall leap to 227 million from the year 2015 (425 million) to 2045 (629 million) [1]. The zones that are most prone to diabetes across the world are southeast Asia and Western Pacific, followed by North America. Diabetes mellitus (DM) is a metabolic disorder causing hyperglycemia, increased urine secretion, delayed healing, and eventual renal damage. DM is classified into two types, *i.e.*, Type 1 DM (T1DM) and Type 2 DM (T2DM). In T1DM, the pancreas is inefficient in producing insulin, secreted by specialized cells present in islets of Langerhans, whereas in T2DM, the body shows an impaired response toward the amount of insulin secreted. T1DM is of two types, 1A and 1B. 1A is immune-mediated diabetes wherein obliteration of β-cells occurs and 1B is idiopathic diabetes [2, 3]. T2DM is associated with lowering insulin sensitivity and deterioration of its secretion from pancreatic β-cells leading to dysregulation of glucose levels in the blood [4, 5]. In addition, a gestational type of [1] diabetes mellitus also exists. Fig. (**1**) provides a summary of different types of diabetes with their complications. Maintaining blood sugar levels within physiological limits is essential to avoid diabetic complications. This is achieved by scheduled administration of insulin, diet management, and physical activity. Parenteral injection of insulin is a massive deterrent to its widespread use. Insulin administration techniques are regularly being advanced and optimized *via* the development of numerous devices and formulations. However, safe, precise, controlled, and sustained drug or insulin delivery is far from reality, stemming an aggressive search for new alternatives for optimum and timely delivery *via* various routes [6-8]. By overwhelming the pharmacokinetic and biopharmaceutical barriers associated with plant-derived antidiabetic medications, nanotechnology is the best approach for enlightening compliance and clinical effectiveness [9]. Numerous recent reviews have highlighted the applications of nanocarriers and nanotechnology for optimizing pharmacotherapy *via* the use of plant-based antidiabetic medications, use of nanocarriers for augmenting bioavailability, co-delivery of genes and peptides, topical delivery of drugs to reduce some diabetic complications, oral, buccal and nasal delivery of insulin, new approaches that are incorporating nanocarriers inside micelles, and many more. However, an

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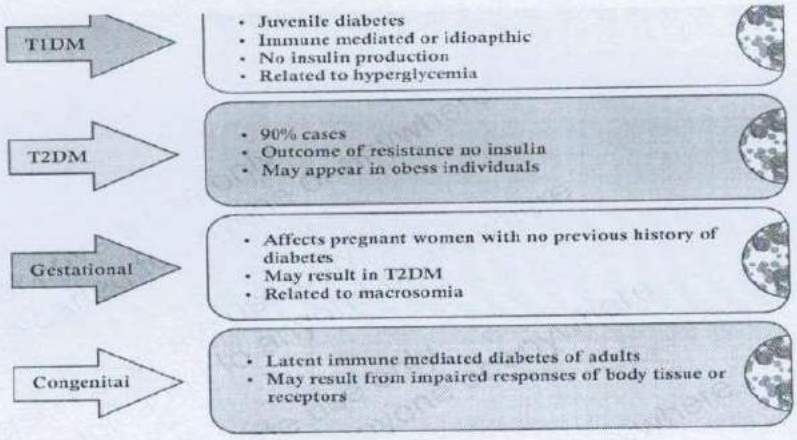

Fig. (1). Type of diabetes and their complications. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

compilation of all such approaches involving the use of nanocarriers for the betterment of pharmacotherapy of diabetes for a glance comparative reading that will help researchers in the field to broaden further the horizons of their translational research [10-21].

2. CURRENT CHALLENGES OF ANTIDIABETICS

Antidiabetics are readily available in the market as tablets or capsules (Table **1**). Antidiabetic agents control blood sugar levels throughout the day, including during meals. However, these drugs' common side effects are nausea, upset stomach, and increased bowel movement. These side effects contribute to poor quality of life for diabetic patients. A prolonged effect on the targeted site of action is the essential requirement for drug efficacy [22-23]. On consumption, the drug is released in the systemic circulation that eventually reaches the target site. For a sustained effect, dosage regimen compliance is required. Commonly prescribed antidiabetics are metformin/biguanides, sulfonylurea, and thiazolidinediones. Metformin use is associated with burping, belching, upset stomach, dizziness, and renal malfunctions. The use of sulfonylurea derivatives causes skin rash and gastric irritation. Likewise, thiazolidinediones cause weight gain, risk of anemia, and risk of liver disease. Injections of insulin and peptides are also available, but the phobia of pricking the skin acts as a barrier [24-28].

Nanocarriers, if utilized as dosage forms, may overcome all these complications and side effects. Nanocarriers provide targeted action, sustained drug release, avoidance of first-pass effect, increased therapeutic window, and many more [29].

3. NOVEL DRUG DELIVERY SYSTEM

The limitations of the current pharmacotherapy are a stimulus for researchers [illegible] more precise systems. Furthermore, changes to the features of already-existing antidiabetic drugs have avoided or altered the hurdles that have previously limited their use. Various nanocarriers are already available in the pharmaceutical market, and new carriers are continuously emerging [30, 31]. Fig. (**2**) describes the existing nanocarriers and their structural description, mechanism, function, and application in diabetes. A novel drug delivery system (NDDS) ensures safe and precise delivery of antidiabetics to the targeted sites for the scheduled time to enhance therapeutic efficacy and patient compliance and consequently improve control over diabetes. Table **2** enlists novel drug delivery systems with their key features and advantages.

3.1. Microspheres

Microspheres, also known as microparticles, comprise a vital fraction of NDDS. They are carrier systems typically made up of free-flowing powders. Microspheres are tiny, *i.e.*, less than 200 micrometers, and have a large surface-to-volume ratio. Microspheres are biodegradable and consist of protective materials such as waxes, polymers, and modified natural products such as starch, gum, proteins, and fats. The drug is dissolved or dispersed throughout the particle matrix. They possess interfacial and colloidal properties [31, 32].

Microparticles are valuable tools for parenteral, oral, topical, and nasal routes. Literature documents the formulation of magnetic microparticles, solid lipid microparticles, biological microparticles, metal-based and polymeric microparticles. The efficacy of Glipizide and Gliclazide was enhanced using the concept of microencapsulation. Furtado *et al.* [32] formulated poly (fumaric-co-sebacic anhydride) microspheres to increase the life of the drug span *via* the oral route. Recently, a very effective formulation, Afrezza (MannKind Corporation), received marketing approval from the

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Table 1. Antidiabetics with their functions and side effects.

Antidiabetics	Function	Side Effects
Metformin	Reduces production of glucose in liver and increases insulin sensitivity	Gastric complications, diarrhoea, improper bowel movement, decreased appetite, fatigue, nausea
Thiazolidinediones (TZDs)	Lowers body's resistance to insulin	Edema of ankles and wrists, susceptible to anaemia, chances of liver diseases increases
Sulfonylureas	Stimulates release of insulin in beta cells of pancreas	Upset stomach, weight gain, skin complications like rash, itching, redness, *etc.* Poses major risk for cardiovascular mortality
Metglinides	Induce insulin secretion in presence of glucose right after meal consumption	Weight gain, lower back pain, disturbed gastrointestinal functioning, provides short term action
Alpha glucosidase inhibitors	Extends duration of carbohydrate digestion and decreases absorption of glucose	Flatulence, nausea, upset stomach
DPP-4 inhibitors	Increases insulin secretion, decreases blood sugar level	Skin rashes, runny nose, coughing, nausea, abdominal pain
SGLT-2 inhibitors	Decrease or completely inhibit reabsorption of glucose *via* kidney resulting in elimination of excess glucose through urine	Urinary tract infections, risk of hypoglycemia, kidney complications

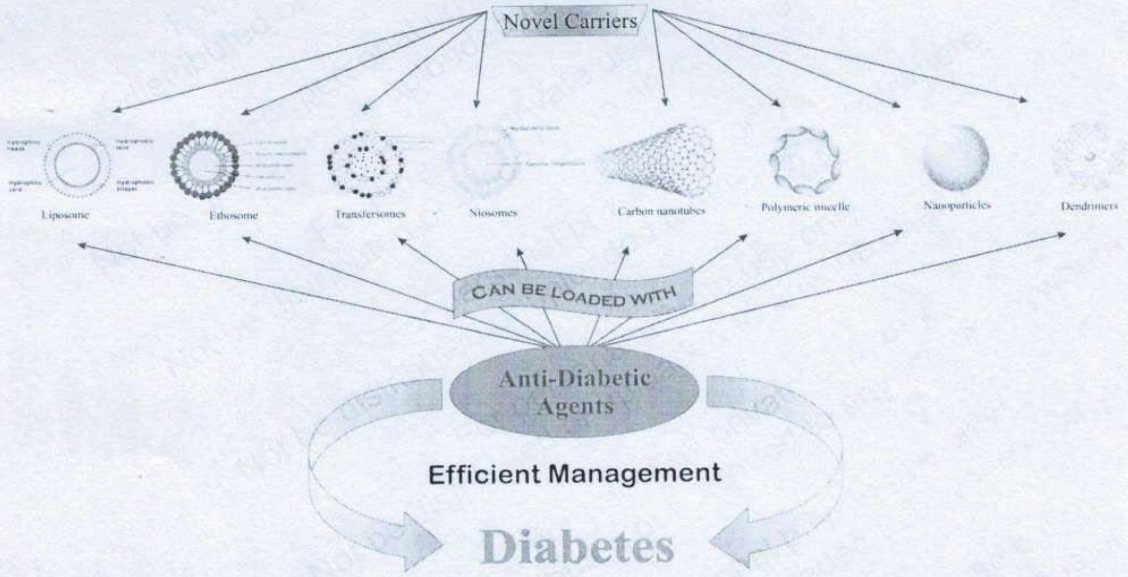

Fig. (2). Classification of novel carriers for the effective management of diabetes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

FDA to control meal-time blood sugar spikes. It is a very fast-acting inhalable insulin prepared as microspheres. Clinically, it lowers glycated hemoglobin (HbA1c) levels in patients with type 1 and type 2 diabetes. *In vitro* study of mucoadhesive microspheres of Repaglinide showed prolonged controlled action and gastric residence time. Additionally, mucoadhesive microspheres are not degraded in the stomach and directly reach the absorption site enabling improvement in absorption [33, 34]. Microspheres are also suitable for the development of an oral formulation of insulin [35]. Microspheres containing insulin are proficient enough to maintain stable plasma glucose levels (about 100 mmol/L), up until 15 h of administration.

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Table 2. Novel drug delivery systems with salient features.

Carrier	Drug	Features
Microparticles	Insulin	• Safeguarding protein stability • Lengthened lifespan • Prolonged release • For oral/nasal route • No GIT degradation • Enhanced stability
	Glimepiride	• Enhanced oral bioavailability • Sustained release • Increased gastric residence time • Improved patient compliance
	Glipizide	• Prolonged release • Improved *in vivo* hypoglycaemic activity • Superior drug entrapment efficiency • Long lasting effect • Greater adheration to mucus when sodium alginate along with Carbopol 934 used
	Exenatide	• Prolonged release • Decreased blood sugar level in diabetic rats
	Repaglinide	• Sustained release • Increased retention in GIT
Nanoparticles	Insulin	• Enhanced intestinal absorption • Hypoglycemic activity observed in diabetic rats
	Glibenclamide	• Enhanced dissolution rate • Enhanced bioavailability
	Repaglinide	• Sustained release • Improved patient compliance • Reduced dosing frequency
Transfersomes	Metformin	• Enhanced bioavailability
Liposomes	Insulin	• Enhanced stability • Prolonged release
Niosomes	Nateglinide and glimeperide	• Improved oral bioavailability, enhanced rate and extent of hypoglycaemic activity
Ethosomes	Repaglinide	• Improved bioavailability, reduced dose and dosing frequency
	Glimepiride and gliclazide	• Very effective when loaded into transdermal vehicle

The mechanisms involved in the release of microencapsulated drugs are diffusion for reservoir type and erosion or diffusion for matrix-type formulations. The transcellular route is the primary transport mechanism of the microparticulate systems reported in the literature. The size and nature (hydrophilic/hydrophobic) of particles serve as the limiting factor for transport through biological membranes. Transport of microparticles through the paracellular route does not occur because the average diameter of the tight junctions is 3.9-8.4°A. Thus, microparticles typically cross the biological membranes *via* some transcellular pathways, *i.e.*, endocytosis (carrier- or receptor-mediated) [36-38].

