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Pharmacology Annual Volume 2025

*Edited by Cristina Manuela Drăgoi
and Alina Crenguta Nicolae*



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IntechOpen Book Series

Pharmaceutical Science

Volume 16

Aims and Scope of the Series

Pharmaceutical science focuses on the design, synthesis, formulation, targeting, distribution, safety, and efficacy of active compounds as potential therapeutics. It is a large interdisciplinary discipline that aims to integrate the basic principles of physical and organic chemistry, biochemistry, biology, and engineering to discover, develop, and characterize active compounds and to optimize the formulation and delivery of drugs in the body for offering new and improved safe and efficacious therapies against human diseases. The research areas covered by the pharmaceutical sciences range from medicinal chemistry and pharmaceutical technology to pharmacology and toxicology, which represent the preliminary phases of drug development. Medicinal chemistry involves the design and synthesis of pharmaceuticals as well as the isolation of active agents from natural sources. Computer-aided strategies are increasingly involved in this drug discovery process. Pharmaceutics is a multidisciplinary science that examines the relationships between drug formulation, delivery, distribution, and clinical outcomes. Modern clinical approaches are increasingly relying on controlled release strategies and drug delivery and targeting systems, including nanotechnological platforms (nanomedicine). Pharmacology is the science of drug action in biological systems. Pharmacologists also make drugs as tools to explore aspects of cell and tissue functions. Toxicology is the study of the adverse effects of active agents on living organisms and the ecosystem, including the prevention and amelioration of such adverse effects. This book series includes volumes on Drug Discovery, Delivery, and Pharmacology. Their overall aim is to present the latest research in the whole path of drug discovery and development from different points of view of this multidisciplinary and dynamic field.

Meet the Series Editor



Prof. Rosario Pignatello is a Full Professor of Pharmaceutical Technology and Legislation at the University of Catania, Italy. He is the Director of the Department of Drug and Health Sciences. He has nearly 30 years of experience in the research and development of innovative formulations for the controlled release and targeting of bioactive molecules, through chemical approaches as well as nanotechnological carriers, aimed at treating different disorders.

Prof. Pignatello has coauthored about 180 papers and edited a series of textbooks on biomaterials and their application in medicine. The main areas of his research are polymeric and lipid-based micro- and nanoparticles as modified drug delivery systems; vesicular nanocarriers (liposomes, micelles); lipophilic prodrugs and conjugates; synthesis and evaluation of new polymeric biomaterials for drug delivery and tissue regeneration. In particular, Prof. Pignatello works actively in the field of ocular drug delivery, leading the Research Centre for Ocular Nanotechnology, within the NANOMED Centre (Centre for Nanomedicine and Pharmaceutical Nanotechnology) at the University of Catania.

Meet the Topic Editor



Cristina Manuela Drăgoi, Ph.D., is a pharmacist and associate professor at the Department of Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania. She has professional experience in oxidative stress mechanisms, in assessing drug effects on biochemical markers, and in identifying predictive biomarkers for metabolic diseases. Her scientific research focuses on revealing circadian entrainment’s actions on major physiological and pathological processes, and on determining its modulatory effects on different aspects of hormone synthesis, healthy ageing mechanisms, and nutrition and medicine administration. This captivating subject of applied biochemistry in drug research is the core of her scientific projects, as evidenced by her authorship of several books, book chapters, and research articles.

Meet the Associate Topic Editor



Alina Crenguța Nicolae, Ph.D., is an associate professor at the Department of Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy “Carol Davila”, Bucharest, Romania. She completed her Ph.D. thesis on the biochemical mechanisms underlying multidrug resistance, a subject that remains a major pillar of her work. She is passionate about neuropharmacology. Her research projects conducted so far focus on the individual neuro-biochemical profile, correlated with age, gender, race, metabolic and genetic profiles, as well as on the establishment of predictive biomarkers that trigger the response to pharmacotherapy and on the modulation of multidrug resistance mechanisms at the level of the blood-brain barrier. Mrs. Nicolae is a pharmacist specializing in clinical pharmacology, a field encompassing drug properties, functions, sources, synthesis, and drug design; molecular and cellular mechanisms; signal transduction and cellular communication; molecular diagnostics; interactions; chemical biology; therapy; medical applications; and antipathogenic capabilities.

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Preface

The field of pharmacology continues to evolve at the intersection of molecular science, clinical application, and biotechnological innovation. This volume brings together a collection of contemporary studies and reviews that reflect the multifaceted nature of drug research and development, encompassing both traditional therapeutic approaches and cutting-edge pharmacological strategies.

The selected chapters illustrate the dynamic interplay between natural products, synthetic agents, and physiological targets. From exploring the antifungal potential of medicinal plant-derived compounds to assessing the role of silver nanoparticles in metabolic disorders, the volume offers insight into emerging interventions grounded in rigorous experimental frameworks. The discussion of corticosteroid pharmacotherapy revisits a cornerstone of clinical medicine, while innovative formulation techniques to enhance the bioavailability of aromatase inhibitors address the challenges of drug delivery and precision therapy. Finally, the intriguing role of oxytocin as a modulator of opioid receptors expands our understanding of neuropharmacological interactions and opens new avenues for translational research.

Taken together, these contributions reflect the depth and extent of modern pharmacological inquiry. We hope that this volume will serve as a valuable reference for researchers, clinicians, and students engaged in the ongoing pursuit of therapeutic excellence.

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Section 1

Natural Compounds and Innovative Therapies

Chapter 1

Medicinal Plant Derived Natural Compounds against Human Pathogenic Fungi: A Study, Challenges, and Future Scenarios

Banu Naziya and Hayat Sakander

Abstract

Plants are highly rich in essential bioactive medicinal components. Synthetic medications can have side effects compared to antifungal drugs derived from medicinal plant sources. This study was aimed to seek plant-based drugs that are nontoxic in nature and have a competent role in reducing pathogenic fungal infections in humans. In the current study, a literature search was carried out using different databases and searching techniques along with inclusion and exclusion of research articles, theses, and book chapters. The study revealed the use of phytochemicals and minimum doses required to antagonize pathogenic fungal growth. The antioxidant potency of medicinal plants is directly linked with their antifungal activity. The study exploited that the bioactive compounds found in medicinal herbs have various therapeutic properties such as antioxidant, anti-inflammatory, anti-obesity, antinausea, anti-emetic, antidiabetic, and anticancer activities. With regard to antifungal mechanism delivered by these metabolites, they showed reduction of pathogenic fungal growth and can disrupt plasma membrane permeability, prevent toxin/hyphal accumulation, and cause cell wall lysis. Thus, the report provides comprehensive information on medicinal plant derived metabolites and their use in antifungal drug preparation that has much consequence for researchers, herbal medicators, pharmacists, and clinicians.

Keywords: medicinal plants, human pathogenic fungi, phytochemicals/bioactive metabolites, therapeutics, mechanism

1. Introduction

Medicinal herbs are the first source from which bioactive metabolites are derived, possessing a wide variety of therapeutic properties and used from very ancient times. Most of the world's population, especially in India, is interested more toward the use of traditional plant-based medicines mainly to overcome primary health care issues [1]. If the medicinal properties of these traditional herbs could be scientifically validated, folklore rights would be justified. Modern pharmaceuticals and nutraceuticals are largely dependent on medicinal plants. Ayurvedic medical systems such as unani

and siddha serve as a storehouse of potent drugs in which over 2600 plant species were reported to be highly useful for medication purposes. Approximately 25% of the recommended medicines are derived directly from the plant source. Over the last few decades, curiosity has been raised in medicinal plant-based research to treat a wide variety of infections caused by pathogenic microbes [2].

It was observed that, medicinal herbs associated with beneficial microbes deliver antimicrobial activity and provide protection from clinical infections [3, 4]. The key principle that lies in inducing the defense systems is the presence of active secondary metabolites like peptides, essential oils, tannins, flavonoids, glycosides, saponins, phenols, and aldehydes [5]. These secondary metabolites have pharmaceutical properties that can be used to treat various noxious infections caused by viruses, bacteria, and fungi (**Figure 1**) [6].

Fungal infections are mostly neglected. There must be a raising of awareness, and funding in research needs to be increased. More than 1.5 million people are at risk of contracting fungus, making invasive fungal diseases a source of worry for the scientific community [7]. Data reported from the United States suggests that approximately 666,235 cases have been diagnosed as fungal infections costing around \$6.7 billion respectively [8, 9]. Among the tested infections, *Aspergillus*, *Pneumocystis*, and *Candida* were found to be highest (76.3%) with a cost of 81.1%. In India, around 41% of the population, that is, ~60 million Indians, are afflicted with serious fungal diseases [10]. As per the report of the World Health Organization (WHO; FPPL1-Fungal Priority Pathogen List), there are around 19 fungal pathogens that cause high rates of mortality [11].

Skin fungal infections are quite prevalent and most seriously deadly. The skin, nail, hair, and stratum corneum may become more susceptible to infections, causing

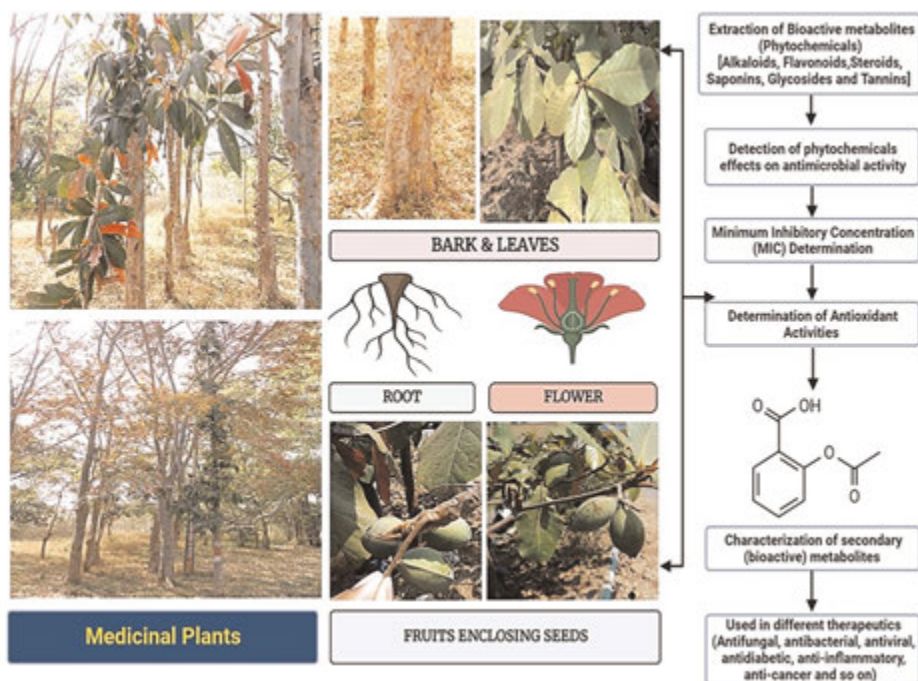


Figure 1.
A general overview on the pharmacological utility of the medicinal plants.

dermatophytosis, inflammatory reaction, and considerable discomfort. Although there are control techniques available, they are only partially efficient in preventing dermatophytic infections in humans [12, 13]. A significant challenge in diagnosing fungal infection is identifying the respective symptoms and suggesting appropriate medications. Also, it is now clear that using specific drugs to treat a fungal disease requires both money and time. The important fungal diseases along with synthetic drugs being administered were listed in **Table 1**.

Artificial antifungal medications are known to have side effects including allergies, rashes, itching, and swelling (particularly on the face, tongue, or throat), as well as serious dizziness, nausea, and liver inflammation [22]. In the current situation, there is a rapid emergence of therapeutic failure due to the development of resistance in pathogenic strains to these synthetic medications, which has constrained researchers to develop novel broad-spectrum nontoxic antifungal medicines [26]. To avoid pathogens developing antibiotic resistance, using novel compounds that are not based on existing synthetic medications could be a good strategy for the management of human diseases [27]. Since 1926, scientists have studied the efficiency of plants in suppression of pathogenic bacteria, which is crucial to human health. The antifungal action of medicinal herbs has not been thoroughly investigated, and only a few findings have been published [5, 12, 15, 28–30]. Therefore, the present article highlights the importance of bioactive metabolites present in medicinal plants and their potential effect in the suppression of fungal infections in humans.

2. Methodology

The literature search has been carried out using search engines like Google Scholar, and the results were scanned for their alternative words, phrases, and subject headings. Around 300 to 350 articles were searched with the help of physical and electronic literature to find out the lacunae and the relevant information regarding the therapeutic use of medicinal plants in relation to the control of human pathogenic fungi. Also, various other techniques involved in performing a literature search such as medical subject heading (MeSH) term search, Boolean operators, phrase search, field search, and truncation search were explicated in this article. Also, the search strategy has been refined, repeated, and a record of searched articles have been reserved.

3. Results

Around 100 articles and relevant abstracts or articles were chosen to define the goal, identify research lacunae, and determine the significance of chapter writing. Around 80 articles were chosen from Google Scholar, 10 from PubMed, and 20 from Scopus, and the remaining articles were left as the data was irrelevant or insufficient. From the data obtained, phytochemicals belonging to specific families and the plant parts from which phytochemicals are obtained along with their medicinal value have been briefed in **Table 2**. The role of phytochemicals specifically in relation to antifungal effects toward the deadliest pathogens has been specified in **Table 3** along with the inhibition zones in detail. Finding the active medicinal extracts at minimal concentration of usage that can target the pathogen growth is essential; hence detailed information in this regard has been listed in **Table 4**. The medicinal plant boost antioxidant capacity, total phenol, total flavonoid content, lower fungal infection and

Chronic disease	Infectious agents	Symptoms	Affected parts	Synthetic drug used	Reference
Cryptococcosis	<i>Cryptococcus gattii</i> , <i>Cryptococcus neoformans</i> , <i>Cryptococcus bacillisporus</i> , <i>Cryptococcus</i> <i>deuterogattii</i> , <i>C. tetragattii</i> & <i>C. decagattii</i>	Fever, Headache, Neck stiffness, Cough, Rashes & Blisters.	Skin, Lungs, Central Nervous System & Spinal cord	Fluconazole, Amphotericin B, Flucytosine & Corticosteroids.	[14, 15]
Candidiasis	<i>C. albicans</i> & <i>C. auris</i>	Cracking, Soreness, Itching & Rashes	Skin, Vagina & Mouth	Nystatin & Miconazole.	[16]
<i>Tinea capitis</i> & <i>Tinea corporis</i> (Ringworm)	<i>Microsporum canis</i> <i>Trichophyton</i> <i>mentagrophytes</i> & <i>T. tonsurans</i>	Hair loss, Dry scaly rashes, Itching, & Swollen red spots.	Hair & Skin	Terbinafine, Griseofulvin, Itraconazole, & Fluconazole.	[17]
Blastomycosis	<i>B. dermatitidis</i> , <i>B. gilchristii</i> , <i>B. helices</i> & <i>B.</i> <i>percurus</i>	Lesions, Joint pain, Breath shortness, Fever & Chest/Back pain.	Skin, Lungs, Bones, Joints & Central Nervous System	Itraconazole, Voriconazole, & Liposomal Amphotericin B.	[18, 19]
Coccidioidomycosis	<i>C. immitis</i> & <i>C. posadasii</i>	Muscle/Joint pain, Rashes, Breathing issues & Fatigue.	Skin, Lungs, Bones & Joints	Fluconazole & Liposomal Amphotericin.	[20]
Sportotrichosis	<i>Sporothrix schenckii</i>	Lesions with papules & nodules, Cough, Shortness of breath, Chest pain & Fever.	Skin & Lungs	Itraconazole, Fluconazole, Ketoconazole, Terbinafine & Amphotericin B.	[21]
Paracoccidiomycosis	<i>P. brasiliensis</i> , <i>P. americana</i> , <i>P. restrepiensis</i> , <i>P. venezuelensis</i> & <i>P. lutzii</i>	Cough, Dyspnea, Sputum & Ulcerated oral lesions.	Skin & Mucous membranes	Itraconazole, Sulfanilamides, Cotrimoxazole & Amphotericin B.	[22]
<i>Tinea barbae</i> & <i>Tinea unguium</i>	<i>T. rubrum</i> & <i>T. mentagrophytes</i>	Ring shaped rashes.	Beard/Hair	Terbinafine, Itraconazole, Fluconazole & Griseofulvin.	[23]
		White/yellow-brown spot under the tip of your fingernail or toenail.	Nails	Terbinafine, Itraconazole, Fluconazole, Efinaconazole & Tavaborole or Ciclopirox.	[24]
<i>Tinea pedis</i>	<i>Trichophyton rubrum</i> , <i>T. mentagrophytes</i> & <i>Epidermophyton floccosum</i>	Inflammation & Itchiness.	Legs	Terbinafine, Itraconazole, Fluconazole, Benzylamine, Ciclopirox, Tolnaftate & Amorolfine.	[25]

Table 1.
A brief overview of the major fungal diseases, including their drug administration.

Plants name	Family	Uses	Part used	Extract used	Phytochemicals	Reference
<i>Antidesma madagascariense</i> , & <i>Erythroxylum macrocarpum</i>	Phyllanthaceae & Erythroxylaceae	Can be used for dysentery, fever, body pain & skin infections. The plants are good for diabetes & rheumatism.	Leaves & Twigs	Crude water extract	Alkaloids, flavonoids, tannins, leucoanthocyanins, phenols, saponins & steroids were present.	[31]
<i>Bauhinia racemose</i> , <i>Lawsonia inermis</i> , <i>Datura stramonium</i> , <i>Datura metel</i> & <i>Withania somnifera</i>	Fabaceae, Lythraceae & Solanaceae	Relieves headache, fever, skin diseases, tumors, hemorrhagic, cardio-inhibitory, hypotensive, pain, diarrhea, chronic bronchitis, asthma, skin diseases, worm infestation, toothache, anxiety & cancer.	Leaves	Methanol	Alkaloids, flavonoids, saponins, steroids & tannins were observed.	[6]
<i>Vitis vinifera</i> cv. Sultana <i>V. vinifera</i> cv. Roumy Ahmer <i>Zizyphus spina-christi</i> <i>Punica granatum</i> cv. baladi & <i>Ficus carica</i> cv. sultani	Vitaceae, Rhamnaceae, Punicaceae & Moraceae	Relieves itching, burning of piles, release discomfort & heaviness of legs.	Leaves	Crude extracts	Tannins, resins, alkaloids, carbohydrates, flavonoids, resins, phenols & glycosides were present.	[32]
<i>Carica papaya</i>	Caricaceae	Cure dyspepsia, piles, psoriasis, ringworm, hypoglycemia, hypolipidemia & lowering blood pressure.	Leaves	Crude extracts	Flavonoids, alkaloids, saponins, cardiac glycosides, anthraquinones, carbohydrates & proteins were observed.	[33]
<i>Citrullus colocynthis</i>	Cucurbitaceae	Treat gastrointestinal disorders, anti-diabetic, anti-inflammatory, anthelmintic & anti-cancerous.	Seeds	Aqueous, Hydro-methanol, Ethylacetate & n-Butanol	Terpenoids, tannins, flavonoids & coumarins were present.	[34]
<i>Calendula officinalis</i> , <i>A. arabica</i> , <i>Altheae officinalis</i> , <i>Ginkgo biloba</i> , <i>Juglans regia</i> , <i>Ocimum basilicum</i> , <i>S. nigrum</i> , <i>Hypericum perforatum</i> , <i>Urtica dioica</i> & <i>Anagalis arvensis</i>	Asteraceae, Rubiaceae, Malvaceae, Ginkgoaceae, Juglandaceae, Lamiaceae, Solanaceae, Clusiaceae, Urticaceae & Primulaceae	Treat Gingivitis, episiotomy healing, chronic prostatitis, vaginal candidiasis, dermatitis (Diaper), leg & stomach ulcers, leucorrhoea, inflammation, blood disorders, memory issues, arthritis, eczema, cough,	Leaves	Methanol	Alkaloids, flavonoids, terpenoids, oils, phenolic compounds, proteins, saponin, steroids, tannins, glycosides, & reducing sugars were documented.	[35]

Plants name	Family	Uses	Part used	Extract used	Phytochemicals	Reference
		constipation, worms, kidney malfunctions, pneumonia, tumor, tonsillitis, obesity, diabetes milletus, hypertension & skin rashes.				
<i>Lantana camara</i> , <i>Citrus limonum</i> , <i>Azadirachta indica</i> & <i>Hibiscus Rosasinensis</i>	Verbenaceae, Rutaceae, Meliaceae & Malvaceae	Relieves cough, fever, malaria, chickenpox, cancer, high blood pressure, irregular menstruation, ulcer, tumor & inflammation	Leaves	Aqueous, Ethanol & Methanol	Tannins, alkaloids, terpenoids, cardiac glycosides, saponins, phlobatanins & flavonoids were found.	[36]
<i>Terminalia arjuna</i> , <i>Terminalia tomentosa</i> , <i>Terminalia catappa</i> , <i>Terminalia chebula</i> , <i>Persia americana</i> , <i>Psoralea corylifolia</i> , <i>Polyalthia longifolia</i> & <i>Syzygium jambos</i>	Combretaceae, Lauraceae, Fabaceae, Annonaceae & Myrtaceae	Treat heart failure, ischemic, cardiomyopathy, atherosclerosis, anemia, ulcers, hemorrhages, bronchitis, diarrhea, gastritis, leprosy wounds, skin diseases, scabies, constipation, diabetes, hypertension, inflammatory conditions, pyrexia & rheumatism.	Bark, Leaf, Fruit, Meso-carp & Seeds	Aqueous, Methanol & Ethyl acetate	Alkaloids, flavonoids, terpenoids, tannins, phenols, proteins, steroids & carbohydrates were present.	[37]
<i>Pimenta dioica</i>	Myrtaceae	Cure obesity, digestion problem, menstrual cramps & abdominal pain.	Leaves	Petroleum Ether	Tannins, terpenoids, flavonoids steroids & coumarins were recorded.	[13]
<i>Pistacia lentiscus</i>	Anacardiaceae	Utilized in the management of respiratory, skin & gastrointestinal problems.	Aerial parts	Ethanol	Leuco-anthocyanins, condensed tannins, gallic tannins, saponoside, coumarins & flavonoids were reported.	[38]
<i>Ricinus communis</i>	Euphorbiaceae	Used to treat abdominal disorders, arthritis, chronic backache, sciatica, constipation, expulsion of placenta, gallbladder pain, period pain, rheumatism, & sleeplessness.	Leaves	Aqueous, Methanol & Ethanol	Tannins, saponins, terpenoids & flavonoids were found.	[39]

Plants name	Family	Uses	Part used	Extract used	Phytochemicals	Reference
<i>Leptadenia hastata</i>	Apocynaceae	Relieves hypertension, sexual impotence, trypanosomosis & acute rhinopharyngitis.	Stem Bark	Aqueous & Ethanol	Carbohydrates, steroids, terpenoids, phenols, tannins, alkaloids, flavonoids, proanthocyanidins & glycosides showed their presence in the extracts.	[40]
<i>Cyrtomium caryotideum</i>	Dryopteridaceae	Act as anti-pyretic & anti-cancer.	Whole Plant	Methanol, Chloroform & Ethanol	Carbohydrates, flavonoids, phlobatannins, alkaloids, saponins, tannins, phenols, terpenoids & cardiac glycosides were reported.	[29]
<i>Pistacia lentiscus</i> <i>Artemisia herba-alba</i> & <i>Citrullus colocynthis</i>	Anacardiaceae, Asteraceae & Cucurbitaceae	Can be used in the treatment of respiratory, skin & gastrointestinal disorders i.e., indigestion, gastroenteritis & intestinal parasites. Cure jaundice, cough, cold, anxiety, measles & muscle weakness.	Seeds	Methanol & Aqueous	Alkaloids, flavonoids, tannins & saponins were found in the extracts.	[11]
<i>Teucrium polium</i>	Lamiaceae	Used for gastrointestinal disorders, inflammations, diabetes & rheumatism.	Plant material	Methanol	Alkaloids, sugar, flavonoids, limonoid, terpenoids, lipids & phenols were found.	[41]
<i>Curcuma longa</i> <i>Prosopis africana</i> & <i>Psorospermum febrifugum</i>	Zingiberaceae, Fabaceae & Hypericaceae	Used in the treatment of anorexia, wounds, biliary disorders, cough, diabetics, hepatic disorders, rheumatism, fever, stomach pain, chest pain, sinusitis, gonorrhea, & kidney disease.	Stem Bark, Leaves & Rhizome	Methanol	Cardiac glycosides, saponins, steroids, flavonoids, terpenoids, phenols & tannins were present.	[42]
<i>Rumex abyssinicus</i> , <i>Polysphaeria aethiopica</i> , <i>Lippia adoensis</i> , <i>Cucumis pustulatus</i> , <i>Cirsium englerianum</i> , <i>Discopodium penninervium</i> & <i>Euphorbia depauperata</i>	Polygonaceae, Rubiaceae, Verbenaceae, Cucurbitaceae, Asteraceae, Solanaceae & Euphorbiaceae	Treat eczema, gonorrhea, syphilis, pneumonia, scabies, skin infection & superficial mycosis.	Leaf, Bark & Root	Methanol & Aqueous	Saponins, tannins, alkaloids, terpenoids, anthraquinones, phenols, cardiac glycosides & flavonoids were observed.	[43]

Plants name	Family	Uses	Part used	Extract used	Phytochemicals	Reference
<i>Moringa oleifera</i> , <i>Boswellia dalzielii</i> , <i>Rumex crispus</i> , <i>Ocimum gratissimum</i> , <i>Leptadenia hastata</i> , <i>Breynia nivosa</i> & <i>Cassia alata</i>	Moringaceae, Burseraceae, Polygonaceae, Lamiaceae, Asclepiadaceae, Euphorbiaceae & Leguminosae	Good source to treat diarrhea, malaria, vomiting, inflammation, arthritis, gastrointestinal disorders, liver protection, cancer, anemia, hypertension, rhinopharyngitis, tooth infections & ringworm.	Leaf, Stem & Root	Methanol	Alkaloids, anthraquinones, flavonoids & tannins were reported.	[44]
<i>Acer cappadocicum</i>	Sapindaceae	Treat appetite loss, hair loss, anemia & cell damage can be managed.	Leaves	n-Butanol, Chloroform, Ethyl acetate, Methanol & n-Hexane.	Alkaloids, tannins, steroids, phytosteroids, glycosides & quinones were documented.	[45]
<i>Rauwolfia vomitoria</i>	Apocynaceae	Cure fever, intestinal diseases, liver problems, mental illness, hemorrhoids, hypertension, snakebite, cholera & jaundice	Leaves	Ethanol	Tannins, Saponins, Steroids, Glycosides, Alkaloids, & Flavonoids were found.	[46]
<i>Brucea antidysenterica</i> , <i>Aloe vera</i> & <i>Justicia schimperiana</i>	Simaroubaceae, Liliaceae & Acanthaceae	Dysentery, fever, skin injuries, digestive problems, headache & liver disease are treated & act as anthelmintic.	Leaves	Methanol	Alkaloids, Phenols, tannins, saponins & flavonoids were obtained.	[47]
<i>Teucrium stocksianum</i>	Lamiaceae	Diarrhea, cough, jaundice & abdominal pain are treated.	Leaves	Methanol & Ethanol	Alkaloid, tannin, saponins, anthraquinone, steroid, phlobatannin, terpenoid, flavonoids, glycoside, & reducing sugar were present.	[48]
<i>Azadiracta indica</i>	Meliaceae	Treat skin disease, Loss of appetite, cure wounds/Ulcer, Intestinal wounds. Act as hypoglycemic, antiarthritic & antipyretic.	Flowers	Petroleum Ether (PE), Acetone (AC), & Hydroalcohol (20%)	Polyphenols, flavonoids & glycosides were seen.	[49]

Table 2.