3.2. Microcapsules

Microcapsules are an emerging category of nanocarriers. They are microencapsulated in a structure loaded with drugs inside and may be coated with specific antibodies for targeting [33, 38]. There are various types of microcapsules, such as microcapsules with a solid center; microcapsules with a nonsolid center; microcapsules with a solid micro- or nano-

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field; microcapsules with nonsolid micro- or nano- field; microspheres with a mixture of encapsulated components.

Microcapsules are widely used in islet cell transplantation. Microencapsulation safeguards new cells against immune attacks due to biopolymers used for joint, immune isolation. Generally, islet cells are incorporated inside sodium alginate microcapsules, which facilitate the transfer of healthy cells. Microcapsules are still in their budding stage and a lot of progress is yet to be made in this area concerning the treatment of diabetes [39-41]. Secondly, a lot of clinical applications are evident for antibiotics, anticancer drugs, and topical formulations. In the case of antidiabetics, the applications of microcapsules are limited to a few and wide scope is there for implementation in clinics.

3.3. Liposomes

Liposomes are small lipid vesicles made up of phospholipids and cholesterol, and they are generally spherical. Phospholipids, an essential ingredient of liposomes, are nontoxic, biocompatible, and biodegradable. The liposome is a sphere-shaped vesicular system that consists of a lipid bilayer, which transports drugs to different biological compartments. These are formulated using phospholipids like phosphatidylcholine, and a few others so long as they are attuned to the lipid bilayer structure. A liposome design may employ surface ligands for attaching to unhealthy tissue [42, 43]. Recently, elastic liposomes have been used for the buccal delivery of insulin across porcine tissues [44]. Similarly, Silymarin nanoliposomes are reported to be effective in nephropathy in rats [45]. The liposomal drug delivery approach is widely being explored to successfully deliver peptide types of antidiabetic drugs like GLP-1 and its analogs and insulin. Even embryonic stem cell therapy approaches also use liposomes.

3.4. Ethosomes

Ethosomes are pliable, malleable novel vesicular carriers. Ethosomes deliver active substances into the deep layers of the skin *via* the stratum corneum. They mainly contain ethanol, phospholipids, and water. The interaction between ethanol and phospholipids produces an effect more remarkable than the sum of their individual effects, *i.e.*, a synergistic effect occurs that is highly beneficial in efficient drug delivery across skin. Moreover, the relatively high concentration of ethanol allows vesicles to penetrate through the skin barrier. Enhanced permeation, no toxic raw materials, and high patient compliance, simple drug delivery are the advantages associated with ethosomes [46-48].

A preliminary study with insulin reveals some new opportunities for enhanced drug delivery. *In vivo* findings show transdermal delivery of insulin-loaded ethosomal carriers in normal and diabetic rats lowered blood sugar levels lasting for not less than 8 hours. A study examined eight ethosomal preparations of Gliclazide and a single formulation of hydroethanolic solution for different formulation parameters. Ethosomes were reported to be very efficient drug delivery carriers for topical and systemic delivery [49, 50]. A repaglinide-loaded ethosomal gel was evaluated for vesicular size, shape, stability, entrapment efficiency, and *in vivo* and *ex vivo* tests. The [illegible] gel exhibited improved *in vitro* drug release, which helped in the minimization of dose and dosing frequency, enhanced bioavailability, and improved skin permeation capacity. Similarly, a report on Glimepiride ethosomal nanovesicles performed *ex vivo* and *in vivo* studies, showed that *ex vivo* permeations of ethosomal loaded formulation, were comparatively better than pure drug formulation. Concluding, ethosomes may demonstrate an extraordinary future in glucose-mediated delivery of drugs [51-53]. Ethosomal formulations are preferred for the transdermal delivery of drugs and this is not an exception for antidiabetic drugs. It is successfully evaluated for transdermal delivery of Glimipride and found to be associated with a reduction in side effects.

3.5. Niosomes

Niosomes are non-ionic surfactant vesicles, a hydrating combination of single or double alkyl chains. They come into existence by gathering to form a bunch of these nonionic surfactants in aqueous media. The parameter that makes niosomes exclusive nanocarriers is that they can hold hydrophilic and lipophilic drugs in them due to their amphiphilic properties. Significant advantages of niosomes include cheaper raw materials, stability, and carefree storage. Thus, niosomes are included in a controlled and novel type of drug delivery system. Insulin-containing niosomes were formulated with an external coating of N-trimethyl chitosan (TMC). They were evaluated using a Caco-2 cell monolayer that acted as an intestine mock-up. The entrapment efficiency (EE) parameter and permeability were determined and found to be optimum under different conditions. In addition, researchers have used niosomes for vaginal and oral drug delivery, and drug delivery of insulin [53], oral delivery of metformin [54], and repaglinide The incorporation of drugs within niosomes is very favorable in terms of sustained release and targeted action of the drug, resulting in maximum bioavailability and reduced toxicity [55-57]. Similar to liposomal drug delivery, niosomes are also widely used to deliver peptide drugs, indicating the benefit of protection from enzymatic degradation that they confer. In addition, a reduction in total dose is also possible; hence, this approach is suitable for antidiabetic peptide drugs, phytochemical agents, and metformin.

3.6. Transfersomes

A novel nanocarrier "transfersomes" encapsulates the drug in a vesicular system. The name comes from the Latin 'transfere' and 'soma' which means "carrying body". These are also called elastic liposomes, which consist of edge activators, phospholipids, and ethanol. They are applied nonocclusive (will not seal or block through the area). They have the potential to improve the transdermal delivery of drugs, which are deformable lipid complex molecular aggregates. Due to their flexibility, they can impulsively penetrate the stratum corneum proficiently to squeeze through one-tenth of their diameter channels. The osmotic gradient (driving force) is set up by the water content difference between the viable aqueous epidermis and the relatively dehydrated skin surface (~20% water), allowing the penetration to occur. It can be used to deliver insulin as it possesses stability, high penetration due to the high deformability, high entrapment effi-

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ciency, biocompatibility, biodegradability, suitable for drug molecules having a high molecular weight and wide range of solubility, protection against metabolic degradation [58-60].

Transfersomes containing insulin can deliver the drug through the skin barrier with no changes in the pharmacokinetic and pharmacodynamic properties compared to the injected insulin. A normal blood sugar level lasting less than 16 is achieved using a single noninvasive epicutaneous administration of insulin. Metformin transdermal patches are formulated using transfersomes, which overcome its gastrointestinal side effects, has been overcome using in a [61]. Sodium cholate and phosphatidylcholine were used in the formulation of transferosomes containing metformin. In hyperglycemic rabbits treated with a metformin transfersome patch, the plasma concentration of the drug was extensively higher than in controls. The study showed enhanced antihyperglycemic effect and bioavailability of metformin. Glibenclamide (GBD), an oral hypoglycemic agent, is associated with hypoglycemic episodes and numerous gastrointestinal side effects. These side effects were overcome by using a transferosomal GBD formulation. The histopathological study of GBD-loaded nano-transfersomes revealed loosening and rupture of the stratum corneum, indicating increased penetration *via* zero-order kinetics. *In vivo* study of optimized transfersomal gel has shown a prolonged hypoglycemic effect in alloxan-induced diabetic rats for more than 24 h after transdermal administration [62]. A recent study of ginsenoside Rh1 isolated from red ginseng employed transfersomes, and evaluated its particle size, zeta potential, entrapment efficiency, and shape. It showed higher skin permeation compared to ethosome and conventional liposomes. Consequently, ginsenoside Rh1-loaded transfersomes can act as topical therapeutic effects' potential [63]. Furthermore, nano-transferosomes are also being developed as part of a strategy for the concomitant treatment of hypertensive diabetic subjects. Pioglitazone (PIO) and eprosartan mesylate were formulated together as nano-transferosomes [64].

3.7. Polymeric Nanoparticles

Nanoparticles are particles whose sizes range between 1-100 nanometers and are objects which behave as whole units concerning their transport and properties. These particles have comparatively higher intracellular intake than other systems, making them a carrier system of choice when delivering antidiabetic drugs. In polymeric nanoparticles, the drug is either dispersed in the particles' core or adsorbed on the surface of the nanoparticles. These polymeric nanoparticles are produced by the dispersion of natural macromolecules or synthetic polymers or by the polymerization of synthetic monomers. Some examples of polymers used in the formulation of polymeric nanoparticles are chitosan, polyvinyl alcohol, poly-lactic acid (PLA), polymethylmethacrylate (PMMA). In addition, zwitterionic arginyl-glycyl-aspartic acid-conjugated polypeptide nanoformulation was prepared for doxorubicin to achieve targeted drug delivery in the tumor. Polymeric micelles containing camptothecin and coated with RBC membrane achieved targeting and better efficiency due to nanosizing. To illustrate the absorption effectiveness of these self-emulsifying drug delivery systems a biomimetic membrane assay is also developed to achieve clinical translation [65-69]. According to recent advances, nanoparticles have proved to be of great potential in the treatment of diabetes by behaving as carriers for insulin. Controlled delivery of insulin has been made possible because of biodegradable polymeric nanoparticles. These polymers are biodegradable and have nano-porous membranes containing glucose oxidase. This grafted membrane surrounds the polymer insulin matrix. Controlled insulin delivery in these nanoparticles is based on the simple concept that a rise in blood glucose level alters the nano-porous membrane. Biodegradation of the membrane occurs due to alteration, resulting in subsequent insulin delivery [70, 71].

3.8. Lipid-based Nanoparticles

Solid lipid nanoparticles (SLN) were, for the first time, prepared in 1991. It is used as a substitute carrier method over the conventional colloidal carrier such as emulsion, liposomes, microneedles, dendrimers, *etc*. It is prepared using a mixture of lipids, emulsifiers, and water ranging from 50 to 100 nm. The main reasons for the rising interest in the lipid-based system are enhanced oral bioavailability with reduced plasma profile variation, and improved characterization of lipid excipients. SLN is used for the oral delivery of insulin. Results reported significant hypoglycemic activity [72, 73].

Nanostructured lipid carrier (NLC) is another lipid-based carrier system and a modified form of SLN. They are colloidal carriers and are based on a mixture of solid lipids and liquid lipids that remain solid at the body and room temperature. This nanostructure improves drug loading and drug stability during storage. NLC could be a better alternative to SLN due to superior controlled drug release and better stability profile. NLC has been explored using different routes, like oral, transdermal, ocular, *etc*., for drug delivery with enhanced bioavailability [74]. Silymarin, a mixture of flavonolignans, has recently proven its utility in type II diabetic patients. Silymarin loaded with NLC exhibited a significant down-regulation of blood glucose and improved triglyceride levels [75]. Like numerous drugs, Baicalin, Quercetin, Resveratrol, N-palmitoylethanolamide have been successfully employed with NLC for improved bioavailability, sustained release, and better antidiabetic effects [76].

Nanoemulsions (NEs) are emulsions and are usually made up of oil, water, and emulsifier. They offer numerous benefits over other delivery systems, like higher solubilization capacity, quick onset of action, toxicological safety, and the possibility of large-scale production. NEs have proven their utility for Repaglinide, Insulin, 2,4,6-triphenylaniline and showed significant hypoglycemic effects [77].