List of medicinal plants, their medicinal uses, and their screening for the presence of phytochemicals in various plant extracts.

Plant name	Family	Part taken	Extract Name	Pathogen	Inhibition Zone	Reference
<i>Taxus baccata</i>	Taxaceae	Heart wood	Ethanol	<i>Microsporum canis</i> & <i>Epidermophyton floccosum</i>	21 mm & 23 mm	[50]
<i>Terminalia chebula</i>	Combretaceae	Galls	Aqueous	<i>Trichophyton</i> sp. & <i>Candida</i> sp.	1 mm to 3 mm	[51]
<i>Plagiiochasma appendiculatum</i>	Aytoniaceae	Plant material	Chloroform, Acetone, Aqueous & Ethanol	<i>Aspergillus niger</i> , <i>A. flavus</i> , <i>A. spinulosus</i> , <i>A. terreus</i> , <i>A. nidulans</i> , <i>Trichophyton rubrum</i> & <i>C. albicans</i>	10 mm to 25.67 mm	[52]
<i>Betula papyrifera</i> , <i>Centaurea maculosa</i> , <i>Epilobium angustifolium</i> , <i>Hypericum perforatum</i> , <i>Lythrum salicaria</i> & <i>Rhus glabra</i>	Betulaceae, Asteraceae, Onagraceae, Clusiaceae, Lythraceae & Anacardiaceae	Aerial parts	Aqueous & Ethanol	<i>C. albicans</i>	7 mm to 20 mm	[53]
<i>Terminalia catappa</i>	Combretaceae	Leaves	Methanol	<i>C. albicans</i>	19.33 mm	[27]
<i>Terminalia arjuna</i>	Combretaceae	Bark	Crude extract	<i>T. mentagrophytes</i> , <i>T. rubrum</i> , <i>T. tonsurans</i> , <i>M. fulvum</i> & <i>M. gypseum</i>	3 mm to 25 mm	[54]
<i>Lobelia nicotianaefolia</i> , <i>Lagenandra toxicaria</i> & <i>Remusatia vivipara</i>	Lobeliaceae & Araceae	Rhizome, Leaves, Corm & Roots	Chloroform, Ethanol & Acetone	<i>C. albicans</i>	7 mm to 11.56 mm	[55]
<i>Catharanthus roseus</i>	Apocynaceae	Leaves	Ethanol	<i>Candida albicans</i> , <i>Aspergillus fumigatus</i> & <i>Aspergillus niger</i>	1.1 cm to 2.1 cm	[56]
<i>Terminalia chebula</i> , <i>Terminalia bellerica</i> & <i>Embllica officinalis</i>	Combretaceae & Phyllanthaceae	Fruit	Crude extract	<i>T. rubrum</i> , <i>M. gypseum</i> , <i>C. albicans</i> & <i>Malassezia furfur</i>	15 mm to 42 mm	[57]
<i>Lawsonia inermis</i>	Lythraceae	Leaves	Ethanol & Petroleum ether	<i>C. albicans</i>	16.7 mm to 22.7 mm	[58]
<i>Calendula officinalis</i> , <i>Acacia arabica</i> , <i>Althea officinalis</i> , <i>Ginkgo biloba</i> , <i>Juglans regia</i> , <i>Ocimum basilicum</i> , <i>Solanum nigrum</i> , <i>Hypericum perforatum</i> , <i>Urtica dioica</i> & <i>Anagalis arvensis</i>	Asteraceae, Leguminosae, Malvaceae, Ginkgoaceae, Juglandaceae Lamiaceae, Solanaceae, Clusiaceae, Urticaceae & Primulaceae	Leaves	Methanol	<i>T. mentagrophytes</i> , <i>T. rubrum</i> , <i>T. schoenleinii</i> , <i>M. canis</i> , <i>M. gypseum</i> , & <i>Epidermophyton floccosum</i>	2% to 66.6%	[35]

Plant name	Family	Part taken	Extract Name	Pathogen	Inhibition Zone	Reference
<i>Lantana camara</i> , <i>Citrus limonum</i> , <i>Azadirachta indica</i> & <i>Hibiscus rosasinensis</i>	Verbenaceae, Rutaceae, Meliaceae & Malvaceae	Plant material	Aqueous, Ethanol & Methanol	<i>T. rubrum</i> & <i>C. albicans</i>	7 mm to 13 mm	[36]
<i>Glycyrrhiza glabra</i>	Fabaceae	Plant material	Methanol	<i>C. parapsilosis</i>	10.66 mm	[59]
<i>Leptadenia hastata</i> , <i>Barringtonia racemosa</i> & <i>Barringtonia asiatica</i>	Meliaceae, Lecythidaceae & Meliaceae	Stem Bark	Methanol	<i>Candida tropicalis</i> , <i>Fusarium oxysporum</i> & <i>A. flavus</i>	1.95 mm, 1.8 mm & 5 mm	[40]
<i>Parkia biglobosa</i>	Fabaceae	Bark	Aqueous & Ethanol	<i>Candida albicans</i>	11.60 mm	[60]
<i>Teucrium polium</i>	Lamiaceae	Plant material	Methanol	<i>C. albicans</i> , <i>Cryptococcus neoformans</i> , <i>C. vaginalis</i> , <i>A. fumigatus</i> & <i>A. niger</i>	6 mm to 10.33 mm	[41]
<i>B. dalzielii</i> , <i>R. crispus</i> , <i>O. gratissimum</i> , <i>L. hastate</i> , <i>C. alata</i> , <i>M. oleifera</i> & <i>B. nivosa</i>	Burseraceae, Polygonaceae, Chenopodiace, Lamiaceae, Fabaceae, Moringaceae & Phyllanthaceae	Root & Leaves	Methanol	<i>T. rubrum</i> , <i>T. mentagrophytes</i> & <i>C. albicans</i>	19.43 mm, 24.3 mm	[44]
<i>Pastinca sativa</i> , <i>Carum carvi</i> , <i>Cuminum cyminum</i> , <i>Dacus carota</i> , <i>Centella asiatica</i> , <i>Anthriscus cerefolium</i> , <i>Trachyspermum ammi</i> , <i>Pimpenella anisum</i> & <i>Apium graveolens</i>	Apiaceae	Leaves	Ethanol, Methanol, Hexane & Ethyl Acetate	<i>Aspergillus fumigates</i>	3.5 mm to 19.3 mm	[61]
<i>Plantago lanceolata</i> & <i>Sida ovata</i>	Plantaginaceae & Malvaceae	Leaves	Ethanol	<i>Aspergillus niger</i> & <i>Aspergillus fumigatus</i>	19.6 mm & 20.4 mm	[48]

Table 3.
Antifungal activity of medicinal herbs toward human disease-causing fungi.

Plant name	Family	Part taken	Extract Name	Pathogen	MIC	Reference
<i>Cymbopogon citratus</i> , <i>Lantana</i> sp., <i>Nerium</i> sp., <i>Ocimum basilicum</i> & <i>Olea europaea</i>	Poaceae, Verbenaceae, Apocynaceae, Lamiaceae & Oleaceae	Leaves	Methanol, Aqueous, Diethyl ether, Ethyl acetate, n-Butanol & Chloroform	<i>M. canis</i> , <i>M. gypseum</i> , <i>T. rubrum</i> , <i>T.</i> <i>mentagrophytes</i> & <i>T. verrucosum</i>	1.0 to 175 µg/mL	[62]
<i>Terminalia arjuna</i>	Combretaceae	Bark	Crude extract	<i>T. mentagrophytes</i> , <i>T. rubrum</i> , <i>T.</i> <i>tonsurans</i> , <i>M. fulvum</i> & <i>M. gypseum</i>	75 to 2000 µg/ mL	[54]
<i>Carica papaya</i>	Caricaceae	Leaves	Crude extract	<i>Candida</i> sp., <i>Trichophyton</i> sp. & <i>Microsporum</i> sp.	80 to 320 mg/ mL	[33]
<i>Persea americana</i> , <i>Psidium guajava</i> , <i>Carica papaya</i> , <i>Citrus lemonum</i> , <i>Citrus aurantiifolia</i> & <i>Zingiber</i> <i>officinale</i>	Lauraceae, Myrtaceae, Caricaceae, Rutaceae & Zingiberaceae	Leaves & Fruits	Methanol	<i>A. niger</i> , <i>T. rubrum</i> , <i>A. awamori</i> , <i>Rhodotorula</i> spp., <i>Cryptococcus</i> <i>neoformans</i> , <i>A. carborarium</i> , <i>M. canis</i> & <i>C. albicans</i>	1.56 to 6.25 mg/ mL	[63]
<i>S. chirata</i> , <i>Z. armatum</i> & <i>T. bellerica</i>	Gentianaceae, Rutaceae & Combretaceae	Plant Material	Methanol	<i>C. albicans</i> , <i>C. galbrata</i> , <i>C. tropicalis</i> & <i>C. krusei</i>	10 to 180 mg/ mL	[64]
<i>Juglans regia</i> , <i>Acacia arabica</i> , <i>Calendula officinalis</i> , <i>Ginkgo biloba</i> , <i>Anagalis arvensis</i> , <i>Ocimum basilicum</i> , <i>Hypericum perforatum</i> & <i>Solanum</i> <i>nigrum</i>	Juglandaceae, Leguminosae, Asteraceae, Ginkgoaceae, Primulaceae, Lamiaceae, Clusiaceae & Solanaceae	Leaves	Methanol	<i>E. floccosum</i> , <i>T. mentagrophytes</i> , <i>T.</i> <i>schoenleinii</i> , <i>T. rubrum</i> , <i>M. gypseum</i> & <i>M. canis</i>	0.1 to 12.5 mg/ mL	[35]
<i>Glycyrrhiza glabra</i>	Fabaceae	Plant material	Methanol	<i>Candida parapsilosis</i>	62.5 mg/ mL	[59]
<i>Ricinus communis</i>	Euphorbiaceae	Leaves	Aqueous, Ethanol, Methanol, Petroleum ether & Ethyl acetate	<i>Candida albicans</i>	12.5 to 25 mg/mL	[39]
<i>Guapira graciliflora</i>	Nyctaginaceae	Leaves	Methanol	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. krusei</i> & <i>C. dubliniensis</i>	125 & 250 µg/ mL	[65]

Plant name	Family	Part taken	Extract Name	Pathogen	MIC	Reference
<i>Parkia biglobosa</i>	Fabaceae	Bark & Leaves	Aqueous & Ethanol	<i>Candida albicans</i>	0.2 to 0.7 mg/mL	[60]
<i>Dodonaea viscosa</i> , <i>Plantago lanceolata</i> , <i>Withania samnifera</i> , <i>Pulicaria crispa</i> , & <i>Rumex nervosus</i>	Sapindaceae, Plantaginaceae, Solanaceae, Asteraceae & Polygonaceae	Leaves	Methanol	<i>Candida albicans</i>	5 to 80 mg/mL	[66]
<i>Tephrosia vogelii</i>	Fabaceae	Leaves & Roots	Dichloromethane & Methanol	<i>C. albicans</i> & <i>C. neoformans</i>	1.25 mg/mL	[67]
<i>T. polium</i>	Lamiaceae	Flower	Methanol	<i>C. albicans</i> , <i>Cryptococcus neoformans</i> , <i>C. vaginalis</i> , & <i>A. fumigatus</i>	2.0 to 4.0 mg/mL	[41]
<i>Rumex abyssinicus</i> , <i>Cucumis pustulatus</i> , <i>Discopodium penninervium</i> , <i>Lippia adoensis</i> , <i>Euphorbia depauperata</i> & <i>Cirsium englerianum</i>	Polygonaceae, Cucurbitaceae, Solanaceae, Verbenaceae, Euphorbiaceae, Asteraceae & Fabaceae	Leaf, Bark & Root	Methanol	<i>Trichophyton mentagrophytes</i> & <i>Candida albicans</i>	16 to 512 µg/mL	[43]
<i>Prosopis julifera</i>	Fabaceae	Leaves	Ethanol	<i>G. candidum</i> & <i>C. albicans</i>	0.5 to 2.5 mg/mL	[2]
<i>Myrtus communis</i>	Myrtaceae	Plant material	Methanol	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. keyfr</i> , <i>C. parapsilosis</i> , & <i>C. tropicalis</i>	12.5 to 25 mg/mL	[68]
<i>Chamaecostus cuspidatus</i>	Costaceae	Leaves, Stems & Rhizomes	Ethanol	<i>Trichophyton rubrum</i> & <i>Candida albicans</i>	15.62 to >1000 µg/mL	[69]
<i>Clematis hirsuta</i>	Ranunculaceae	Leaves	Acetone, Chloroform, Ethanol, Methanol & Ethyl acetate	<i>C. albicans</i> , <i>A. niger</i> & <i>A. fumigatus</i>	15 to 60 mg/mL	[70]

Plant name	Family	Part taken	Extract Name	Pathogen	MIC	Reference
<i>Plantago lanceolata</i> & <i>Sida ovata</i>	Plantaginaceae & Malvaceae	Leaves	Methanol	<i>Aspergillus niger</i> , & <i>Aspergillus fumigatus</i>	39.4 & 41.3 mg/mL	[71]
<i>Tristemma mauritianum</i> <i>Crassocephalum bougheyannum</i> & <i>Larvigieria macrocarpa</i>	Mecastomataceae, Compositae & Icacinaceae	Aerial parts, Leaves & Roots	Methanol	<i>C. albicans</i> , <i>C. dubliniensis</i> , <i>C. glabrata</i> & <i>C. tropicalis</i>	64 to 2048 µg/mL	[72]

Table 4.
 Evaluation of medicinal herbs for the minimum dose effective against human pathogenic fungi.

provided protection from oxidative damage. A brief summary of medicinally important herbs that are reported to have antioxidant capacity has been categorized in **Table 5**. Characterization and purification of specific compounds from the crude extracts is an essential step to develop novel antifungal drug therapies. The important source of medicinal plants and specific plant parts from which bioactive compounds are obtained, showing their potential and efficacy in different therapeutic effects has been broadly mentioned in **Table 6**.

4. Discussions

4.1 Assessment of medicinal herbs for the occurrence of their active phytochemicals

The therapeutic benefits of medicinal plants are mainly decided by the amounts of phytochemicals present in them naturally. Plants with valuable phytochemicals can supplement human needs by acting as natural antioxidants and protecting against free radical damage. These chemicals provide protection from infectious diseases and aid in maintaining good color, flavor, and aroma in plants. In addition, they are known to guard plants against diverse environmental pressures such as ultraviolet (UV) exposure, stress, pollution, drought, and pathogenic attacks [100]. Other than pathogens gaining resistance, there is upsurge in the number of opportunistic infections with regard to acquired immunodeficiency syndrome (AIDS), antibacterial and antifungal drug toxicity [101]. Therefore, there is rising pressure on researchers as well as on scientists to seek for alternative, nontoxic, and novel bioactive antifungal drugs. Phytochemicals are good agents possessing bioactive properties, such as antioxidant, antibacterial, antifungal, antidiabetic, and anti-inflammatory. Phenols, tannins, alkaloids, essential oils, flavonoids, resins, and other bioactive substances were chiefly present in from medicinal plants parts, such as, leaves, flowers, bark, seeds, fruits, and roots [13, 19, 30, 102–105]. These phytochemicals possess bioactive properties, namely, antioxidant, antibacterial, antifungal, antidiabetic, and anti-inflammatory. In most of the reports, methanol can act as promising solvent to detect valuable phytochemicals [72, 102, 106, 107]. The solubility of specific plant components is reportedly influenced by the concentration of the solvent used [74]. The presence of phytochemicals, namely, alkaloids, steroids, and tannins in *T. catappa* extract were responsible for projecting antimicrobial activities [27, 108]. In the methanol extract of *Teucrium polium*, 29 phytochemicals were found to be active toward different bacteria, fungi, and viruses in addition to displaying cytotoxic and anti-quorum-sensing properties [41]. Alkaloids, phenols, and flavonoids were discovered in *Aloe vera* leaf extract, but glycosides and saponins were not. An absence or occurrence of phytochemicals in medicinal herbs is attributed to variations in plant growth seasons, soil types, and native geographic areas [109]. Butanol and methanol extracts of *A. cappadocicum* containing steroids, alkaloids, tannins, phytosteroids, glycosides, and quinones delivered antimicrobial, antioxidant, and anticancerous action [103]. The biological and phytochemical properties of various *Clematis* species showed antibacterial and anti-inflammatory properties [110]. Alkaloids, flavonoids, and phenols were reported in medicinal plants, such as *Brucea antidysenterica*, *Aloe vera*, and *Justicia schimperiana* plants [106]. The phytochemicals were effective in controlling pathogen gene expression, constraining pathogens growth, and other metabolic activities [111]. Phytochemicals are likely to link in delivering antifungal and antibacterial

Plant name	Family	Part taken	Extract name	TPC+ TFC (Total phenol & Total flavonoid contents)	Antioxidant effect	Reference
<i>Terminalia chebula</i> , <i>Terminalia belerica</i> & <i>Emblica officinalis</i>	Combretaceae & Euphorbiaceae	Dry fruits	Methanol	—	For superoxide assay, concentrations of 20.5, 40.5, 6.5 & 12.5 µg/mL, is required for 50% inhibition. In Hydroxyl radical assay, 165.5, 71, 155, 151 µg/mL is needed for 50% inhibition. For lipid peroxidation, the concentration of 85.5, 27, 74 & 69 µg/mL is needed for 50% inhibition.	[73]
<i>Swertia chirayita</i> , <i>S. nervosa</i> , & <i>Andrographis paniculata</i>	Gentianaceae & Acanthaceae	Root, Shoot & Combination of Inflorescence + Leaf	Aqueous & Ethanol	Both the plant extracts exhibited highest TPC particularly <i>S. chirayita</i> 5.6 mg/g GAE), followed by <i>S. nervosa</i> (both 4.7 mg/g GAE) & <i>Andrographis paniculata</i> (4.5 mg/g GAE).	The extracts recorded prominent DPPH radical activity. Among all the extracts, <i>S. chirayita</i> recorded best radical scavenging activity of 70%.	[74]
<i>Swertia chirayita</i>	Gentianaceae	Leaves	Aqueous, Methanol, Ethanol & Acetone	Aqueous extract showed highest phenolic content (221 mg GAE/100 g). While ethanol was found to be less effective.	In ferric reducing antioxidant power (FRAP), aqueous extract presented the highest reducing power [162 mg AAE 100 g⁻¹]. Whereas, least reducing power was found in ethanol (142 AAE 100gm⁻¹), acetone (144 mg AAE 100gm⁻¹) & methanol (146 mg AAE/100 g) respectively. In total antioxidant capacity assay (TAC), the extracts presented lowest to the highest activity i.e., 139 mg AAE 100 gm⁻¹(acetone 100%) to 707 mg AAE 100 gm⁻¹ (ethanol 50%). Alpha amylase inhibition & DPPH radical scavenging activities were shown to be dose-dependent, with ranges of 3 to 15 mg per mL & 7 to 21 mg per mL, respectively.	[75]

Plant name	Family	Part taken	Extract name	TPC+ TFC (Total phenol & Total flavonoid contents)	Antioxidant effect	Reference
<i>Limnophila aromatica</i>	Plantaginaceae	Plant material without roots	Methanol, Ethanol & Acetone	The ethanol extracts showed maximum phenolic content (40.5 mg GAE g ⁻¹) & the highest flavonoid content (31.11 mg QE g ⁻¹).	The ethanol extract exhibited potential effect in enhancement of total antioxidant, reducing power & DPPH radical scavenging activities performed.	[76]
<i>Pistacia lentiscus</i>	Anacardiaceae	Plant material	Ethanol	—	At 100 µg L ⁻¹ of concentration, <i>P. lentiscus</i> ethanol extract recorded the considerable reducing capacity (0.816) which reflect the highest phenol content accountable for this activity.	[38]
<i>Oryza sativa</i> varieties namely Suwadal Heeneti, Nilkanda, Maa Wee & Kurulu Thuda.	Poaceae	Whole grains	70% Ethanol & Water (v/v)	—	Plant (Kurulu Thuda variety) presented greatest FRAP (6.79 mmol FeSO ₄ 100 g ⁻¹ bran) & ABTS (13.13 mmol Trolox 100 g ⁻¹ bran) radical scavenging antioxidant properties followed by Nilkanda & Heeneti, respectively.	[12]
<i>Artemisia herba-alba</i> <i>Pistacia lentiscus</i> & <i>Citrullus colocynthis</i>	Asteraceae, Anacardiaceae & Cucurbitaceae	Aerial part, Leaves & Fruit	Aqueous & Methanol	—	The methanol extract of <i>A. herba alba</i> recorded best antioxidant (FRAP, DPPH, ABTS) activities of 6080.67, 1942.80, 1059.05 µM TE g ⁻¹ followed by <i>Pistacia lentiscus</i> & <i>Citrullus colocynthis</i> .	[11]
<i>Pistacia lentiscus</i>	Anacardiaceae	Leaves Twigs & Fruits	Aqueous & Methanol	Plant leaf extract recorded sturdiest phenolic compounds (345.95 to 67.83 mg of GAE g ⁻¹), tailed by the extracts of twigs (302.01 to 48.71 mg GAE/gm) & the fruits (192.68 to 36.45 mg of GAE/gm of extract) respectively.	Plant leaves (aqueous) extract showed the highest TAC with 488.16 mg AA g ⁻¹ of extract. The same aqueous extract present highest DPPH activity with IC50 values ranging between 0.09 to 0.53 mg mL ⁻¹ & from 0.57 to 1.13 mg mL ⁻¹ .	[77]

Plant name	Family	Part taken	Extract name	TPC+ TFC (Total phenol & Total flavonoid contents)	Antioxidant effect	Reference
<i>Ficus benjamina</i> var. <i>comosa</i>	Moraceae	Leaf	Ethanol	Total flavonoid & phenolic components were measured as 4.50 mg (GAE/gm) & 11.20 mg (QE/mg).	The highest radical scavenging activity of 68.27 $\mu\text{g mL}^{-1}$ was found in prepared plant extract.	[78]
<i>Punica granatum</i> <i>Mentha piperita</i> <i>Petroselinum crispum</i> <i>Olea europaea</i> <i>Ruta graveolens</i> <i>Rosmarinus officinalis</i> & <i>Salvia officinalis</i>	Lythraceae & Lamiaceae	Leaves	Hydromethanol	The <i>P. granatum</i> leaf extract recorded highest phenol, ortho-diphenols contents of 199.26 & 391.76 GAE g ⁻¹ DW respectively. <i>M. piperita</i> leaf extract recorded highest flavonoid content of 70.14 mg CATE per g DW respectively.	The <i>P. granatum</i> leaf extract presented antioxidant capacity i.e., ABTS, DPPH and FRAP was found to be 2.14, 2.27, & 2.33 mM Trolox PDW (plant dry weight) respectively.	[79]
<i>Crassocephalum bogheyianum</i> , <i>Tristemma mauritianum</i> & <i>Larvigieria macrocarpa</i>	Asteraceae, Melastomataceae & Icacinaceae	Aerial parts Leaves & Roots	Methanol (200 $\mu\text{g/mL}$)	High TPC & total tannin content (TTC) was found to be in <i>T. mauritianum</i> (110.23 mg GAE/g) & 10.90 mg TAE/g. Whereas, <i>L. macrocarpa</i> recorded highest TFC of 9.16 mg QE/g.	Maximum DPPH radical scavenging activity of 94.69% was found in <i>C. bogheyianum</i> followed by <i>T. mauritianum</i> (93.42%) & <i>L. macrocarpa</i> (74.83%). Among the extracts, <i>L. macrocarpa</i> leaves had the highest reduction power. The capacity to scavenge nitric oxide ranged between 39.87 to 96.09%.	[72]
<i>Teucrium stocksianum</i>	Lamiaceae	Leaves	Methanol & Ethanol (60 $\mu\text{g/mL}$)	—	Highest DPPH scavenging activity of 86.4% & 83.8% was found in the methanol & ethanol extracts. Similar results were observed in reducing power assay which presented 2.38% & 0.965% scavenging activity in methanol & ethanol extracts.	[48]

Table 5.
 The list of therapeutic herbs with antioxidant qualities to protect against fungal infection and oxidative damage.

Plant name	Family	Part taken	Extract name	Bioactive compounds	Therapeutic effect	Reference
<i>Terminalia bellirica</i>	Combretaceae	Fruit	Ethanol	7-hydroxy-3',4'-(methylenedioxy)-flavan	The compound presented highest antifungal activity toward <i>C. albicans</i> in addition to anti-HIV-1 & antimalarial effect.	[80]
<i>Eysenhardtia texana</i>	Fabaceae	Aerial parts	Methanol & Dichloromethane	4,5-dihydroxy 7-methoxy, 6[3methyl-2-butenyl]-(2S)-flavanone	Demonstrated antifungal effect against <i>Candida albicans</i> .	[81]
<i>Rheum emodi</i>	Polygonaceae	Rhizomes	Methanol	Rhein, Physcion, Aloeemodin & Chrysophanol	The compounds showed significant inhibitory effect <i>C. albicans</i> , <i>Cryptococcus neoformans</i> , <i>Trichophyton mentagrophytes</i> & <i>A. fumigatus</i> .	[82]
<i>Gardenia jasminoides</i>	Rubiaceae	Fruit	—	Genipin (geniposide)	Presented anti-inflammatory effect by stopping the NF-kB activation & expression of IFN- γ , iNOS respectively.	[83]
<i>Glycine max</i>	Fabaceae	Flour	Methanol	Saponins	They have anti-inflammatory role by producing proinflammatory mediators.	[84]
<i>Artemisia dracunculus</i>	Asteraceae	Dried aerial parts	Crude extract	Essential oils i.e., α -Pinene, β -Pinene, β -Myrcene, Limonene, cis-Ocimene, cis-allo-Ocimene, trans ocimene, trans-Anethole, Bornyl acetate, γ -Elemene, β -Caryophyllene & Germacrene D were obtained through GC-MS analysis.	All the compounds were reported to possess considerable antifungal effect toward <i>C. albicans</i> , <i>C. lusitaniae</i> , <i>C. glabrata</i> , & <i>C. tropicalis</i> .	[85]
<i>Sapindus saponaria</i>	Sapindaceae	Fruits	Butanol	Triterpene acetylated saponins	Exhibited strong inhibitory effect toward <i>C. parapsilosis</i> .	[86]
<i>Scutellaria baicalensis</i>	Lamiaceae	—	—	Baicalin	The compound stopped <i>C. albicans</i> biofilms formation & elevated the rate of apoptosis thereby exerting a concentration-dependent antifungal effect.	[87]
<i>Toddalia asiatica</i>	Rutaceae	Leaves	Ethyl acetate	Flindersine	The extracted compound shows significant antifungal effect against dermatophytic fungi i.e., <i>T. rubrum</i> , <i>T. mentagrophytes</i> , <i>T. simii</i> , <i>Epidermophyton floccosum</i> & <i>C. albicans</i> .	[88]