3.9. Dendrimers

Dendrimers are a class of synthetic polymeric nanoparticles that are highly branched and have a tree-like structure design. Their diameter is approximately 2-10 nm on a nanometer scale. The most used dendrimers are the series of polyamidoamine (PAMAM). PAMAM possesses very interesting characteristics, which make it superior to classical linear polymers. In one study, PAMAM-treated animals considerably lowered their blood glucose level in comparison with non-treated animals. They also lower diabetes-induced blood-brain barrier permeability [78, 79].

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Fig. (3). Applications of NDDS for diabetes management. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

4. APPLICATIONS OF NDDS FOR DIABETES MANAGEMENT

In recent years, Novel drug delivery systems (NDDSs) are becoming trendy because they deliver palpable assistance in terms of abridged dosing frequency, improved bioavailability, prevention from degradation specifically in contrast to the punitive gastric environment, site specificity and reduced adverse effects [80, 81]. Nanocarriers have been explored for numerous antidiabetic agents as shown in Fig. (3). In summary, the major advantage of these micro- and nanoparticulate systems or carriers is the feasibility of peptide-like drug delivery *via* oral route *e.g.* insulin, and GLP-1 analogs. In addition, an increased rate of absorption due to solubilization for many drugs is possible and demonstrated. Third, but by no means less is targeting, which is being explored by many. These formulations are also handy for islet cell transplantation techniques. So, the preclinical scenario involving NDDS use in diabetes management is promising and the clinical translation is in the incubation phase.

CONCLUSION

The annual economic burden of diabetes is huge and steadily increasing; however, the progress in modern drug delivery is also catching up. The approaches addressed in the review, if successful at the clinical level, may be the game-changer as far as diabetic pharmacotherapy is concerned. However, cost and biosafety are two vital issues that need to be addressed as early as possible for nano- and microparticulate systems. Biocompatibility is the key to the successful implementation of these NDDS. To address this issue, advances in the understanding of cellular pathways handling vesicular nutrient delivery, especially *via* the Golgi apparatus pathway, must be carefully looked into, and an inter-disciplinary approach addressing the pharmacokinetics and toxicokinetics shall pave the way for clinical success of these nanotechnological avenues. The objectives of this review are to compile tactics involving the applications of nanocarriers for the betterment of pharmacotherapy of diabetes and to provide a way ahead for researchers in the field.

FUTURE PERSPECTIVES

With the advancement in nanotechnology, developing new ways of administrating anti-diabetics is feasible. For instance, octreotide acetate microcapsules are available in the market prepared by Novartis. Such advances serve as a basis for many other novel systems. The main concerns associated with the process of NDDS are approval procedures, cost, registration, etc. Providentially, the government is becoming aware of the vastly feasible outcomes from NDDS, but at the same time, not taking any steps regarding its economic impact. Consequently, some flourishing countries

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decided to provide funds. Furthermore, the association between two or more organizations with multidisciplinary expertise should be made. In addition, safeguarding property rights is very significant as it provides large profitable success after being transferred to the pharmaceutical industry. Moreover, testing parameters are required to be reassessed for the assessment of NDDS-based products. Proper guidelines as well as amendments to the earlier documented ones are needed in the future for NDDS. Lastly, the success of NDDS will boost reluctant people to look at them more closely.

LIST OF ABBREVIATIONS

NDDSs	=	Novel Drug Delivery Systems
PAMAM	=	Polyamidoamine
SLN	=	Solid Lipid Nanoparticles
PMMA	=	Polymethylmethacrylate

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Rai, V.K.; Mishra, N.; Agrawal, A.K.; Jain, S.; Yadav, N.P. Novel drug delivery system: An immense hope for diabetics. *Drug Deliv.*, **2016**, *23*(7), 2371-2390. http://dx.doi.org/10.3109/10717544.2014.991001 PMID: 25544604

[2] Veiseh, O.; Tang, B.C.; Whitehead, K.A.; Anderson, D.G.; Langer, R. Managing diabetes with nanomedicine: Challenges and opportunities. *Nat. Rev. Drug Discov.*, **2015**, *14*(1), 45-57. http://dx.doi.org/10.1038/nrd4477 PMID: 25430866

[3] Moghassemi, S.; Hadjizadeh, A. Nano-niosomes as nanoscale drug delivery systems: An illustrated review. *J. Control. Release.* **2014**, *185*, 22-36. http://dx.doi.org/10.1016/j.jconrel.2014.04.015 PMID: 24747765

[4] Poudwal, S.; Misra, A.; Shende, P. Role of lipid nanocarriers for enhancing oral absorption and bioavailability of insulin and GLP-1 receptor agonists. *J. Drug Target.*, **2021**, *29*(8), 834-847. http://dx.doi.org/10.1080/1061186X.2021.1894434 PMID: 33620269

[5] Lee, J.S.; Han, P.; Chaudhury, R.; Khan, S.; Bickerton, S.; McHugh, M.D.; Park, H.B.; Siefert, A.L.; Rea, G.; Carballido, J.M.; Horwitz, D.A.; Criscione, J.; Perica, K.; Samstein, R.; Ragheb, R.; Kim, D.; Fahmy, T.M. Metabolic and immunomodulatory control of type 1 diabetes *via* orally delivered bile-acid-polymer nanocarriers of insulin or rapamycin. *Nat. Biomed. Eng.*, **2021**, *5*(9), 983-997. http://dx.doi.org/10.1038/s41551-021-00791-0 PMID: 34616050

[6] Misra, P.; Upadh[illegible]; Anand, K. A review of the epidemiology [illegible] India. *Diabetes Res. Clin. Pract.*, **2011**, *92*(3), [illegible]

[7] http://dx.doi.org/10.1016/j.diabres.2011.02.032 PMID: 21458875 Ensign, L.M.; Cone, R.; Hanes, J. Oral drug delivery with polymeric nanoparticles: The gastrointestinal mucus barriers. *Adv. Drug Deliv. Rev.*, **2012**, *64*(6), 557-570. http://dx.doi.org/10.1016/j.addr.2011.12.009 PMID: 22212900

[8] Tuomilehto, J.; Lindström, J.; Eriksson, J.G.; Valle, T.T.; Hämäläinen, H.; Ilanne-Parikka, P.; Keinänen-Kiukaanniemi, S.; Laakso, M.; Louheranta, A.; Rastas, M.; Salminen, V.; Aunola, S.; Cepaitis, Z.; Moltchanov, V.; Hakumäki, M.; Mannelin, M.; Martikkala, V.; Sundvall, J.; Uusitupa, M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.*, **2001**, *344*(18), 1343-1350. http://dx.doi.org/10.1056/NEJM200105033441801 PMID: 11333990

[9] Padhi, S.; Nayak, A.K.; Behera, A. Type II diabetes mellitus: A review on recent drug based therapeutics. *Biomed. Pharmacother.*, **2020**, *131*, 110708. http://dx.doi.org/10.1016/j.biopha.2020.110708 PMID: 32927252

[10] Shoaib, A.; Azmi, L.; Pal, S.; Alqahtani, S.S.; Rahamathulla, M.; Hani, U.; Alshehri, S.; Ghoneim, M.M.; Shakeel, F. Integrating nanotechnology with naturally occurring phytochemicals in neuropathy induced by diabetes. *J. Mol. Liq.*, **2021**, *350*, 118189.

[11] Yu, B.; Li, C.; Sun, Y.; Wang, D.W. Insulin treatment is associated with increased mortality in patients with COVID-19 and type 2 diabetes. *Cell Metab.*, **2021**, *33*(1), 65-77. http://dx.doi.org/10.1016/j.cmet.2020.11.014 PMID: 33248471

[12] Muneer, M. Hypoglycaemia. *Adv. Exp. Med. Biol.*, **2021**, *1307*, 43-69.

[13] Singh, A.P.; Biswas, A.; Shukla, A.; Maiti, P. Targeted therapy in chronic diseases using nanomaterial-based drug delivery vehicles. *Signal Transduct. Target. Ther.*, **2019**, *4*(1), 33. http://dx.doi.org/10.1038/s41392-019-0068-3 PMID: 31637012

[14] Dewanjee, S.; Chakraborty, P.; Mukherjee, B.; De Feo, V. Plant-based antidiabetic nanoformulations: The emerging paradigm for effective therapy. *Int. J. Mol. Sci.*, **2020**, *21*(6), 2217. http://dx.doi.org/10.3390/ijms21062217 PMID: 32210082

[15] Nie, X.; Jnr, Chen, Z.; Pang, L.; Wang, L.; Jiang, H.; Chen, Y.; Zhang, Z.; Fu, C.; Ren, B.; Zhang, J. Oral nano drug delivery systems for the treatment of type 2 Diabetes mellitus: An available administration strategy for antidiabetic phytocompounds. *Int. J. Nanomedicine*, **2020**, *15*, 10215-10240. http://dx.doi.org/10.2147/IJN.S285134 PMID: 33364755

[16] Karabasz, A.; Bzowska, M.; Szczepanowicz, K. Biomedical applications of multifunctional polymeric nanocarriers: A review of current literature. *Int. J. Nanomedicine*, **2020**, *15*, 8673-8696. http://dx.doi.org/10.2147/IJN.S231477 PMID: 33192061

[17] Lemmerman, L.R.; Das, D.; Higuita-Castro, N.; Mirmira, R.G.; Gallego-Perez, D. Nanomedicine-based strategies for diabetes: Diagnostics, monitoring, and treatment. *Trends Endocrinol. Metab.*, **2020**, *31*(6), 448-458. http://dx.doi.org/10.1016/j.tem.2020.02.001 PMID: 32396845

[18] Maikawa, C.L.; d'Aquino, A.I.; Lal, R.A.; Buckingham, B.A.; Appel, E.A. Engineering biopharmaceutical formulations to improve diabetes management. *Sci. Transl. Med.*, **2021**, *13*(578), eabd6726. http://dx.doi.org/10.1126/scitranslmed.abd6726 PMID: 33504649

[19] Chariou, P.L.; Ortega-Rivera, O.A.; Steinmetz, N.F. Nanocarriers for the delivery of medical, veterinary, and agricultural active ingredients. *ACS Nano*, **2020**, *14*(3), 2678-2701. http://dx.doi.org/10.1021/acsnano.0c00173 PMID: 32125825

[20] Kankala, R.K.; Lin, X.F.; Song, H.F.; Wang, S.B.; Yang, D.Y.; Zhang, Y.S.; Chen, A.Z. Supercritical fluid-assisted decoration of nanoparticles on porous microcontainers for codelivery of therapeutics and inhalation therapy of diabetes. *ACS Biomater. Sci. Eng.*, **2018**, *4*(12), 4225-4235. http://dx.doi.org/10.1021/acsbiomaterials.8b00992 PMID: 33418821

[21] Souto, E.B.; Souto, S.B.; Campos, J.R.; Severino, P.; Pashirova, T.N.; Zakharova, L.Y.; Silva, A.M.; Durazzo, A.; Lucarini, M.; Izzo, A.A.; Santini, A. Nanoparticle delivery systems in the treatment of diabetes complications. *Molecules*, **2019**, *24*(23), 4209. http://dx.doi.org/10.3390/molecules24234209 PMID: 31756981

[22] Sutradhar, K.B.; Sumi, C.D. Implantable microchip: The futuristic controlled drug delivery system. *Drug Deliv.*, **2016**, *23*(1), 1-11.

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http://dx.doi.org/10.3109/10717544.2014.903579 PMID: 24758139

[23] Pradhan, S.K. Microsponges as the versatile tool for drug delivery system. *Int. J. Res. Pharm. Chem.*, **2011**, *1*(2), 243-258.

[24] Kalra, S. Glucagon-like peptide-1 receptors agonists (GLP1 RA). *J. Pak. Med. Assoc.*, **2013**, *63*(10), 1312-1315. PMID: 24392570

[25] Rathore, B.; Yadav, A.; Nayak, G.; Saraogi, G.K.; Singhai, A.K. A review on microspheres as drug delivery carriers for management of diabetes mellitus. *Int J of Pharm & Life Sci*, **2012**, *3*, 2064-2070.