Plant name	Family	Part taken	Extract name	Bioactive compounds	Therapeutic effect	Reference
<i>Rubia tinctorum</i>	Rubiaceae	Roots	—	Purpurin (1,2,4-trihydroxy-9,10-anthraquinone)	The bioactive compound possessed significant antifungal potential toward different isolates of <i>Candida</i> sp.	[84]
<i>Thymus vulgaris</i>	Lamiaceae	Leaves	—	Thymol & Carvacrol	The compounds presented anti-inflammatory effects via stopping of inflammatory edema & leukocyte migration.	[89]
<i>Maranta arundinace</i>	Marantaceae	Rhizomatous tubers	Ethanol	Propanoic acid & 2-oxo- ethyl ester.	It is said that the compounds have the highest level of antioxidant activity.	[90]
<i>Vitis vinifera</i>	Vitaceae	Leaves	—	Pterostilbene	An active antifungal compound against <i>Candida albicans</i> . An antifungal mechanism acts on the pathogen Ras/cAMP pathway & downregulating genes expression involved in ergosterol biosynthesis.	[91]
<i>Dioscorea alata</i>	Dioscoreaceae	Underground tuber	Hydromethanol	Propanedioic acid & Bis (trimethylsilyl) ester.	It has strong immunomodulatory properties.	[92]
<i>Ipomoea batatas</i>	Convolvulaceae	—	—	Cyanidin acyl glucosides & peonidin acyl glucosides	An antioxidant activity and anti-inflammatory action was noticed in the extract which reduced the amount of cleaved IL-1 β & reducing liver cell inflammation by blocking NLRP3 inflammasome activation.	[15]
<i>Guapira graciliflora</i>	Nyctaginaceae	Leaves	Methanol	Pinitol, Kaempferol-3-O- β -D-galactopyranosyl-(6"à1"")-O- α -L-rhamnopyranoside & rutin	The compound of the plant extracts exhibited antifungal, antiproliferative & antibiofilm activities.	[65]
<i>Gauzuma ulmifolia</i>	Malvaceae	Seeds	Ethanol	2,6-Bis (3,4-methylenedioxyphenyl)-3,7-dioxabicyclo (3.3.0) octane	All the compounds managed oxidative stress & had antihypertensive properties.	[93]
<i>Zingiber officinale</i>	Zingiberaceae	Rhizome	—	6-gingerol, 10-gingerol, 6-shogaol & 10-shogaol	Compounds recognized to possess an antioxidant, anti-inflammatory, anti-obese, anti-nausea, anti-emetic, anti-diabetic, anticancer & antimicrobial activities, respectively.	[94]

Plant name	Family	Part taken	Extract name	Bioactive compounds	Therapeutic effect	Reference
<i>Plumbago zeylanica</i>	Plumbaginaceae	Leaves	Petroleum ether, Ethyl acetate, Methanol & Water	Heneicosane	Outstanding antifungal agent toward <i>Aspergillus fumigatus</i> .	[30]
<i>Rauwolfia vomitoria</i>	Apocynaceae	Leaves	Ethanol	Methenamine, Benzofuran & Methyl urinate	Compounds with anti-fungal, antioxidant, & anti-inflammatory properties.	[46]
<i>Coptis chinensis</i> & <i>Phellodendron chinense</i>	Ranunculaceae	—	—	Berberine	The compound showed potent inhibitory action toward <i>C. albicans</i> by upregulating the expression of core genes i.e., <i>sln1</i> , <i>ssk2</i> , <i>hog1</i> & <i>pbs</i> + induced ROS accumulation. It also stop the chitin synthase gene (<i>chs3</i>) & the β -(1,3) glucan synthase gene (<i>gsc1</i>) expressions to break cytoplasmic integrity & prevent germ tubes, hyphal formations which disturb cell wall integrity.	[95, 96]
<i>Podocarpus lambertii</i>	Podocarpaceae	Leaves	Methanol	Manool & Rimuene	Compounds reported significant antioxidant activity.	[97]
<i>Ipomoea batatas</i>	Convolvulaceae	Root, Leaves & Tubers	Aqueous, Ethanol & Methanol	Phenolic compounds, Anthocyanins, Flavonoids, Coumarins, Sterols, Pectin, Peptides & Glycoproteins	The compounds reported potent antitumor & antiproliferative properties	[98]
<i>Citrullus colocynthis</i>	Cucurbitaceae	Fruits	Methanol	Quercetin, Gallic acid, Vanillic acid, Chlorogenic acid, Syringic acid, Ferulic acid, Cinnamic acid & Sinapic acid.	Strong antioxidant, antidiabetic & haemolytic properties were exhibited by the compounds.	[99]

Table 6.
Efficacy of herbal compounds obtained from the medicinal herbs and their different anti-infectious properties.

actions by altering cell membrane structure, electron flow disruption and proton motive force, and cell coagulation [70]. Thus, medicinally important derived phytochemicals reduced fungal infection via cytotoxicity, reducing virulence, and synergistic activity [112].

4.2 Efficacy of medicinal herbs in delivering antifungal activity and for the minimum inhibitory concentration (MIC) toward human pathogens

Nowadays, fungal infections are huge threat to public health, causing illness and deadly infectious diseases. Mucosal surfaces and skin infections are one of the severe issues in hot and sub-hot countries [113]. Systemic toxicity and other health problems have been associated with synthetic antifungal medications. Developing efficient antifungal treatments could be competitive in treating candidiasis and other human-associated infections [114, 115]. Because drug-resistant pathogens are widespread and extremely severe, chemically sourced antifungal agents have proven to be less effective. Thus, WHO claims that plant extracts and their active components can be used as ancient folk therapies among 80% of global human population known to be dependent on it. Plant-derived chemicals such as phytoalexins, isothiocyanates, allicins, anthocyanins, essential oils, tannins, polyphenols, and terpenoids can be obtained from stem, root, leaf, bark, flower, fruit, and seed extracts [53, 116]. Therefore, in order to minimize the pathogenic infections particularly caused by bacteria and fungi, it is highly essential to screen and check for bioactive-based therapeutic properties. The solvent-based *Nandina domestica* extracts can be antagonistic towards *T. rubrum*, *T. mentagrophytes*, and *M. canis* [117]. In comparison to present artificial drugs, the bioactive compounds obtained from medicinal herbs could be highly antagonistic to pathogenic fungal growth. These statements justify previous reports wherein *Capparis decidua* methanol extract showed greater antifungal effect toward *Candida albicans* (1.33 mm to 43 mm) compared to fluconazole, which recorded only a 34 mm inhibition zone [118]. There are reports comparing antifungal effects with respect to plant-based antifungal drugs and synthetic drugs wherein plant-based medications were proven to have greater efficiency when used at the same concentration. In the *Veronica biloba* ethyl acetate extract, at 10 μ L, 12.3 mm of inhibitory zone was observed, bestowing anti-infectious action. The conventional drug, nystatin, showed a 6.7 mm inhibition zone when used at the same concentration [96]. Likewise, *Plantago lanceolata* and *Sida ovata* medicinal herb ethyl acetate extracts were linked with preventing infections caused by *C. albicans*, *M. furfur*, and *M. globose* showing 15 mm to 28.67 mm inhibition zones, parallel to ketoconazole used [119]. Thus, it has been authenticated from the reports that medicinal herbs serve to upkeep the number of beneficial microbes through symbiotic relationships in delivering antifungal protection [120]. The bioactive compounds can inhibit fungi and show gene variation in pathogens under different environmental pressures [121]. The antifungal protection delivered by the plant-based chemicals is highly specific to the family they belong to, phytochemical occurrence, concentration used, and solvent type used for extraction [29]. One of the mechanisms behind delivering antifungal efficiency could be due to the presence of active chemical compounds disrupting pathogenic cell membrane [68]. Synthetic therapies are poorly preferred and develop drug choice pressure among scientists as pathogens are gaining antibiotic resistance [122]. Therefore, it is advisable to manage antibiotics specifically during true infections, and they should be administered at proper doses mainly to medicinal success.

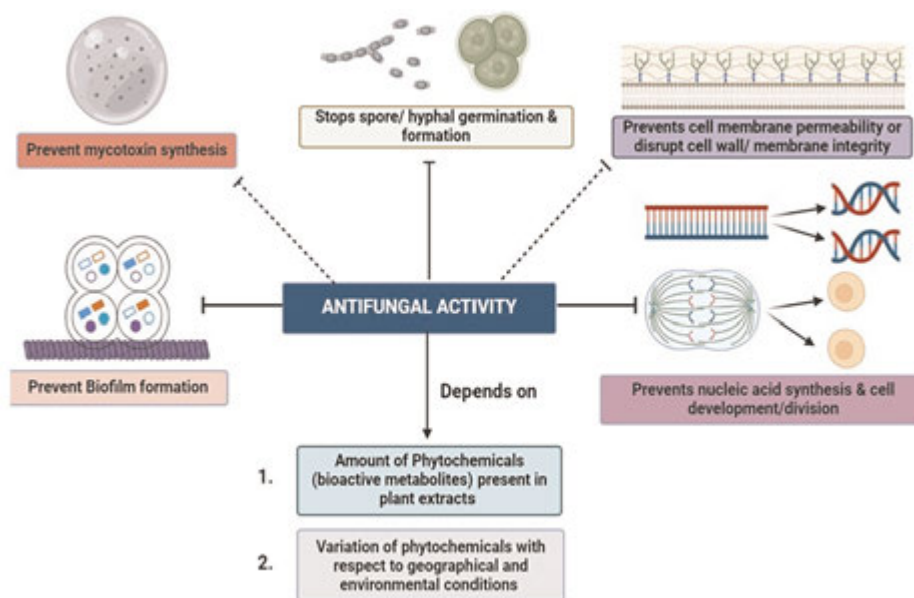


Figure 2.

A general mechanism of the antifungal activity mediated by phytochemicals and/or metabolites derived from plants.

The minimum inhibitory concentration (MIC) or minimum fungicidal concentration (MFC) is the least drug concentration that decreases pathogen colony factor unit (CFU) to a definite value [123]. Ethanol and petroleum ether leaf extracts of *Lawsonia inermis* recorded significant MIC values against *T. mentagrophytes*, *T. verrucosum*, and *T. violaceum* [39]. *Pelargonium zonale* stalks bestowed antifungal properties against *Candida albicans* [124]. For instance, after 48 h of treatment with a methanolic extract of *Glycyrrhiza glabra* at a dose of 62.5 mg/mL, cell deterioration and cytoplasmic damage were observed in pathogenic fungal cells. However, untreated fungal cells were observed to be spherical in nature with smooth surfaces [17]. Hence, MIC is the most preferred method used to detect active compounds constraining bacterial and fungal growth in an antibiotic-free media. It can be used in detection, confirmation, and categorization of any pathogenic isolate to be resistant, and in antifungal drug development [56]. The general mechanism of antifungal action mediated by the phytochemicals has been depicted in **Figure 2**. Overall, medicinal plants serve to be the most efficient source of antifungal protection toward various human pathogenic fungi.

4.3 Antioxidant potential of medicinal herbs and their function in reducing pathogenic fungal growth

The medicinal plants produce secondary metabolites in the leaves and fruits. The plant metabolites are the chemicals that are known to impart defense toward diverse pathogens, thereby improving resistance. Plants' herbal products have anticarcinogenic, anti-inflammatory, anti-atherosclerotic, antibacterial, antifungal, antiviral, antimutagenic, and antiallergic properties [125]. In general, an antioxidant is any material or substance that delays oxidative damage of a target molecule. Free radicals primarily oxidize nucleic acids, proteins, and lipids and cause coronary heart

disease and cancer. The phytochemical compounds of medicinal herbs possess anti-oxidant activity that plays a crucial role in activating antifungal, anti-diabetic and anti-inflammatory activities [126–131]. Phenolic acids, polyphenols, and flavonoids possess antioxidant potential known to scavenge free radicals, H_2O_2 , peroxide/lipid peroxy, thereby inhibiting the oxidative pathways [31]. DPPH (2,2-diphenyl-1-picrylhydrazyl) is one of the most rapid, sensitive, and methods to assess the antioxidant potential of plant extract. However, a reducing power assay is used to measure the reductive capability of antioxidants present in the plant extracts. The reports documented that the methanol extracts of *X. kraussiana* possess the highest antioxidant capacity and are a potent radical scavenger based on the solvents' polarity [132]. When an extract donates a hydrogen atom, DPPH may lose its free radical characteristics and change color from purple to yellow, confirming DPPH activity [32]. The findings showed *Citrullus colocynthis* seed extracts had a prevention effect by reducing DPPH radicals [i.e., IC₅₀ values of 500, 580, and 350 $\mu\text{g/mL}$] in aqueous, hydro-methanolic, and ethyl acetate extracts [34]. In *Flacourtia indica* methanolic root extract, at 100 $\mu\text{g/mL}$, over 92% of the DPPH radical scavenging activity with an IC₅₀ value of 42.73 $\mu\text{g/mL}$ was found. Furthermore, strong antioxidant and reducing power assays were checked in a dose-dependent manner [89]. Primarily, the study claims that gallic acid is chiefly accountable for the reduction ability apart from the presence of flavonoids and phenolic acids. A study evidenced that shifting in the sample color from yellow to different green and blue shades is mostly dependent on active compound concentration. The antioxidant activity is chiefly due to the redox nature of phenolic compounds. Also, Fe^{3+} to Fe^{2+} transformations could be one of the reasons for strong reducing power primarily to the occurrence of phenolic compounds having hydrogen donating capacity [133]. Hydrogen peroxide (H_2O_2) and nitric oxide (NO) assays were generally performed to test antioxidant capacity in medicinal plants as they can directly inactivate enzymes by oxidizing thiol groups and damaging cell membranes. NO has been linked to inflammation, cancer, and other pathological disorders. Under aerobic environments, NO is an extremely unstable species because the plant biometabolites possess the tendency to offset NO synthesis and are very useful in reducing negative consequences of abundant NO generation in the human body [90]. The methanolic extracts of *Acacia hockii* reported higher hydroxyl radical scavenging activities (81.52% inhibition) when compared to extracts of *Vernonia lasiopus* (73.93% inhibition) and *Caesalpinia volkensii* (66.99% inhibition) [134]. According to [135], the methanol extract of *Torilis leptophylla* had substantial EC₅₀ values, namely, 41 $\mu\text{g/mL}$ (DPPH), 10 $\mu\text{g/mL}$ (ABTS), 10.7 $\mu\text{g/mL}$ (phosphomolybdate), and 8 $\mu\text{g/mL}$ (TLC) in numerous tests examined. Nonetheless, ethyl acetate extract was found to have comparatively reduced levels of superoxide radicals (57 $\mu\text{g/mL}$) and H_2O_2 radicals (68 $\mu\text{g/mL}$), indicating potential antioxidant properties of *Torilis leptophylla*. It was revealed that methanolic extracts tend to stop lipid oxidation. Also, it was evidenced that the presence of certain compounds, such as gallic acid, 1,2,3,4,6-pentagalloyl glucose, 5-O-galloyl, 3,5-di-O-galloyl, and 3,4,5-tri-O-galloyl, was strongly responsible for antioxidant action [136, 137]. The *Artemisia herba alba*, *Ruta chalapensis* and *Peganum harmala* provided anti-inflammatory and anticancer activities [138]. As per the investigations, it was reported that antioxidant and anti-inflammatory properties have been found in significant concentrations in an essential oil of *P. lentiscus* [88]. The aqueous extract of Propolis contains polyphenols which are responsible to possess antioxidant property [139]. Moreover, an antioxidant effect of polyphenols was mostly attributed to their ability to decrease oxides and permit polyphenols to act as singlet oxygen suppressors, reducing agents, and