[26] Lopes, M.; Simões, S.; Veiga, F.; Seiça, R.; Ribeiro, A. Why most oral insulin formulations do not reach clinical trials. *Ther. Deliv.*, **2015**, *6*(8), 973-987. http://dx.doi.org/10.4155/TDE.15.47 PMID: 26272222

[27] Singh, M.N.; Hemant, K.S.Y.; Ram, M.; Shivakumar, H.G. Microencapsulation: A promising technique for controlled drug delivery. *Res. Pharm. Sci.*, **2010**, *5*(2), 65-77. PMID: 21589795

[28] Buder, B.; Alexander, M.; Krishnan, R.; Chapman, D.W.; Lakey, J.R.T. Encapsulated islet transplantation: Strategies and clinical trials. *Immune Netw.*, **2013**, *13*(6), 235-239. http://dx.doi.org/10.4110/in.2013.13.6.235 PMID: 24385941

[29] Devadasu, V.R.; Alshammari, T.M.; Aljofan, M. Current advances in the utilization of nanotechnology for the diagnosis and treatment of diabetes. *Int. J. Diabetes Dev. Ctries.*, **2018**, *38*(1), 11-19. http://dx.doi.org/10.1007/s13410-017-0558-1

[30] DiSanto, R.M.; Subramanian, V.; Gu, Z. Recent advances in nanotechnology for diabetes treatment. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, **2015**, *7*(4), 548-564. http://dx.doi.org/10.1002/wnan.1329 PMID: 25641955

[31] Krol, S.; Ellis-Behnke, R.; Marchetti, P. Nanomedicine for treatment of diabetes in an aging population: State-of-the-art and future developments. *Nanomedicine*, **2012**, *8*(Suppl. 1), S69-S76. http://dx.doi.org/10.1016/j.nano.2012.05.005 PMID: 22640905

[32] Furtado, S.; Abramson, D.; Burrill, R.; Olivier, G.; Gourd, C.; Bubbers, E.; Mathiowitz, E. Oral delivery of insulin loaded poly(fumaric-co-sebacic) anhydride microspheres. *Int. J. Pharm.*, **2008**, *347*(1-2), 149-155. http://dx.doi.org/10.1016/j.ijpharm.2007.06.039 PMID: 17707601

[33] Wong, C.Y.; Al-Salami, H.; Dass, C.R. Microparticles, microcapsules and microspheres: A review of recent developments and prospects for oral delivery of insulin. *Int. J. Pharm.*, **2018**, *537*(1-2), 223-244. http://dx.doi.org/10.1016/j.ijpharm.2017.12.036 PMID: 29288095

[34] Wu, J.Z.; Williams, G.R.; Li, H.Y.; Wang, D.X.; Li, S.D.; Zhu, L.M. Insulin-loaded PLGA microspheres for glucose-responsive release. *Drug Deliv.*, **2017**, *24*(1), 1513-1525. http://dx.doi.org/10.1080/10717544.2017.1381200 PMID: 28975813

[35] Xing, X.; Zhao, X.; Ding, J.; Liu, D.; Qi, G. Enteric-coated insulin microparticles delivered by lipopeptides of iturin and surfactin. *Drug Deliv.*, **2018**, *25*(1), 23-34. http://dx.doi.org/10.1080/10717544.2017.1413443 PMID: 29226733

[36] Zhang, H.; Wang, W.; Li, H.; Peng, Y.; Zhang, Z. Microspheres for the oral delivery of insulin: Preparation, evaluation and hypoglycaemic effect in streptozotocin-induced diabetic rats. *Drug Dev. Ind. Pharm.*, **2018**, *44*(1), 109-115. http://dx.doi.org/10.1080/03639045.2017.1386197 PMID: 28956663

[37] Wang, J.; Tabata, Y.; Morimoto, K. Aminated gelatin microspheres as a nasal delivery system for peptide drugs: Evaluation of *in vitro* release and *in vivo* insulin absorption in rats. *J. Control. Release*, **2006**, *113*(1), 31-37. http://dx.doi.org/10.1016/j.jconrel.2006.03.011 PMID: 16707188

[38] Zheng, J.; Yue, X.; Dai, Z.; Wang, Y.; Liu, S.; Yan, X. Novel iron-polysaccharide multilayered microcapsules for controlled insulin release. *Acta Biomater.*, **2009**, *5*(5), 1499-1507. http://dx.doi.org/10.1016/j.actbio.2009.01.017 PMID: 19231303

[39] Llacua, L.A.; Hoek, A.; de Haan, B.J.; de Vos, P. Collagen type VI interaction improves human islet survival in immunoisolating microcapsules for treatment of diabetes. *Islets*, **2018**, *10*(2), 60-68. http://dx.doi.org/10.1080/19382014.2017.1420449 PMID: 29521546

[40] Dufrane, D.; Gianello, P. Macro- or microencapsulation of pig islets to cure type 1 diabetes. *World J. Gastroenterol.*, **2012**, *18*(47), 6885-6893. http://dx.doi.org/10.3748/wjg.v18.i47.6885 PMID: 23322985

[41] Mooranian, A.; Negrulj, R.; Al-Salami, H.; Morahan, G.; Jamieson, E. Designing anti-diabetic β-cells microcapsules using polystyrenic sulfonate, polyallylamine, and a tertiary bile acid: Morphology, bioenergetics, and cytokine analysis. *Biotechnol. Prog.*, **2016**, *32*(2), 501-509. http://dx.doi.org/10.1002/btpr.2223 PMID: 26748789

[42] Agrawal, A.K.; Harde, H.; Thanki, K.; Jain, S. Improved stability and antidiabetic potential of insulin containing folic acid functionalized polymer stabilized multilayered liposomes following oral administration. *Biomacromolecules*, **2014**, *15*(1), 350-360. http://dx.doi.org/10.1021/bm401580k PMID: 24283460

[43] Bi, R.; Shao, W.; Wang, Q.; Zhang, N. Spray-freeze-dried dry powder inhalation of insulin-loaded liposomes for enhanced pulmonary delivery. *J. Drug Target.*, **2008**, *16*(9), 639-648. http://dx.doi.org/10.1080/10611860802201134 PMID: 18982512

[44] Bashyal, S.; Seo, J.E.; Keum, T.; Noh, G.; Lamichhane, S.; Lee, S. Development, characterization, and *ex vivo* assessment of elastic liposomes for enhancing the buccal delivery of insulin. *Pharmaceutics*, **2021**, *13*(4), 565. http://dx.doi.org/10.3390/pharmaceutics13040565 PMID: 33923670

[45] Chen, Y.; Chen, L.; Yang, T. Silymarin nanoliposomes attenuate renal injury on diabetic nephropathy rats *via* co-suppressing TGF-β/Smad and JAK2/STAT3/SOCS1 pathway. *Life Sci.*, **2021**, *271*, 119197. http://dx.doi.org/10.1016/j.lfs.2021.119197 PMID: 33577847

[46] Mishra, D.; Mishra, P.K.; Dabadghao, S.; Dubey, V.; Nahar, M.; Jain, N.K. Comparative evaluation of hepatitis B surface antigen-loaded elastic liposomes and ethosomes for human dendritic cell uptake and immune response. *Nanomedicine*, **2010**, *6*(1), 110-118. http://dx.doi.org/10.1016/j.nano.2009.04.003 PMID: 19446655

[47] Dubey, V.; Mishra, D.; Jain, N.K. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. *Eur. J. Pharm. Biopharm.*, **2007**, *67*(2), 398-405. http://dx.doi.org/10.1016/j.ejpb.2007.03.007 PMID: 17452098

[48] Dubey, V.; Mishra, D.; Dutta, T.; Nahar, M.; Saraf, D.K.; Jain, N.K. Dermal and transdermal delivery of an anti-psoriatic agent *via* ethanolic liposomes. *J. Control. Release*, **2007**, *123*(2), 148-154. http://dx.doi.org/10.1016/j.jconrel.2007.08.005 PMID: 17884226

[49] Bodade, S.S.; Shaikh, K.S.; Kamble, M.S.; Chaudhari, P.D. A study on ethosomes as mode for transdermal delivery of an antidiabetic drug. *Drug Deliv.*, **2013**, *20*(1), 40-46. http://dx.doi.org/10.3109/10717544.2012.752420 PMID: 23311652

[50] Touitou, E.; Godin, B.; Weiss, C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Dev. Res.*, **2000**, *50*(3-4), 406-415. http://dx.doi.org/10.1002/1098-2299(200007/08)50:3/4<406::AID-DDR23>3.0.CO;2-M

[51] Ahmed, T.A.; El-Say, K.M.; Aljaeid, B.M.; Fahmy, U.A.; Abd-Allah, F.I. Transdermal glimepiride delivery system based on optimized ethosomal nano-vesicles: Preparation, characterization, *in vitro*, *ex vivo* and clinical evaluation. *Int. J. Pharm.*, **2016**, *500*(1-2), 245-254. http://dx.doi.org/10.1016/j.ijpharm.2016.01.017 PMID: 26775063

[52] M. Abdulbaqi, I.; Darwis, Y.; Abdul Karim Khan, N.; Abou Assi, R.; Ali Khan, A. Ethosomal nanocarriers: The impact of constituents and formulation techniques on ethosomal properties, *in vivo* studies, and clinical trials. *Int. J. Nanomedicine*, **2016**, 2279. http://dx.doi.org/10.2147/IJN.S105016

[53] Ning, M.; Guo, Y.; Pan, H.; Yu, H.; Gu, Z. Niosomes with sorbitan monoester as a carrier for vaginal delivery of insulin: Studies in rats. *Drug Deliv.*, **2005**, *12*(6), 399-407. http://dx.doi.org/10.1080/10717540590968891 PMID: 16253956

[54] Sankhyan, A.; Pawar, P.K. Metformin loaded non-ionic surfactant vesicles: Optimization of formulation, effect of process variables and characterization. *Daru*, **2013**, *21*(1), 7