hydrogen donors [140]. Several rice cultivars were measured; the Attakkar variety exhibited the greatest total antioxidant capacity (TAC), measuring $0.70 \text{ mmol } 100 \text{ g}^{-1}$ FW. ferric reducing power Assay (FRAP) activity of 0.561 to $0.695 \text{ mmol}/100 \text{ g}$ fresh weight (FW) DPPH (26.07 to 53.66 mg/mL FW) and reducing power (33.49 to 40.81 mg/mL), respectively [16]. Root extracts of *Flacourtia indica* and ascorbic acid (standard) both exhibited capable NO scavenging action at $200 \text{ }\mu\text{g/mL}$ concentration, suppressing more than 57% and 91% with IC₅₀ values of $172.41 \text{ }\mu\text{g/mL}$ and $95.56 \text{ }\mu\text{g/mL}$ [89]. Ethanol extracts of *Arthrocnemum indicum* recorded the uppermost amounts of total polyphenol (303.67 mg gallic acid equivalent (GAE)/g) and flavonoid (55.33 mg CE/g) contents compared to acetone and hexane extracts. The strong DPPH scavenging ability of ethanolic extract (IC₅₀ = $7.17 \text{ }\mu\text{g/mL}$) does not significantly differ from the standard used (IC₅₀ = $0.70 \text{ }\mu\text{g/mL}$), which thus demonstrates the extract's strong antioxidant activity [141, 142]. According to many research studies, apart from providing protection toward type-2 diabetes and reactive oxygen species (ROS) generation, the antioxidant activities shown by medicinal herbs have a strong relation in delivering antifungal action toward many human pathogenic fungi and also operates as protective shields against many diseases [16, 105, 142–144]. The importance of medicinal herbs with potent antioxidant activities has been shown in the **Table 5**.

4.4 Characterization and purification of medicinal herbs derived bioactive metabolites

From the herbal plants huge source of bioactive compounds possessing therapeutic and nutraceutical importance were isolated and characterized [145]. Target-directed isolation of active molecules on a chromatogram is possible through the use of bioautography. The growth inhibition of microorganisms has been traditionally tested using the bioautographic technique chiefly to identify the antimicrobial compounds present in the extracts chromatographed on a thin-layer chromatography (TLC) layer [146]. TLC is one of the analytical techniques that plays a dominant and significant role in offering quality control of herbal medicines and biologically derived food and cosmetic products [56]. TLC can be utilized to maintain the compound's identity and purity by comparing the retardation (R_f) value of an unknown compound with the R_f value of an identified compound. Further tests include viewing the plate under a UV light source or spraying phytochemical screening reagents, which alter the color according to the phytochemical's concentration present in an extract [147]. The bioactive compound Indolidizine obtained from *Prosopis glandulosa* has anti-infective and antiparasitic property [148]. Bioautography on the TLC plates wherein *Myrcia splendens* leaf extracts revealed the presence of an antioxidant compounds, which was evidenced by the white bands when tested with DPPH solution [149]. It is obvious that from *Plumbago zeylanica* extract [ethylacetate/distilled water at 19:1 (v/v) ratio], ten different compounds were eluted using TLC, among which the fifth compound recorded more distinguished R_f value followed by the third compound [51]. TLC performed in medicinal plants extracts such as *J. schimperiana*, *C. macrostachyus*, *A. gumifera*, *Clematis hirsuta*, *T. dregaeha*, *C. abyssinicum*, and *T. rhynchocarpum* showed distinct spots of flavonoids, alkaloids, and phenols with different R_f values. The presence of these compounds has been correlated with strong antifungal and antibacterial medications [75]. Liquid chromatography–mass spectrometry (LCMS) and gas chromatography mass spectroscopy (GCMS) are novel and effective methods for identifying the bioactive compounds that are unknown in plant extracts by

utilizing high performance liquid chromatography (HPLC)'s effective separation capabilities and mass spectrometry's precise structural characterization [55, 150]. In LCMS, the most effective interface is electro-spray ionization (ESI). The following bioactive compounds, namely, β -caryophyllene, caryophyllene oxide, α -humulene, linalool, linalooloxide (furanoid), bornyl acetate, camphor, α -thujone, β -thujone, 1,8-cineole, α -pinene p-cymene, and eugenol, were characterized and purified in the *A. absinthium* and *Artemisia vulgaris* extracts. These compounds showed antifungal protection effect toward *C. albicans* and *Aspergillus niger* [151]. The following bioactive compounds have been characterized using GCMS in *Centella asiatica* methanol extracts include cis-vaccenic acid (91%), tetradecanoic acid (86%), 5-hydroxymethylfurfural (88%), and n-hexadecanoic acid (99%) that were found to be have significant antifungal activity toward *C. albicans* and *A. niger* [152]. HPLC is an applied technique used to detect bioactive constituents. It permits methodical outlining of complex plant samples and focuses chiefly on the identification and purification of compounds having respectable retention time. However, HPLC could only provide a limited amount of structural, spectrum, and peak information by comparing the sample with reference/standard compounds [55, 153]. HPLC analysis of *A. indicum* shoot extracts reported 19 and 18 different phenolic and flavonoid compounds, respectively, including trans-ferulic acid, *p*-coumaric acid, rutin, cirsiolol, 4,5-di-O-caffeoylquinic acid, hyperoside (quercetin3-O-galactoside), protocathechuic acid, acacetin, and cirsiolol having antioxidant, antimicrobial and antidiabetic properties [142]. Interestingly, *C. auris* growth was inhibited at very low concentration of plant extract used. The combination treatments i.e., plant based carvacrol were combined with synthetic fluconazole, amphotericin B, nystatin and caspofungin were found to be promising in delivering anti-infectious activity. Interestingly, drugs showed synergistic and additive effects showing 68%, 64%, 96% and 28% inhibition, respectively [154]. Different polyphenol compounds, namely flavan-3-ol, gallotannin, ulopterol, osthenol, colchiside, punicalagin, lambertianin, ellagic acid, and gallagic acid, were purified and characterized to be used in anti-infectious therapy [155]. Nuclear magnetic resonance (NMR) spectroscopy is a potent and quick tool for both navigating the target isolation process and identifying bioactive components. NMR spectroscopy played an important role in the isolation and identification of siderol obtained from *Sideritis euboea*, which has proven to be a remarkable cytotoxic and anticancerous effect [86, 156]. Salicylic acid, a bioactive compound, was isolated from *Cleome viscosa* (seeds) and characterized using mass spectroscopy, infrared (IR) spectroscopy, and ¹H and ¹³C NMR spectroscopy [157]. Upon IR and NMR spectrometry analyses, in *A. racemosus* and *C. occidentali* extracts, two most important bioactive compounds, namely, saponin and hydroxy anthraquinone, have been reported [158]. Also, the plant extracts showed minimal to no hemolytic activity, which makes them excellent for use in alternative antifungal therapies [159]. In addition, there have also been studies that highlight the synergistic use of medicinal plant extracts in conjunction with synthetic antifungal drugs that have reduced pathogen toxicity and enhanced antifungal properties [27, 160, 161].

4.5 The role of medicinal plant-based bioactive metabolites and how they work

Scientists from all over the world has been investigating natural therapeutic compounds in the last few decades. The foundation for finding novel therapeutic compounds is laid by ethnomedical surveys of plants. Over time, substantial research has been conducted to support the application of herbs in the field of pharmacology [48].

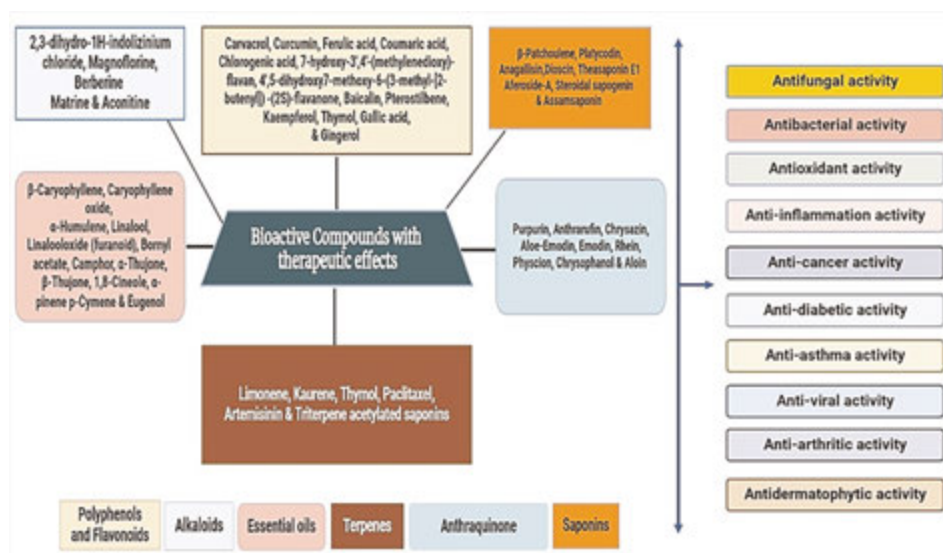


Figure 3.

An outline of the bioactive compounds types found in medicinal plants and their therapeutic uses.

Plant secondary metabolites and bioactive compounds are the other names for phytobioactives that are chiefly present in medicinal herbs and are not required for basic breakdown processes but yet are considered to be the essential elements in providing anti-infectious protection to human health [162]. Also, secondary metabolites, namely, essential oils, phenols, flavonoids, alkaloids, saponins, terpenoids, and anthraquinones (**Figure 3**) have remarkable potential as medical treatments including against SARS-CoV-2 protease [163]. It was observed that the antioxidant property is associated with anti-fungal activity [105].

Plant polyphenols (present in vegetables, fruits, and seeds) are naturally occurring secondary metabolites produced from L-phenylalanine via the phenylpropanoid pathway. They are distinguished by the presence of one or more phenolic groups with a wide variety of structures. The main structural types of polyphenols are flavonoids, saponins, steroids, phenolic acids, lignans, glycosides, stilbenes, tannins, and coumarins [164]. Polyphenols directly or indirectly play a vital role in antifungal, antioxidant, and many other biological activities. For example, quercetin is a flavonoid found in many medicinal plants, particularly apples and onions. It has potent antioxidant properties because it inhibits lipid peroxidation and neutralizes ROS. Additional polyphenols, like curcumin (turmeric) and resveratrol (grapes), also have strong antioxidant qualities and support the health advantages of polyphenols [165, 166]. In addition, coumaric acid has a stronger antioxidant, antifungal potential to treat the symptoms of hyperlipidemia and inhibit cells that cause cancer [142]. Previous research on the antioxidant activity of ferulic acid is mainly related to the stabilization of resonance and the elimination of free radicals. Ferulic acid is reported to be a potent anticancerous, neuroprotective agent and has a vital role in cardiovascular and kidney structure improvement [167].

Antioxidant polyphenols are becoming more crucial in the prevention and handling of different diseases, especially concerned with oxidative stress, cancer, cardiovascular disease, inflammation, neurodegenerative diseases, and age-related degenerative diseases [168]. Research has shown that the antifungal properties of

thymol and carvacrol derived from the Lamiaceae plant members disrupt the integrity of *C. albicans* cell membrane and stop ergosterol synthesis, causing pathogen cell death [169]. Curcumin present in a medicinal herb (*Curcuma longa*) displayed anti-inflammatory, anticarcinogenic, and antioxidant properties by reducing oxidative damage and preventing myocardial apoptosis (JAK2/STAT3 signal pathway) [170]. Baicalin (a flavon glycoside) stops accumulation of *C. albicans* cells by upregulating the CAP1, SOD2, and TRR1 redox related genes, which increase Ca^{2+} amount in the cytosol, causing cell structure disruption that lead to apoptosis [87]. Isoflavonol glycosides [genistein 7-a-L-6-deoxytalopyranoside (talosin-A) and genistein 4,7-di-a-L-6-deoxy-talopyranoside (talosin-B)] present in *Kitasatospora kifunensis* demonstrated potent antifungal properties toward different fungi such as *Aspergillus niger*, *C. albicans*, and *Cryptococcus neoformans* with MIC values ranging from 3 to 15 mg/mL [44, 171]. An antioxidant effect of gingerol on human intestinal epithelial cells is due to inhibition of Keap1-Nrf2 protease, facilitating Nrf2's transport to the nucleus, raising Nrf2 target genes expression and reducing glutathione levels, thereby lowering ROS concentrations. Additionally, gingerol decreases prostaglandin (E2) and NO production [161, 172, 173]. Extractions of *Morus alba* containing quercetin recorded more prominent inhibitory effect toward *Aspergillus fumigatus*. Quercetin-treated mice pointedly lower fungal load in the corneal tissue in comparison to control [79].