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http://dx.doi.org/10.1186/2008-2231-21-7 PMID: 23351604
[55] Leyva-Gómez, G.; Del Prado-Audelo, M.L.; Ortega-Peña, S.; Mendoza-Muñoz, N.; Urbán-Morlán, Z.; González-Torres, M.; González-Del Carmen, M.; Figueroa-González, G.; Reyes-Hernández, O.D.; Cortés, H. Modifications in vaginal microbiota and their influence on drug release: Challenges and opportunities. *Pharmaceutics*, **2019**, *11*(5), 217. http://dx.doi.org/10.3390/pharmaceutics11050217 PMID: 31064154
[56] Pardakhty, A.; Varshosaz, J.; Rouholamini, A. *In vitro* study of polyoxyethylene alkyl ether niosomes for delivery of insulin. *Int. J. Pharm.*, **2007**, *328*(2), 130-141. http://dx.doi.org/10.1016/j.ijpharm.2006.08.002 PMID: 16997517
[57] Hasan, A.A.; Madkor, H.; Wageh, S. Formulation and evaluation of metformin hydrochloride-loaded niosomes as controlled release drug delivery system. *Drug Deliv.*, **2013**, *20*(3-4), 120-126. http://dx.doi.org/10.3109/10717544.2013.779332 PMID: 23651102
[58] Mishra, D.K.; Dhote, V.; Mishra, P.K. Transdermal immunization: Biological framework and translational perspectives. *Expert Opin. Drug Deliv.*, **2013**, *10*(2), 183-200. http://dx.doi.org/10.1517/17425247.2013.746660 PMID: 23256860
[59] Dubey, V.; Mishra, D.; Nahar, M.; Jain, N.K. Vesicles as tools for the modulation of skin permeability. *Expert Opin. Drug Deliv.*, **2007**, *4*(6), 579-593. http://dx.doi.org/10.1517/17425247.4.6.579 PMID: 17970662
[60] Mishra, D.; Garg, M.; Dubey, V.; Jain, S.; Jain, N.K. Elastic liposomes mediated transdermal delivery of an anti-hypertensive agent: Propranolol hydrochloride. *J. Pharm. Sci.*, **2007**, *96*(1), 145-155. http://dx.doi.org/10.1002/jps.20737 PMID: 16960826
[61] Malakar, J.; Sen, S.O.; Nayak, A.K.; Sen, K.K. Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery. *Saudi Pharm. J.*, **2012**, *20*(4), 355-363. http://dx.doi.org/10.1016/j.jsps.2012.02.001 PMID: 23960810
[62] Kanchan Kohli, V.M. Nano-carrier for accentuated transdermal drug delivery. *J. Dev. Drugs*, **2013**, *3*(2). http://dx.doi.org/10.4172/2329-6631.1000121
[63] Choi, J.H.; Cho, S.H.; Yun, J.J.; Yu, Y.B.; Cho, C.W. Ethosomes and transfersomes for topical delivery of ginsenoside Rh1 from red ginseng: Characterization and *in vitro* evaluation. *J. Nanosci. Nanotechnol.*, **2015**, *15*(8), 5660-5662. http://dx.doi.org/10.1166/jnn.2015.10462 PMID: 26369134
[64] Ramkanth, S.; Anitha, P.; Gayathri, R.; Mohan, S.; Babu, D. Formulation and design optimization of nano-transferosomes using pioglitazone and eprosartan mesylate for concomitant therapy against diabetes and hypertension. *Eur. J. Pharm. Sci.*, **2021**, *162*, 105811. http://dx.doi.org/10.1016/j.ejps.2021.105811 PMID: 33757828
[65] Lin, W.; Ma, G.; Yuan, Z.; Qian, H.; Xu, L.; Sidransky, E.; Chen, S. Development of zwitterionic polypeptide nanoformulation with high doxorubicin loading content for targeted drug delivery. *Langmuir*, **2019**, *35*(5), 1273-1283. http://dx.doi.org/10.1021/acs.langmuir.8b00851 PMID: 29933695
[66] Malhotra, S.; Dumoga, S.; Joshi, A.; Mohanty, S.; Singh, N. Polymeric micelles coated with hybrid nanovesicles enhance the therapeutic potential of the reversible topoisomerase inhibitor camptothecin in a mouse model. *Acta Biomater.*, **2021**, *121*, 579-591. http://dx.doi.org/10.1016/j.actbio.2020.11.049 PMID: 33285325
[67] Hedge, O.J.; Höök, F.; Joyce, P.; Bergström, C.A.S. Investigation of self-emulsifying drug-delivery system interaction with a biomimetic membrane under conditions relevant to the small intestine. *Langmuir*, **2021**, *37*(33), 10200-10213. http://dx.doi.org/10.1021/acs.langmuir.1c01689 PMID: 34379976
[68] Rezaei-kelishami, A.; Yavarmanesh, M.; Sadeghi, Z.; Palizban, A.; Parandin, R. Control [illegible] efficient and treatment of diabetes using modern drug delivery systems and special properties of nanoparticles. *J. Biol. Todays World*, **2014**, *3*(9). http://dx.doi.org/10.15412/J.JBTW.01030905
[69] Nahar, M.; Mishra, D.; Dubey, V.; Jain, N.K. Development, characterization, and toxicity evaluation of amphotericin B–loaded gelatin nanoparticles. *Nanomedicine*, **2008**, *4*(3), 252-261. http://dx.doi.org/10.1016/j.nano.2008.03.007 PMID: 18502187
[70] Bayat, A.; Dorkoosh, F.A.; Dehpour, A.R.; Moezi, L.; Larijani, B.; Junginger, H.E.; Rafiee-Tehrani, M. Nanoparticles of quaternized chitosan derivatives as a carrier for colon delivery of insulin: *Ex vivo* and *in vivo* studies. *Int. J. Pharm.*, **2008**, *356*(1-2), 259-266. http://dx.doi.org/10.1016/j.ijpharm.2007.12.037 PMID: 18289808
[71] Cetin, M.; Atila, A.; Sahin, S.; Vural, I. Preparation and characterization of metformin hydrochloride loaded-Eudragit® RSPO and Eudragit® RSPO/PLGA nanoparticles. *Pharm. Dev. Technol.*, **2013**, *18*(3), 570-576. http://dx.doi.org/10.3109/10837450.2011.604783 PMID: 21864098
[72] Sarmento, B.; Martins, S.; Ferreira, D.; Souto, E.B. Oral insulin delivery by means of solid lipid nanoparticles. *Int. J. Nanomedicine*, **2007**, *2*(4), 743-749. PMID: 18203440
[73] Ansari, M.J.; Anwer, M.K.; Jamil, S.; Al-Shdefat, R.; Ali, B.E.; Ahmad, M.M.; Ansari, M.N. Enhanced oral bioavailability of insulin-loaded solid lipid nanoparticles: Pharmacokinetic bioavailability of insulin-loaded solid lipid nanoparticles in diabetic rats. *Drug Deliv.*, **2016**, *23*(6), 1972-1979. http://dx.doi.org/10.3109/10717544.2015.1039666 PMID: 26017100
[74] Mishra, D.K.; Shandilya, R.; Mishra, P.K. Lipid based nanocarriers: A translational perspective. *Nanomedicine*, **2018**, *14*(7), 2023-2050. http://dx.doi.org/10.1016/j.nano.2018.05.021 PMID: 29944981
[75] Piazzini, V.; Micheli, L.; Luceri, C.; D'Ambrosio, M.; Cinci, L.; Ghelardini, C.; Bilia, A.R.; Di Cesare Mannelli, L.; Bergonzi, M.C. Nanostructured lipid carriers for oral delivery of silymarin: Improving its absorption and *in vivo* efficacy in type 2 diabetes and metabolic syndrome model. *Int. J. Pharm.*, **2019**, *572*, 118838. http://dx.doi.org/10.1016/j.ijpharm.2019.118838 PMID: 31715362
[76] Puglia, C.; Santonocito, D.; Ostacolo, C.; Maria Sommella, E.; Campiglia, P.; Carbone, C.; Drago, F.; Pignatello, R.; Bucolo, C. Ocular formulation based on palmitoylethanolamide-loaded nanostructured lipid carriers: Technological and pharmacological profile. *Nanomaterials*, **2020**, *10*(2), 287. http://dx.doi.org/10.3390/nano10020287 PMID: 32046269
[77] Akhtar, J.; Siddiqui, H.H.; Fareed, S.; Badruddeen, ; Khalid, M.; Aqil, M. Nanoemulsion For improved oral delivery of repaglinide. *Drug Deliv.*, **2016**, *23*(6), 2026-2034. http://dx.doi.org/10.3109/10717544.2015.1077290 PMID: 27187792
[78] Karolczak, K.; Rozalska, S.; Wieczorek, M.; Labieniec-Watala, M.; Watala, C. Poly(amido)amine dendrimers generation 4.0 (PAMAM G4) reduce blood hyperglycaemia and restore impaired blood–brain barrier permeability in streptozotocin diabetes in rats. *Int. J. Pharm.*, **2012**, *436*(1-2), 508-518. http://dx.doi.org/10.1016/j.ijpharm.2012.06.033 PMID: 22721855
[79] Labieniec, M.; Watala, C. PAMAM dendrimers - diverse biomedical applications. Facts and unresolved questions. *Open Life Sci.*, **2009**, *4*(4), 434-451. http://dx.doi.org/10.2478/s11535-009-0056-7
[80] Zhao, R.; Lu, Z.; Yang, J.; Zhang, L.; Li, Y.; Zhang, X. Drug delivery system in the treatment of diabetes mellitus. *Front. Bioeng. Biotechnol.*, **2020**, *8*, 880. http://dx.doi.org/10.3389/fbioe.2020.00880 PMID: 32850735
[81] Dash, T.K.; Konkimalla, V.B. Poly-ε-caprolactone based formulations for drug delivery and tissue engineering: A review. *J. Control. Release*, **2012**, *158*(1), 15-33. http://dx.doi.org/10.1016/j.jconrel.2011.09.064 PMID: 21963774

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Spinacia Oleracea- An Overview of Its Chemical, Nutritional, Phytochemical and Pharmacological Profile

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Abstract

Nowadays, natural medications and excipients are becoming increasingly popular. There are benefits to using natural elements versus synthetic onesbecause they are non-toxic, more cheap, and easily accessible. One of the most significant sources of medications comes from plants. The therapeutically significant essential oils and secondary metabolites found in medicinal plants are abundant. Since the dawn of human civilization, people have employed plant-based medicines to treat a variety of diseases. Spinach is a member of the Amaranthaceae family of eating angiosperms(*Spinacia oleracea*).Spinach is frequently recognised as a functional food because of its diverse nutritional composition, which includes vitamins and minerals as well as phytochemicals and bioactives that support health in ways other than nutrition. Water makes up the majority of spinach, with trace amounts of protein, carbohydrate, and fat. Due to the presence of biological tannins and phenolic functional phytochemicals such as terpenoids, glycosides, steroids, flavonoids and alkaloids, the plant demonstrates its medical value against a variety of human ailments. The anti-cancer, hypoglycemic, hypolipidemic, neuroprotective, and hepatoprotective qualities of spinach are result of their biological activity. With an emphasis on its phytochemical and pharmacological profile, this review analyses the chemical and nutritional composition of *Spinacia oleracea*.

Keywords- Phlobatannins, flavonoids, anti-cancer, thylakoid, steatosis

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I. INTRODUCTION

Nowadays, natural medications and excipients are becoming increasingly popular. Due to their less costly and toxic-free, and readily available, natural materials certainly have benefits over synthetic ones. Additionally, they can be altered to produce materials specifically for the medication delivery system that will allow them to compete with the synthetic agents that are now on the market (Edwin et al., 2007). One of the most significant sources of medications comes from plants. The therapeutically significant essential oils and secondary metabolites found in medicinal plants are abundant.In addition to being affordable, effective, and readily available, medicinal plants' major benefits for therapeutic usage in treating various illnesses includes safety (Prakash and Gupta, 2005). The utilization of plant-based medicines to treat various illnesses is as old as human civilisation and has been used in all historical cultures. By trial and error, the early man began to discern between beneficial and dangerous or deadly plants. Using knowledge of the indigenous flora, religion, and culture, tribal people created a well-defined herbal pharmacopoeia. The basis for traditional medicine around the world is the steady development and transmission of knowledge about medicinal plants (Khan et al., 2014).An edible flowering plant belonging to the Amaranthaceae family is spinach (*Spinacia oleracea*). Up to 30 cm is the maximum height of this plant. In temperate zones, spinach may endure the winter.The leaves are alternate, simple, and ovate to triangular - based, varying in size from about 2-30 cm long and 1-15 cm broad. The larger leaves are at the plant's base and small leaves arehigher on the flowering stem. The unassuming, yellow-green, 3–4 mm in diameter flowers develop into a small lumpy, dry, hard fruit cluster that is 5–10 mm across and contains a number of seeds (Olasupo et al., 2018).

Traditional Uses

The plant is delicious, cooling, carminative, laxative, alexipharmic, useful for blood and brain problems, asthma, leprosy, and biliousness, and it also promotes "kapha" (Ayurveda). It has been utilised to treat urinary calculi. It has been demonstrated in tests to have hypoglycemic effects. The leaves are calming, emollient, healthful and they have been employed in the treatment of febrile disorders.They are also beneficial for urinary concretion, lung and bowel inflammation, thirst, joint discomfort, lumbago, colds and sneezes, sore eyes, ring worm, scabies, and leucoderma.Seeds are soothing and laxative. They have been employed to treat jaundice, liver inflammation, and respiratory difficulties. The herb is prescribed for urolithiasis (Gaikwad et al., 2010).

Chemical and Nutritional composition of *Spinacia oleracea*

Spinach is a versatile plant that can be eaten raw or cooked. Spinach is primarily composed of water, with low quantities of protein, starch, and lipid. Mono- and poly-unsaturated fatty acids make up the majority of the lipid fraction, with trace amounts of saturated fatty acids. A 100 g portion of spinach has 2.2 g of fibre, which satisfies 8.8% of the RDA for a 2,000 kcal diet.

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When compared to other regularly eaten leafy green vegetables, the vitamin and mineral makeup is far more varied. For instance, 100 g of spinach has significant quantities of magnesium, potassium, and iron. That is significantly more than lettuce, cabbage, and broccoli. Spinach contains adequate vitamin K, vitamin A, folate, and vitamin C in 100 g (Roberts and Moreau, 2016).

Spinach's nutrient makeup, including the amounts of pro-vitamin A,β-carotene, vitamin C, vitamin E, folate, and phylloquinone (vitamin K1), might vary depending on the variety, growth method, leaf size, and length of storage. The average vitamin C concentration ranged from 24.57 to 48.49 87 mg/100 g FW, respectively, in 27 types of spinach. Similar to this, the cultivar Lazio had much greater levels of γ- and α-tocopherol than Samish. When compared to spinach grown in the subtropics during the winter solstice, spinach cultivated in the subarctic during the summer solstice showed much greater levels of folate and tocopherol (Koh et al., 2012; Lester et al., 2010).Spinach planted in the fall had leaves with higher levels of manganese, zinc, copper, magnesium, calcium and potassium compared to spinach grown in the winter (Citak and Sonmez, 2009). The manner of production has an additional impact on nutrient composition; for example, spinach grown organically had about 31% more vitamin C than spinach grown conventionally. The nutritional content of spinach is also influenced by conditions after harvest. For instance, the quantities of folate and vitamin C were reduced when fresh spinach was boiled or steamed (Delchier et al., 2012).

Phytochemical screening of *Spinacia oleracea*

To ascertain the phytochemicals in spinach, a study was done. The results of a phytochemical examination on spinach plant extracts revealed the existence of substances that are helpful in the medication sector, cleaning up groundwater, and getting rid of heavy metals from agricultural products (Olasupo et al., 2018). Plant leaf extract was observed and screened to reveal phytochemicals such as flavonoids, tannins, saponins, terpenoids, and phlobatannins. According to Saif et al. (2016) and Ebrahiminezhad et al. (2017), the variety and combination of solvents, like ethyl acetate, acetone, water, and alcohols, considerably influence the extraction of phenolics.In contrast to the ethyl acetate extract of the same vegetable, the aqueous extract had a high concentration of flavonoids, phlobatannins, and terpenoids. While the concentration of tannins and saponins was high in the ethyl acetate extract of spinach leaves, they were completely missing in the aqueous extract. The anti-oxidative property of the spinach leaf extract was due to the presence of phenolic chemicals.

The presence of phytochemicalswas discovered using phytochemical screening. In contrast to the leaves extract of ethyl acetate, the aqueous extract of the plant was rich in flavonoids, phlobatannins, and terpenoids. Similarly, the ethyl acetate extract of spinach leaves had a high amount of tannins and saponins but the aqueous extract did not (Fayyaz et al., 2022). In another study phytochemical screening of methanolic extract of *Spinacia oleracea* leaf displayed the presence of saponin, tannin and alkaloids (Shaheen et al., 2018).A study's

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exploratory phytochemical examination showed that several active components, including sugars, proteins, amino acids, lipids, oils, steroids, terpenoids, glycosides, alkaloids, tannins, and other phenolic compounds, were present in distinct extracts (Singh et al., 2017). Flavonoids have the ability to fight cancer, allergies, and free radicals. To extract flavonoids from various types of herbal plants, 50% acidified methanol by 1.2 M HCl was utilized. Although the mechanism of action of flavonoids is not fully understood, phenolics of plant origin have long been recognized for their wide variety of biological functions.Tannins help keep an animal's body healthy and functioning properly. Phlobatannins have pharmacological and biological effects and have anti-tumor properties. Terpenoids, on the other hand, can be used to treat asthma, coughing, and fever. In a different investigation, the extraction of phenolic compounds using an organic solvent contained phytochemicals (Rana et al., 2020).

To determine the existence of phytochemical ingredients, a study is undertaken. Both fresh and dried leaves of Spinacia oleracea underwent cold maceration. ethanol and water, two polar solvents, are used in this method. The impact of drying and solvent on extractability is examined in this study. Fresh leaves are more extractable than dried leaves are. Additionally, ethanol is less extractable in the solvents than water. Drying has little effect on the phytochemical components of fresh and dried Spinacia oleracea leaves (Rao et al., 2015).The results of phytochemical screening on various spinach leaf extracts were reported in a different study. In contrast to other extracts such as water, ethanol, and chloroform, ethyl acetate extract did not include any biomolecules such as carbohydrates, proteins, or glycosides. Acetone was the only exception (1:1). The steroid only manifests in ethanol extract. In addition to ethanol, ethyl acetate, and chloroform, tannins and other phenolic compounds were also found in acetone (1:1). Acetone(1:1) extracts are an acidic substance that is found in water and ethyl acetate but not in ethanol or chloroform. Water, ethanol, and chloroform:acetone (1:1) extracts all contained vitamin A, but ethyl acetate did not. Water, ethyl acetate, and chloroform all contain vitamin C, however ethanol does not contain any acetone (1:1) extracts.Inorganic substances found in spinach leaves include calcium, magnesium, salt, potassium, and iron (Apeksha et al., 2019).

The presence of phytochemicals was examined in Spinacia oleracea. Phitobatamin is present in the crude extract of Spinacia oleracea in aqueous extract but not in ethanol or ethyl acetate. Saponin is present in the crude extracts of aqueous and ethyl acetate but not in that of ethanol. While ethanol and ethyl acetate crude extracts don't contain any terpenes, aqueous extract does.In contrast to aqueous ethyl acetate crude extract, ethanol crude extract contains steroids (Olasupo et al., 2018). In the phytochemical analysis of S. oleracea leaves, alkaloids, tannins, glycosides, terpenoids, and flavonoids were among the notable phytochemicals that were discovered. In most assays, S. oleracea aqueous extract was negative for cardiac glycosides, glycosides and carbohydrates but positive for amino acids, protein, terpenoids, reducing sugar, flavonoids, saponins and reducing sugar. The plant leaves' methanolic extract showed that (in the majority of the tests) steroids, saponins, terpenoids, flavonoids, phenolic compounds and

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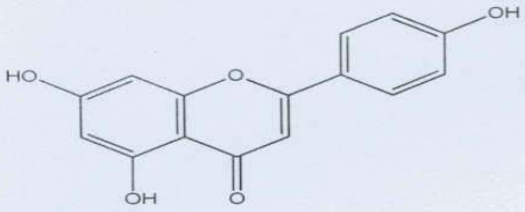
tanninswere present, while the remainder of the tests produced negative results (Kumar and Patwa, 2017).



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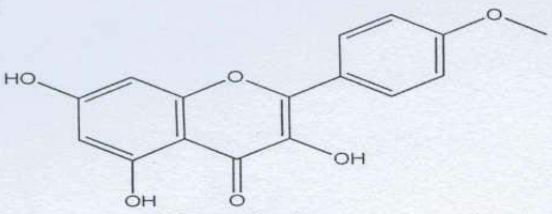

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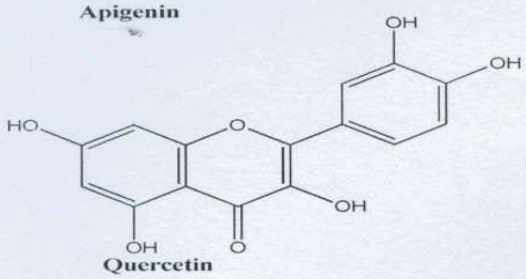

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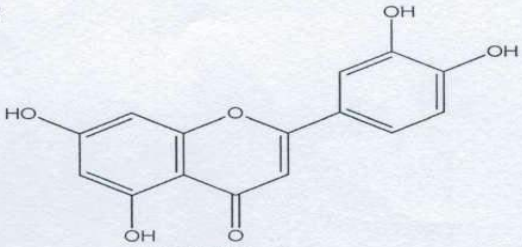

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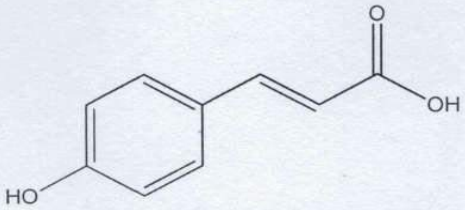
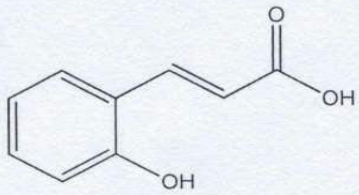
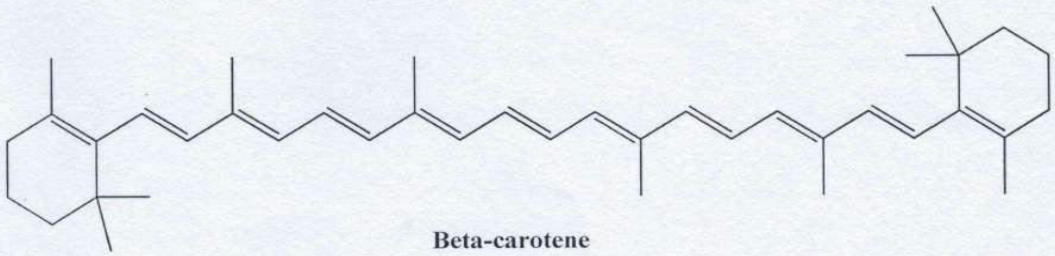


p-Coumaric acid



o-Coumaric acid



Beta-carotene

Fig 1 showing various chemical compounds found in leaves of *Spinacia oleracea*

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II. PHARMACOLOGICAL PROFILE OF *SPINACIA OLERACEA*

Antioxidant properties

In addition to being vital for energy supply, detoxification, chemical signalling, and immune function, free radicals are continuously produced in the human body and are linked to a number of illnesses, including diabetes, rheumatoid arthritis, high blood pressure, urinary tract problems, bronchial asthma, and non-healing wounds. Free radicals can start the oxidation of biomolecules like DNA, proteins, lipids, and amino acids, which can harm cells and cause a variety of disorders (Rao et al., 2015). Numerous ailments, including cancer,age-related disorders, cataracts, and Parkinson's disease, are caused by oxidative stress, which results from an imbalance between antioxidants and reactive oxygen species and damages cells. Antioxidants help treat a variety disease, carcinoma, and inflammatory diseases, by reducing oxidative stress in cells. Protective properties against oxidative stress can be found in phenolic compounds, particularly flavonoids. According to this study, the extract's ability to act as an antioxidant.

A DPPH assay revealed antioxidant activity in the ethanolic extract of SO. It was discovered that the proportion of inhibition was the same at all concentrations of plant extract. The plant extract scavenged and captured the free radicals in a concentration-dependent manner. The ethanolic extract of SO's inhibitory concentration (IC50) value for DPPH is displayed at a concentration of 50 g/ml. This value represents the percentage inhibition of scavenging activities. Nitric oxide was moderately dose-dependently inhibited by SO's ethanolic extract, with an IC50 of 60 g/ml giving a 56.66% inhibition. The ethanolic extract of SO's ability to trap LPO radicals with an IC50, or an IC50 of 1500 g/ml, as a result of its anti-lipoperoxidation free radicals to prevent peroxidation, was demonstrated (Damle and Jadhav, 2018).