Nitrogenous secondary metabolites, particularly cyanogenic glycosides (CNG), contain α -hydroxynitrile composed of an aglycone component with a sugar moiety attached. CNGs are extremely toxic substances because they hydrolyze and release hydrogen cyanide that cause acute cyanide poisoning. Other metabolites include alkaloids and nonprotein amino acids. Nonprotein amino acids, which are significant nitrogen stores in plants, have antifungal, antibacterial, and anticancer properties aiding plants' resistance power [71]. Alkaloids are organic bases with heterocyclic nitrogen rings and have specific pharmacological properties. An indoleazine alkaloid (2,3-dihydro-1H-indolizinium chloride) extracted from *Dryopteris enneaphylla* (a fern) had half-maximal inhibitory concentrations with IC₅₀ values 0.3 μ g/mL and 4.0 μ g/mL against *A. fumigatus* and *C. neoformans*, respectively [174]. Additionally, magnoflorine can harm the cell membrane of *T. rubrum* causing nucleic acid leakage from fungal cells, decreasing squalene epoxidase, 14- α -lanosterol demethylase activities and lowering ergosterol amounts in a pathogen's hyphae [175]. Berberine (an isoquinoline alkaloid) present in chinese medicinal herbs recorded anti-neurodegenerative agency by preventing the most significant pathogenic enzymes from activating, inducing autophagy, reducing intracellular oxidative stress neuroinflammation and shielding neurons from apoptotic cell death [95, 176]. An alkaloid named matrine is reported to reduce inflammation via stopping NF- κ B signaling mechanism in mouse airway epithelial cells, decreasing suppressors of cytokine signaling expression, ROS expression and other inflammation factors [177]. The digestion of these nonprotein amino acids and alkaloids is complex and is affected by plant hormones and stress conditions [161]. Therefore, from the reports, it is clear that nitrogenous compounds possess antifungal, antiviral, antibacterial, cytotoxicity, carcinogenic, or mutagenic properties [42, 71, 176].

Terpenoids (isoprenoid compounds) are synthesized in plastids via the deoxyxylulose-5-phosphate pathway and in the cytoplasm through the mevalonate pathway. It is known that over 80,000 different medicinal herbs contain terpenoids. As per the report, kaurene, which was separated from Asteraceae family members, exhibits strong anti-cluster induction activity against *Candida albicans* [178]. In the meantime,

limonene can cause *C. albicans* to undergo apoptosis by causing damage to DNA, oxidative and nucleolar stress, fungal cell wall/membrane destruction, and metacaspase-dependent pathway [179, 180]. Two of the most well-known medications are terpene-based, namely, artemisinin (an antimalarial), and paclitaxel, (an anticancer drug) obtained from *Ficus benjamina* var. *comosa* [78]. There have been reports of a broad range of biological activities exhibited by terpenes including antifungal, analgesic, food preservative, antibacterial, anticancer, and anti-inflammatory properties [181].

Saponins are surface active compounds having five- or four-ring triterpenes with cardioprotective potential and can be involved in calcium homeostasis, regulation of energy metabolism, inflammation stopping, and oxidative stress. An anti-inflammatory property of saponins obtained from the soybean is due to prevention of NF- κ B (IB) inhibitor degradation and NF- κ B activation, which reduced cyclooxygenase-2, nitric oxide synthase, human macrophage chemoattractant protein-1 (MCP-1), and TNF in Lipopolysaccharide (LPS)-stimulated cells production [43]. β -patchoulene reduced sepsis-induced neuroinflammation and microglia activation while improving peripheral immune function [61]. Another saponin found in *Platycodon grandiflorus* is platycodin D, which has antioxidant properties as well as anti-inflammatory and anti-atherosclerotic properties [73]. Likewise, anagallisin reported to be a potent drug toward the infection caused by *C. albicans* [182]. Dioscin and atheroside-A recorded significant fungicidal activity, causing significant changes in the appearance and accumulation of fungal (*C. albicans*) cells [69]. Theasaponin E1 and assamsaponin extracted from the seeds of *Camellia sinensis* displayed antifungal activity toward the resistant *C. albicans* strains. The antifungal efficacy is linked with increased permeability and disruption of cell membrane structure of *C. albicans* by targeting membrane-bound sterols [183]. Steroidal saponins are most commonly recognized as precursors for steroidal hormone synthesis. The traditional belief that saponins are poor drug candidates and were generally thought to have surfactant properties similar to detergents has been contested in recent years. As per [119], six steroidal sapogenins isolated from *Polygonatum zanlanscianense*, *Agave americana*, *Polianthes tuberosa*, and *Dioscorea parviflora* reported antifungal activity toward opportunistic pathogens, namely, *C. albicans*, *C. krusei*, *Candida glabrata*, *Cryptococcus neoformans*, and *A. fumigatus*. The inhibitory effect was connected with the presence of aglycone moieties and the number and structure of monosaccharide units in sugar chains. A great deal of patents regarding the medicinal uses of steroidal saponins obtained from *Tribulus terrestris* have been reported, particularly treating fungal infections and hepatitis [184].

Anthraquinones are aromatic compounds composed of a 9,10-dioxoanthracene core substituted with benzene rings having phenolic, OH, and aliphatic groups. Emodin (an anthraquinone) unveiled antifungal effect toward *Candida albicans*. *Rheum emodi* rhizomes contain rhein, physcion, aloe-emodin, and chrysophanol, which have documented potent inhibitory effect toward *C. albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, and *Aspergillus fumigatus* (25–250 μ g/mL MIC values) [49]. High levels of anthraquinone aglycones found in *Senna alata* leaves and *C. fistula* pod pulp were found to be antagonistic against various clinical strains of dermatophytes. The antagonistic mechanism of anthraquinones is due to their ability to disrupt the 1,3- β -D-glucans present in the pathogenic fungal cell walls, thereby stopping (1,3)- β -D-glucan synthase activity and finally leading to death of the pathogenic fungi [185]. Purpurin obtained from the madder root tends to strongly antagonize *C. albicans*, and the potential connection in delivering antifungal effect is due to the ability of purpurin in depolarizing mitochondrial membrane, which checks cell death in eukaryotic cells [43].

Apart from the secondary metabolites mentioned previously, essential oils have drawn attention toward antifungal drug development. Essential oils are volatile compounds that are aliphatic and aromatic in nature [45, 186]. *Rhododendron tomentosum* oil not only inhibits *Candida parapsilosis* but also has antifungal properties that influence *Saccharomyces cerevisiae* cell membrane permeability [84]. *A. niger* and both azole-sensitive and azole-resistant *Candida albicans* were susceptible to the antifungal effects of *Melaleuca alternifolia* essential oil, which is primarily pinene-4-alcohol [187]. In addition, *Bucheurum rigidum* essential oil can also have antifungal effects by altering the structure of *Candida albicans*, *Candida neoformans*, and *Candida rubrum* [183]. *In vitro* growth of *Candida albicans* was completely halted by 0.25 mg/mL of oregano oil. Furthermore, it exhibited concentration-dependent inhibition of both mycelium and spore germination [24]. Reports have been shown that essential oils possess potent antifungal effects toward *Candida* and *Aspergillus* spp., *Microsporum canis*, *M. gypseum*, *T. rubrum*, and *T. mentagrophytes* [161, 188]. The primary mechanism through which essential oils inhibit fungal cell growth and mycotoxin synthesis is by disrupting intracellular electron transport chains and cell membrane permeability that led to intracellular metabolic disorders [49]. Research has found that rosemary (*Rosmarinus officinalis*) essential oil specifically targets ergosterol synthesis in order to halt *A. flavus* toxin formation [189, 190]. *Melaleuca alternifolia* essential oil shows synergistic antifungal activity along with itraconazole or ketoconazole toward *Trichophyton rubrum* [135]. For instance, *C. glabrata* metabolic activity was greatly decreased when oregano and winter savory essential oils were combined with the synthetic antifungal medication clotrimazole and cause organellar disorder at low and high concentrations, respectively [14, 191]. An antifungal mechanism of the important bioactive metabolites is denoted in **Figure 4**. Therefore, from the foregoing

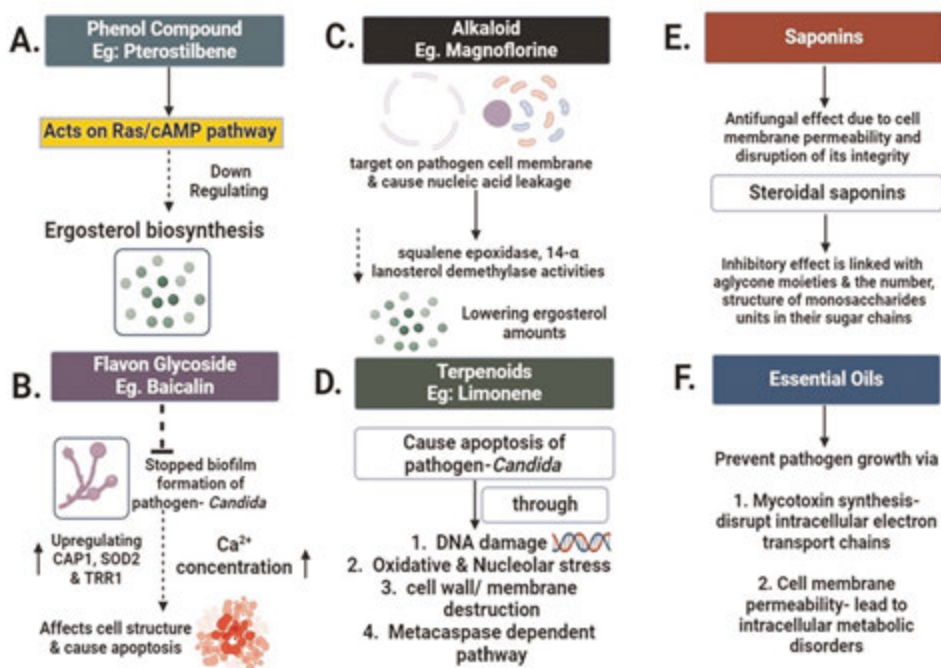


Figure 4.
A schematic depiction of antifungal mechanism mediated by major bioactive metabolites.

reports, it is very clear that pure active compounds were found to be dynamic toward diverse therapeutics from antifungal to anticancerous and anti-inflammatory.

5. Future scenarios

Given the critical role that medicinal herbs play a pivotal role in improving the health of the world's population. More research studies should be performed to use them as synthetic drug substitutes for reduction of diseases caused by fungal infections in humans. In the near future, India and other developing nations should continue to see a demand for medicinal and aromatic plant-derived drugs. Utilizing the distinctive Indigenous knowledge of medicinal herbs holds great promise for developing affordable solutions and screening natural products for drug development, healing fungal infections, inflammations, cancer, and other human ailments. As the host plant produces a variety of biologically active compounds, the microbes linked with medicinal herbs also produce these compounds, so a more thorough analysis of microbiome properties as a possible source of novel antimicrobials could be crucial. Furthermore, the studies will aid in clarifying the pathways and mechanisms of newly discovered bioactive substances derived from medicinal plants to create novel anti-fungal drugs. Moreover, research into plant tissue culture is warranted as it presents a promising substitute for synthesis of medicinally significant plant-based anti-infectious compounds.

6. Conclusions

This article briefs about the current status of clinical antifungal treatments for common superficial or deep fungal infections, and host-immunomodulatory mechanisms of bioactive metabolites. Medicinal herbs are rich in phytochemicals (alkaloids, phenol, flavonoids, anthraquinones, saponins, and so on), which play a reflective role in preventing and reducing the growth of human pathogenic fungi. The antifungal activity delivered by plant chemicals is highly specific, and it is mainly dependent on the type and family of plant species, the content of plant chemicals, and also environmental conditions. The different antioxidant and anti-inflammatory activities shown by bioactive metabolites are associated in delivering the antifungal protection from noxious pathogens. The article throws light on the list of the most important and deadliest diseases that have reason to be treated via plant-based drugs rather than the direct use of synthetic drugs. However, there are also reports suggesting the combined use of plant bioactive metabolites and synthetic drugs at appropriate doses. The article presents a detailed explanation of the antifungal medication types and their targets delivered by plant-based bioactive metabolites. The different mechanisms through which bioactive metabolites of medicinal herbs deliver antifungal effects include (1) prevention of fungal mycotoxin synthesis; (2) inhibition of fungal biofilms formation and the destruction of existing ones; (3) reduction in fungal spore count and hyphae growth; (4) inhibition of ergosterol synthesis, disruption of cell membrane permeability, and encouragement of cell wall lysis and destruction; and (5) modification of cellular DNA replication and disruption of the cell cycle. Additional role of secondary metabolites of plants are also mentioned in the article, namely, they are used in the treatment of cancer, arthritis, dermatophytic infections, diabetes, and other metabolic disorders. Consequently, there are many opportunities for the development of novel

antifungal medications using plant secondary metabolites. The problem associated with this type of research is that the use of plant secondary metabolites in antifungal and immunomodulatory medications is still in its early stages. Clinical usage of these drugs is still a long way off, and clinical references for these drugs are extremely difficult to find. There is much uncertainty regarding the best techniques for obtaining plant secondary metabolites that are broad spectrum and antifungal in nature. How these metabolites affect the immune system of the host, and whether or not they can be used as antifungal drugs in clinical settings, need to be studied in a precise manner. Every one of these issues needs to be addressed in the immediate future.