The chemical makeup of some of these antioxidant compound is given in the current work. Water was used to extract the spinach leaves, and an acetone:water (1:9) solution was used to extract the 20,000 g supernatant, which contained the antioxidant activity. Three distinct tests were used to test the produced fractions, and each one demonstrated antioxidant activity. The current work provides definitive evidence for the first time that the aqueous spinach leaf extract contains both flavonoids and derivatives of p-coumaric acid as antioxidants. The components that were separated in the current work come from two groups of phenolics.

Anti-inflammatory properties

An unchecked immune reaction to injury or infection known as inflammation plays a role in the emergence of major chronic illnesses like cancer. Despite their effectiveness, pharmaceutical interventions for inflammation are linked to unfavourable side effects, and inflammation-mediated illnesses (Gregor et al., 2011). The immunoreactivity of inducible nitric oxide synthase

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and cyclooxygenase-2 was evaluated in groups of male Wistar rats that included those who were injected with lipopolysaccharide following pretreatment with either natural antioxidant from spinach. The inducible nitric oxide synthase and cyclooxygenase-2 scores were positively correlated in all treatment groups, indicating a significant link between these two parameters. The findings suggest that NAO and apocynin may have therapeutic value in preventing liver damage brought on by clinical endotoxemia, which is known to be accompanied by oxidative stress (Lomnitski et al., 2000). Similar to this, LPS-related lesions in the liver, thymus, spleen, eyes, and adrenal gland were less severe in New Zealand rabbits given NAO (10 mg/kg, i.p.) for 8 consecutive days before being challenged with LPS (10 mg/kg, i.p). (Lomnitski et al., 2000). P-coumaric acid (100 mg/kg/day; IP injection) significantly decreased TNF- expression in synovial tissue in arthritic Wistar rats (Pragasam et al., 2013).

Anti-cancer properties

Cancers are any of the many diseases that are characterized by the expansion of abnormal cells that divide uncontrollably, have the ability to penetrate, and cause harm to good physiological tissue. A lower risk of various malignancies is linked to diets high in vegetables, especially dark green vegetables. In a case-control study with 6,888 cases and 9,428 controls, women who consumed more raw spinach (more than 52 servings annually) had a 45% lower risk of breast cancer. Lutein, a carotenoid, linked to a lower risk of breast cancer.In a pooled analysis of 14 prospective cohort studies from North America and Europe, spinach consumption was linked to an 11% decreased risk of colon cancer at one serving (73 g) to one-half serving per day. Out of the 12 vegetables that were evaluated in this study, only spinach showed a discernible reduction in the risk of colon cancer.

The chemoprotective properties of spinach have been investigated in both cancer cell and animal models. Wistar rats on diets enriched with heme, a hyperproliferative chemical included in red meat, were used in further research into the antiproliferative characteristics of whole spinach. Consuming spinach dramatically reduced the growth of colon epithelial cells brought on by heme, probably by interfering with heme metabolism.

Hypoglycemic profile

In numerous animal experiments, the hypoglycemic effect of chemicals produced from spinach has been investigated. After giving Wistar rats an alloxan monohydrate dose to permanently destroy pancreatic beta-cells and impair insulin production, researchers then gave the animals an oral dose of 70% spinach ethanolic extract every day for 12 days. They found that the treated animals' plasma glucose levels were significantly lower than those of the untreated animals (Kumar and Loganathan, 2010).

Studies in cell culture show that spinach extracts have insulin-like and insulin-sensitizing properties. Park et al. examined the effects of either juicing or extracting fresh spinach with ethanol on the growth of 3T3-L1 pre-adipocytes. The growth of adipocytes was chosen to

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emphasise the critical role that GLUT4 plays in insulin-mediated glucose absorption. They found that ethanol extract and spinach juice both induced 3T3-L1 cell differentiation, both in the absence of insulin and even more so in the presence of insulin (Park et al., 2012).

Thylakoids were discovered to bind to intestinal mucosa and decrease intestinal permeability, which in turn reduced the transfer of methyl-glucose through the brush border of rat intestinal portions in vitro. This implies that reduced glucose absorption is most likely what causes the insulin response to be attenuated (Montelius et al., 2011). In comparison to a meal containing a placebo, a meal consisting 5 g of thylakoids drastically increased 2-hour postprandial glucose level in overweight and obese men and women (Rebello et al., 2015).

Hypolipidemic profile

In a study on rodents, the lipid-lowering potential of spinach phytochemicals was examined in type 1 diabetic Wistar rats administered a daily intake of 70% ethanolic spinach extract. When compared to control diabetic rats in this study, the rats given spinach extract showed a 62.3% decrease in serum triglycerides. Spinach extracts restored diabetic rats' plasma triglycerides to those seen in non-diabetic rats (Kumar and Loganathan, 2010).

It has been demonstrated that thylakoid membranes derived from spinach leaves can lower blood lipids. Similar findings showed that thylakoid-enriched high-fat meals dramatically reduced blood triglycerides and free fatty acids in two animal models, Sprague-Dawley rats and apoE-deficient mice (Albertsson et al., 2007; Kohnke et al., 2009).

Neurological profile

In a study, researchers investigated how the spinach extract guarded from I/R injury to the spinal cord. Evaluations of histological, biochemical, and neurological function were done 72 hours following the ischemia. At 72 hours following spinal cord ischemia, the mean motor deficit index scores of the spinach extract groups were considerably lower than in the NS group. Additionally, the spinach extract groups exhibited considerably lower plasma levels of malondialdehyde and greater plasma levels of overall antioxidative capability when compared to the NS group. The groups treated with spinach extract displayed significantly more normal motor neurons than the NS group (Farjah et al., 2018).

24 adult male Holtzman strain albino rats were utilised in a distinct experiment. Rats from the control, SO-treated Control, PTZ-induced experimental epileptic group, and SO-pretreated PTZ-induced experimental epileptic group were used in this study. The epileptic model was set up by giving PTZ intraperitoneally at a dose of 40 mg/kg body weight. Before pretreatment with SO leaf extract, there were appreciable decreases in the seizure score, ictal phase, serum NO level, LPO levels, and DNA fragmentation rate. SOD, CAT, and GSH activity in various brain areas, as well as interictal phase activity, were all noticeably higher in the SO pretreated PTZ triggered group (Mukherjee et al., 2015).

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Hepatoprotective profile

Researchers investigated the effects of dietary carotenoids from spinach on the inflammatory and oxidative stress indicators, liver lipid profile, and hepatic transcriptome and metabolomics profiles in Sprague-Dawley rats with steatosis brought on by a high-fat diet. The overexpression of peroxisome proliferator activated receptors was primarily responsible for the changed and increased expression of genes involved in the metabolism of fatty acids, cholesterol, and the fatty liver state. Animals given diet H had hypoaminoacidemia in terms of liver metabolites, particularly for the glucogenic amino acids. Despite no changes in the symptoms of oxidative stress and inflammation, consuming spinach altered the liver's lipid metabolism, which must be taken into consideration when treating steatosis with diet(Torales et al., 2019a).

The accumulation of fat in liver cells, which has a negative impact on health, is a defining feature of non-alcoholic fatty liver disease. Studies on both humans and animals point to a role for the gut microbiota in the pathophysiology of NAFLD. The effects of high-fat meals on certain intestinal bacterial populations and the byproducts of their metabolism, such as short-chain fatty acids and microbial phenolic catabolites, were examined by researchers to see if spinach supplementation may mitigate these effects. A high-fat diet had an effect on the gut microbiota, the pattern of SCFAs, and the phenolic gut microbial catabolites in addition to increasing the risk of NAFLD and dislipaemia.By improving the readings of the steatosis biomarker, boosting Lactobacillus levels, lowering fasting glucose, total and LDL cholesterol, and reducing the development of excess liver cholesterol, spinach supplementation may be able to counteract some of the effects of a high-fat diet (Torales et al., 2019b).

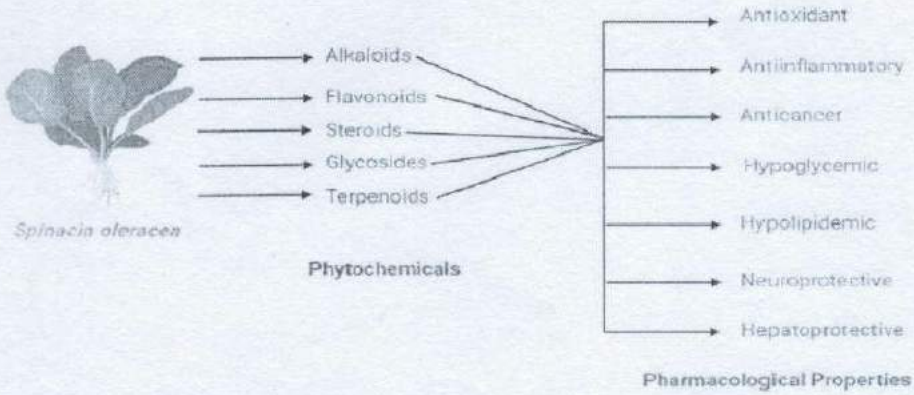

Fig 2 showing overview of phytochemical and pharmacological properties of *Spinacia orelacea*

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III. CONCLUSION

Antioxidants and vitamin A, one of the fat-soluble vitamins, are both abundant in spinach. The extra amount, which is retained in the liver and fatty tissues, is crucial for warding off disease and infection. Vitamin A is required by the body for growth and repair; it also functions as an antioxidant to guard cells against oxidative stress. For healthy skin, mucous membranes, and the tissue that lines the intestines, airways, and other organs, as well as for regular cell growth and division, the development of bone and teeth, and normal vision, vitamin A is crucial. It can help in the prevention of some dangerous diseases and is extremely readily available.The phytochemical examination of S. oleraceae found that the various extracts included a variety of phytochemicals, including tannins, flavonoids, alkaloids, saponins, and terpenoids, among others. We came to the conclusion that Spinacia oleracea is an excellent, nutrient-rich leafy vegetable that can be employed as a therapeutic and curative medicine for many oxidative stress-induced disorders as a result of the presence of these phytochemicals. Studies on both people and animals suggest that substances produced from spinach may help people with their obesity, hyperglycemia, and hypertriglyceridemia, three conditions that are part of the metabolic syndrome.From a molecular perspective, spinach's active ingredients would exert their effects via upregulating satiety hormones, increasing insulin sensitivity, and interacting with the digestive enzymes that break down lipids and carbohydrates. Animal and cell models were used in the vast majority of the studies in this analysis. Even though these studies are quite insightful, there are still few that have examined the relationship between spinach diet and human illness development. To learn more about these actions, more investigation should be done. The objective of this review is to support ongoing study on this plant.

References

1. Abinaya S, Kavitha HP, Prakash M, Muthukrishnaraj A (2021) Green synthesis of magnesium oxide nanoparticles and its applications: A review. Sustain Chem Pharm. https://doi.org/10.1016/j.scp.2020.100368
2. Albertsson PA, Köhnke R, Emek SC, et al. Chloroplast membranes retard fat digestion and induce satiety: effect of biological membranes on pancreatic lipase/co-lipase. The Biochemical Journal. 2007 Feb;401(3):727-733.
3. Apeksha M Dalvi, Sayali P Nilakh, Akshada S Borhade, Vidya N Alhat (2019). Phytochemical studies on various extracts and moisturizing use of spinach leaf. *International Research Journal of Engineering and Technology*, *06*(05), 1379–1384.
4. Bergman, Margalit, et al. "The antioxidant activity of aqueous spinach extract: chemical identification of active fractions." *Phytochemistry* 58.1 (2001): 143-152.
5. Citak, S., &Sonmez, S. (2009). Mineral Contents of Organically and Conventionally Grown Spinach (Spinacea oleracea L.) during Two Successive Seasons. *Journal of Agricultural and Food Chemistry*, *57*(17), 7892–7898. https://doi.org/10.1021/jf900660k

INDORE INSTITUTE OF PHARMACY IQAC INDORE

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6. De Vogel, Johan, et al. "Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon." *Carcinogenesis* 26.2 (2005): 387-393.
7. Delchier, N., Reich, M., & Renard, C. M. G. C. (2012). Impact of cooking methods on folates, ascorbic acid and lutein in green beans (Phaseolus vulgaris) and spinach (Spinacea oleracea). *LWT - Food Science and Technology*, *49*(2), 197–201. https://doi.org/10.1016/j.lwt.2012.06.017
8. Ebrahiminezhad A, Zare-Hoseinabadi A, Berenjian A, Ghasemi Y (2017) Green synthesis and characterization of zero-valent iron nanoparticles using stinging nettle (Urtica dioica) leaf extract. Green Process Synth 6(5):469–475. https://doi.org/10.1515/gps-2016-0133
9. Edwin J, Edwin S, Dosi S, Raj A, G. S. (2007). Application of hibiscus leaves mucilage as suspending agent. *Indian Journal of Pharmaceutical Education and Research*, *41*(4), 373–375.
10. Elvira-Torales, L. I., et al. "Spinach consumption ameliorates the gut microbiota and dislipaemia in rats with diet-induced non-alcoholic fatty liver disease (NAFLD)." *Food & function* 10.4 (2019): 2148-2160b.
11. Elvira-Torales, Laura Inés, et al. "Ameliorative effect of spinach on non-alcoholic fatty liver disease induced in rats by a high-fat diet." *International journal of molecular sciences* 20.7 (2019): 1662a.
12. Farjah GH, Mohammad Pour M, Khadem-Ansari MH, Karimipour M, Pourheidar B. Protective effect of aqueous spinach (*Spinacia oleracea L.*) extract on spinal cord ischemia-reperfusion injury in rats. Vet Res Forum. 2018 Spring;9(2):187-191.
13. Fayyaz, Bakhtawar, Misbah Batool Zahra, and Muhammad Saleem Haider. "Screening of Phenolic Compounds from Spinach (*Spinacia oleracea*), Green Synthesis of Iron-Nanoparticles and Determination of its Anti-Microbial Effect on Escherichia coli." (2022).
14. Freudenheim, Jo L., et al. "Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients." *JNCI: Journal of the National Cancer Institute* 88.6 (1996): 340-348.
15. Gaikwad Priyanka Subhash, Shete Rajkumar Virbhadrappa, K. O. (2010). *Spinacia oleracea* Linn: A pharmacognostic and pharmacological overview. *International Journal of Research in Ayurveda and Pharmacy*, *1*(1), 78–84.
16. Gregor, Margaret F., and Gökhan S. Hotamisligil. "Inflammatory mechanisms in obesity." *Annual review of immunology* 29.1 (2011): 415-445.
17. Khan, Farhat Ali, Muhammad Zahoor, Naseem Ullah, Shazeb Khan, Muhammad Khurram, S. K. and J. A. (2014). A general introduction to medicinal plants and silybum marianum. *Life Science Journal*, *11*(9), 471–481.
18. Khoddami A, Wilkes MA, Roberts TH (2013) Techniques for analysis of plant phenolic compounds. Molecules 18(2):2328–2375. https://doi.org/10.3390/molecules18022328

INDORE INSTITUTE OF PHARMACY IQAC INDORE

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INDORE (M.P.)

19. Koh, E., Charoenprasert, S., & Mitchell, A. E. (2012). Effect of Organic and Conventional Cropping Systems on Ascorbic Acid, Vitamin C, Flavonoids, Nitrate, and Oxalate in 27 Varieties of Spinach (*Spinacia oleracea* L.). *Journal of Agricultural and Food Chemistry*, *60*(12), 3144–3150. https://doi.org/10.1021/jf300051f
20. Kohnke R, Lindqvist A, Goransson N, Emek SC, Albertsson PA, Rehfeld JF, Hultgardh-Nilsson A, Erlanson-Albertsson C. Thylakoids suppress appetite by increasing cholecystokinin resulting in lower food intake and body weight in high-fat fed mice. *Phytother Res.* 2009;23(12):1778–1783.
21. Koushik, Anita, et al. "Fruits, vegetables, and colon cancer risk in a pooled analysis of 14 cohort studies." *Journal of the National Cancer Institute* 99.19 (2007): 1471-1483.
22. Kumar, Ramesh, and Roshni Patwa. "Study of phytochemical constituents and antioxidant activity of *Spinacia oleracea* L. of Bundelkhand Region." *Int. J. Life. Sci. Scienti. Res* 4.1 (2018): 1599-1604.
23. Lester, G. E., Makus, D. J., & Hodges, D. M. (2010). Relationship between Fresh-Packaged Spinach Leaves Exposed to Continuous Light or Dark and Bioactive Contents: Effects of Cultivar, Leaf Size, and Storage Duration. *Journal of Agricultural and Food Chemistry*, *58*(5), 2980–2987. https://doi.org/10.1021/jf903596v
24. Lomnitski, Liat, et al. "Effects of apocynin and natural antioxidant from spinach on inducible nitric oxide synthase and cyclooxygenase-2 induction in lipopolysaccharide-induced hepatic injury in rat." *Pharmacology & toxicology* 87.1 (2000): 18-25.
25. Lomnitski, Liat, et al. "The prophylactic effects of natural water-soluble antioxidant from spinach and apocynin in a rabbit model of lipopolysaccharide-induced endotoxemia." *Toxicologic pathology* 28.4 (2000): 588-600.
26. Longnecker, Matthew P., et al. "Intake of carrots, spinach, and supplements containing vitamin A in relation to risk of breast cancer." *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 6.11 (1997): 887-892.
27. Mondal Mukherjee, M., Sinha, S., Maity, S., Sathian, B., & Guha, D. (2015). Effect of Spinach (Spinacea oleracea) on DNA fragmentation in pentylenetetrazole induced experimental epileptic rat model. *Medical Science*, *3*(3), 265-273.
28. Montelius, C., Gustafsson, K., Weström, B., Albertsson, P., Emek, S., Rayner, M., &Erlanson-Albertsson, C. (2011). Chloroplast thylakoids reduce glucose uptake and decrease intestinal macromolecular permeability. *British Journal of Nutrition, 106*(6), 836-844.
29. Mrinalini C Damle, S. K. J. (2018). AN IN VITRO STUDY OF ANTIOXIDANT CAPACITY AND RADICAL SCAVENGING EFFECT OF *SPINACIA OLERACEA* LEAF EXTRACT. *Asian J Pharm Clin Res*, *11*(11), 324–328.

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30. N. J. Kumar, P. Loganathan, "Hypoglycemic effect of Spinacia oleracia in alloxan induced diabetic rats," *Global Journal of Biotechnology & Biochemistry* , vol. 5, no. 2, pp. 87-91, 2010.
31. Olasupo AD, A. A. and O. O. (2018). Phytochemical Analysis and Antibacterial Activities of Spinach Leaf. *American Journal of Phytomedicine and Clinical Therapeutics*, *6*(2:8).
32. Park, JH., Kim, RY. & Park, E. Antidiabetic activity of fruits and vegetables commonly consumed in Korea: Inhibitory potential against α-glucosidase and insulin-like action *in vitro* . *Food Sci Biotechnol* **21**, 1187–1193 (2012).
33. Pragasam, Samuel Joshua, Vijayalakshmi Venkatesan, and MahaboobKhan Rasool. "Immunomodulatory and anti-inflammatory effect of p-coumaric acid, a common dietary polyphenol on experimental inflammation in rats." *Inflammation* 36.1 (2013): 169-176.
34. Prakash, P., & Gupta, N. (2005). Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian Journal of Physiology and Pharmacology*, *49*(2), 125–131. http://www.ncbi.nlm.nih.gov/pubmed/16170979
35. Rana A, Yadav K, Jagadevan S (2020) A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity. J Clean Prod 122880. https://doi.org/10.1016/j.jclepro.2020.122880
36. Rao, K. N. V., et al. "Preliminary phytochemical screening of *Spinacia oleracea* L." *World Journal of Pharmacy and Pharmaceutical Sciences* 4.6 (2015): 532-551.
37. Rebello, Candida J., et al. "Acute effects of a spinach extract rich in thylakoids on satiety: a randomized controlled crossover trial." *Journal of the American College of Nutrition* 34.6 (2015): 470-477.
38. Roberts, J. L., & Moreau, R. (2016). Functional properties of spinach (*Spinacia oleracea* L.) phytochemicals and bioactives. *Food & Function*, *7*(8), 3337–3353. https://doi.org/10.1039/C6FO00051G
39. Saif S, Tahir A, Chen Y (2016) Green synthesis of iron nanoparticles and their environmental applications and implications. Nanomaterials 6(11):209. https://doi.org/10.3390/nano6110209
40. Sharif Mohammad Shaheen et al. (2018), Phytochemical profiling and evaluation of antioxidant and antidiabetic activity of methanol extract of Spinach (*Spinacia oleracea* L.) leaves. Int J Pharm Sci & Scient Res. 4:1, 24-27
41. Singh N, Tailang M and Mehta SC: Phytochemical screening and immunomodulatory activity of different extract of *Spinacia oleracia*leaves. Int J Pharm Sci Res 2017; 8(2): 878-82.doi: 10.13040/IJPSR.0975-8232.8(2).878-82.
42. De Vogel, Johan, et al. "Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon." *Carcinogenesis* 26.2 (2005): 387-393.

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Research Article

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW QUINAZOLINONE DERIVATIVES AS ANTI DIABETIC AGENTS

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ABSTRACT: In this study, a library of new 4-[6-chloro-2-arylaminoethyl-4-oxoquinazolin-3(4H)-yl] benzoic acid (Q_1-Q_{16}) were synthesized and evaluated for their *in vivo* anti-diabetic activity. All the compounds were prepared in a multistep process involving the initial preparation of 5-chloro-N-acetyl anthranilic acid which was converted to 4-[6-chloro-2-methyl-4-oxoquinazolin-3(4H)-yl] benzoic acid. This resulted intermediate undergoes mannich base reaction in the presence of formaldehyde and different aromatic amine to produce different quinazolinone derivatives in good yield. The structures of the synthesized compounds were establishedon the basis of elemental analysis and spectroscopic studies (FTIR, ^{1}HNMR and Mass) and the purity of the compounds was determined by TLC. All the synthesized compounds were subjected to Oral Glucose Tolerance Test (OGTT) to gain preliminary information regarding the anti-diabetic activity. They were assessed for anti-diabetic actionusing glibenclamide as the standard. Among the test compounds Q_2, Q_6 and Q_{10} were significant in their anti-diabetic activity in comparison with standard while the remaining tested compounds had shown good to moderate activity.

Keywords: Alloxan monohydrate, Anthranilic acid, Anti-diabetic activity, Glibenclamide, OGTT, Quinazolinone

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INTRODUCTION:

Diabetes Mellitus (DM)is severe and very common disease affecting the populations all around the world. It has been observed that around 25% of the total world population facing this problem now days. Due to defect in metabolism of carbohydrate, diabetes may arisewhich ultimately leads to decrease the level of insulin in blood or decline the sensitivity

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Evaluation of In vitro Antioxidant, Antimicrobial and Cytotoxicity Activities of Orthosiphon Pallidus Royle

DOI: https://doi.org/10.37285/ijpsn.2023.16.3.3

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ABSTRACT

Objective: The objective of present research work was to evaluate the *in vitro* antioxidant, antimicrobial and cytotoxicity activity of *Orthosiphon pallidus* Royle.

Methods: Total phenolic and flavonoid content of hydroalcoholic extract (50:50) of *Orthosiphon pallidus* Royle hydroalcoholic extract (OPRHE) was estimated and the *in vitro* antioxidant activity was determined using ABTS radical scavenging assay and metal chelating assay. Antimicrobial activity was investigated

How to Cite

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