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Authors contributions

The first author and corresponding author, B. Naziya (B.N.), came up with the concept, gathered data, and contributed in writing, formal analysis, editing, and software design of the manuscript. The second author, H. Sakander (H.S.), visited medicinal gardens, gathered pictures of medicinal plants, collected information from chapters and articles, and helped in the reference formatting of the manuscript.

Declaration of conflicting interest

B. Naziya and H. Sakander have no conflicts of interest.

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Impact of Aqueous Leaf Extract of *Punica granatum* and Synthesized Silver Nanoparticles against Streptozotocin Induced Diabetes in Rats

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Abstract

Punica granatum leaves are known for various therapeutic properties, but lack proper documentation. The present study was undertaken to study the antidiabetic impact of aqueous leaf extract of *Punica granatum* and silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum* against streptozotocin induced diabetes in rats. Aqueous leaf extract of *Punica granatum* was prepared, phytochemical screening of the extract revealed the presence of various phytochemicals—alkaloid, tannin, saponin, total phenol and flavonoids. The aqueous leaf extract was employed to synthesize silver nanoparticles. Synthesized silver particles were characterized using different techniques such as UV-visible spectrophotometer (UV-Vis), Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM), dynamic light scattering analysis (DLS), and zeta potential analysis (ZP). The characterization revealed the nanoscale (size ≤ 100 nm) of the synthesized silver particles, thus called as nanoparticles. Both the aqueous leaf extract and the synthesized nanoparticles were administered in streptozotocin-induced-diabetic rats to assess their antidiabetic effect. The results revealed that both the aqueous leaf extract of *Punica granatum* and the synthesized silver nanoparticles had significant antidiabetic activity against streptozotocin induced diabetes in rats. It was further found that the silver nanoparticles had enhanced antidiabetic activity as compared to the extract alone.

Keywords: streptozotocin, glibenclamide, *Punica granatum*, silver, green nanoparticles, diabetes, medicinal impact

1. Introduction

Plants have considerable nutritional value and have been a primary source of medicines throughout human history [1, 2]. During the past 30 years, up to 50% of all medications have been derived directly or indirectly from plant sources. 35,000–70,000 plant species have been tested for therapeutic purpose so far. Plants,

particularly those having ethnopharmacological use, served as the major source of medicine for early drug development [3].

Herbs have traditionally been a key component in pharmaceutical preparations. As individuals work to maintain their health, herbal medications are growing in popularity throughout emerging nations. Large flora, however, are still awaiting research to confirm their therapeutic effects [4, 5]. The treatment of many ailments involves the use of various plant components, including leaves, bark, fruits, roots, and seeds. Plants can also provide natural antimicrobials [5].

Nettle, dandelion, chickweed, neem and Tulsi are just a few examples of plants that are frequently found surrounding human settlements and are utilized as herbal remedies [6]. In addition to sheep and other animals, non-human primates, monarch butterflies, and other creatures also use plant parts as medicines when they are unwell [7]. Plant samples found in ancient burial sites are one form of evidence suggesting that Paleolithic humans were familiar with herbal medicine. For instance, Shanidar IV, a 60,000-year-old Neanderthal burial site in northern Iraq has produced significant amounts of pollen from eight plant species, seven of which are currently utilized as herbal treatments [8]. The development of new paths for herbal drugs might result from the intersection of rich information from several traditional medical systems. Ayurvedic medicine, as described in the Atharva Veda, the Rig Veda, and the Sushruta Samhita, has been practiced for thousands of years and employs hundreds of pharmacologically active herbs and spices, including curcumin-containing turmeric. One of the oldest and most prevalent traditional medicinal systems is ayurveda. The history of this conventional medical system is still not fully explored [9, 10]. On the clay tablets found in ancient Sumeria, hundreds of medicinal herbs, including myrrh and opium, are recorded. More than 800 plant remedies, including aloe, cannabis, castor bean, garlic, juniper, and mandrake, are listed in the ancient Egyptian Ebers Papyrus [7].

Punica granatum (Pomegranate) is a small tree which measures between five and eight meters tall and mainly found in Iran, the Himalayas and Northern India [11], China, USA and throughout the Mediterranean region. All the plant parts of *Punica granatum* such as seed, juice, peel, leaf, flower, bark, and root possess interesting pharmacological and toxicological activities [12].

The pomegranate tree is a long-lived tree that may survive for more than 200 years. The leaves are glossy, and the flowers are red, white, big, or variegated, with tubular calyxes that eventually turn into fruit. The pomegranate fruit has a grenade form, a deep red, leathery skin, and a crown-shaped calyx. A tiny quantity of sour and crimson liquid surrounds the seeds, which are separated by a white membrane pericarp [13].

Pomegranate has been consumed by people from all over the world from ancient times (4000–3000 BC). Pomegranate blossoms are associated with life, permanence, well-being, femaleness, fertility, wisdom, immortality, and purity [14]. It has also been utilized as folk medicine in numerous cultures, dating back to at least 1550 BC in the Egyptian Eber Papyrus [15]. According to recent study, pomegranate contains chemical components such as tannins, alkaloids, organic acids, flavonoids. The chemical components of pomegranate vary depending on the section of the fruit, seed, leaf, or peel [16]. Numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, with a focus on the treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections, antibiotic resistance, and UV radiation-induced skin damage. Infant brain ischemia, male infertility, Alzheimer's illness, arthritis, and obesity are other possible uses [13].

Nanotechnology entered the picture during the past vicennial, when research into the therapeutic benefits of plant extracts was at its zenith. Nanotechnology is a science, engineering and technology that deals with particles at the nanoscale, or at or below 100 nm. On December 29, 1959, physicist Richard Feynman gave a discussion titled “There’s plenty of room at the bottom” at a conference of the American Physical Society at the California Institute of Technology. It was a long time before the word “Nanotechnology” was coined. His presentation focused on how scientists would be able to manage and control individual atoms and molecules. Professor Norio Taniguchi created the term nanotechnology later in his research [17, 18]. The notion of synthesis of metallic nanoparticles mediated by plant extracts obtained from plant components such as leaves, roots, fruit peels, flowers, and whole plant, etc., utilizing polar or non-polar extraction medium began to gain momentum in the previous decade [19–22]. This concept gained traction and was widely investigated by workers. Several metals, including silver, zinc, copper, iron, and others, can be used to create plant-mediated nanoparticles. The synthesized nanoparticles must then be characterized for properties such as size, shape, and stability using a variety of techniques such as scanning electron microscopy (SEM), ultraviolet visible spectrophotometer (UV-Vis), Fourier transform infrared spectroscopy (FT-IR), dynamic light scattering analysis (DLS), and zeta potential analysis [23, 24]. Many workers have reported that the silver nanoparticles synthesized using plant extracts have enhanced alleviating effect as compared to extract alone on various parameters of mammalian model in laboratory conditions [25–28].

Considering the above apprehensions, this work was undertaken to synthesize, and characterize silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum* (AgPg_{aq}), and assessing its medicinal impact on mammalian model.

2. Methodology

2.1 Plant materials

Punica granatum fresh tender leaves were obtained in the Ranchi area of India’s Jharkhand state. The plant leaves were washed with deionized water, disinfected for 5 minutes with a 0.1% HgCl₂ solution, dried in the shade for 15 days, and ground to a fine powder [29]. 50 g of sieved powder was subject to extraction using Soxhlet device with 350 ml distilled water. The extraction was carried out without interruption for 4 days. The obtained extract was filtered and concentrated using a rotary flash evaporator set at 45°C. The pure extract was kept at room temperature in airtight containers for further studies [30].

2.2 Preliminary phytochemical screening

Preliminary phytochemical screening assays were performed on *Punica granatum* aqueous leaf PG_{aq} extract using previously published standards [31].

2.3 Quantitative phytochemical screening

2.3.1 Alkaloid determination

For alkaloid determination, 200 ml of 10% acetic acid in ethanol and the sample was weighed into a 250 ml beaker. The extract was concentrated to one-fourth of its original

volume in a hot water bath after being filtered and let to stand in a covered beaker for four hours. Until the precipitation was finished, concentrated ammonium hydroxide was applied drop by drop to the extract. Until the precipitate settled, the entire solution was let to stand. The precipitate was collected, cleaned with dilute ammonium hydroxide, and filtered. After thorough drying, alkaloid residue was gathered, weighed, and its percentage was measured and expressed in mg/g of plant extracts [32].

2.3.2 Tannin determination

After making slight modifications, the analyses of the tannin concentration in the aqueous leaf extract of *Punica granatum* was carried out in accordance with the International Pharmacopoeia [33] and Mulani et al. [34]. A 1 L conical flask was filled with 25 ml of the extract, 25 ml of indigo solution, and 750 ml of distilled water, the solution was made to 1 L using 0.1 N aqueous solution of KMnO_4 . The blue colored solution changed to green color. *Standard indigo carmine solution preparation: 6 g indigo carmine was dissolved in 500 ml of distilled water by heating, after cooling, 50 ml of 95–97% sulfuric acid was added, the solution was diluted to 1 L and then filtered.*

The tannin content was calculated as percentage and expressed as mg/g of plant extract.

$$\text{Tannin\%} = \frac{(V - V_0) \times 0.004157 \times 250 \times 100}{g \times 25} \quad (1)$$

Where V is the volume of 0.1 N aqueous solution of KMnO_4 exhausted in titration (ml), V_0 is volume of 0.1 N aqueous solution of KMnO_4 for titration of the blank sample (ml). 0.004157 is tannin equivalent in 1 ml of 0.1 N aqueous solution of KMnO_4 ; g is mass of the sample taken for the analysis (g); 250 is the volume of volumetric flask (ml).

2.3.3 Saponin determination

Obadoni and Ochuko's [35] published work was used to calculate saponin concentration in the aqueous leaf extract of *Punica granatum*. In a conical flask, 20 g of each ground sample was placed, and 100 ml of 20% aqueous ethanol was added. The flask was then heated for 4 hours on a hot water bath with continual stirring at around 55°C . the mixture was then filtered, and the residue was extracted once again using 200 ml of 20% aqueous ethanol. On a hot water bath at around 90°C , the combined extract was reduced to 40 ml. the concentrate was put into a 250 ml separator funnel, 20 ml diethyl-ether was added, and vigorous shaking was performed. The aqueous layer was saved, but the ether layer was not. The purifying procedure was carried out once again. N-butanol (60 ml) was added. The mixed n-butanol extracts were rinsed with 10 ml of 5% aqueous sodium chloride in triplicate. In a water bath, the residual solution was heated. The samples were dried in an oven after evaporation, weighed, and the saponin concentration was determined as a percentage and represented as mg/g of plant extract.

2.3.4 Total phenol determination

Folin-Ciocalteu's reagent technique was used to assess the quantity of total phenol content in aqueous leaf extracts of *Punica granatum* [36]. 0.5 ml of extract and 0.1 ml

(0.5 N) Folin-Ciocalteu's reagent were combined and incubated at room temperature for 15 minutes. The absorbance at 760 nm was measured after 2.5 mL of saturated sodium carbonate solution was added and incubated for 30 minutes at room temperature. A positive control was gallic acid. Total phenol levels are given in milligrams per gram of extracted components (mg/g).

2.3.5 Flavonoids determination

Flavonoids concentration was determined following previously published work [37]. At room temperature, 10 g of sample was extracted with 100 ml of 80% aqueous methanol. The whole solution was filtered using #42 Whatman filter paper (125 nm). The filtrate was then transferred to a crucible and evaporated until dry over a water bath, and the weight and percentage amount were computed and represented in mg/g of plant extracts.

2.4 Nanoparticle synthesis

Biomolecules found in plant extracts can convert metallic ions to nanoparticles in a single step. This method is rather simple and may be performed at ambient temperature and pressure. The use of plant extracts in the production of metallic nanoparticles is eco-friendly [38]. Plant extracts can be prepared using a variety of solvents (polar or non-polar). Secondary metabolites found in plant extracts, which include alkaloids, phenolic components, terpenoids, tannins, saponins and others are responsible for reduction of metallic ions [39–41].

Silver nanoparticles were synthesized in this work utilizing aqueous *Punica granatum* leaf extract.

2.5 Characterization of synthesized nanoparticles

Several characteristics, including shape, size, capping materials, stability, etc., must be assessed for the synthesized nanoparticles. In this work, synthesized silver nanoparticles were subjected to UV-visible spectrophotometer (UV-Vis), scanning electron microscope (SEM), Fourier transform infrared spectrophotometer (FT-IR), dynamic light scattering (DLS) and zeta-potential analysis.

2.5.1 UV-visible spectrophotometer analysis

The fundamental idea of a UV-visible spectrophotometer is measuring the quantity of light absorbed and scattered by a sample (extinction, defined as the total of absorbed and scattered light). In order to measure the UV/visible light beam's intensity both before and after it passes through the sample, a sample is positioned between a light source and a photodetector. To estimate the wavelength dependent extinction spectrum of the samples, these results are compared at each wavelength. The data is often shown as an extinction function of wavelength. To make sure that unexpected characteristics from the buffer are not included in the extinction-spectrum of the samples, each spectrum is background corrected using a buffer blank [42].

UV-visible spectrophotometers are effective tools for recognizing, describing, and studying nanomaterials because the optical characteristics of silver nanoparticles are sensitive to size, shape, concentration, aggregation state and refractive index close to the surface [43].

In this study the reduction of silver ions was monitored by measuring UV-visible spectrum of the reaction medium 5 hours after diluting a small aliquot of the sample with distilled water. UV-visible spectral analysis was performed using Perkin, Lambda 25 UV-visible spectrophotometer (USA).

2.5.2 Scanning electron microscope (SEM) analysis

Analysis performed using a scanning electron microscope may be utilized to look at the surface morphology of generated nanoparticles. The powder of the nanoparticles is first obtained and then coated with a heavy metal layer to produce SEM pictures. Once in the SEM step, the coated nanoparticles are bombarded with electron beams, which results in release of secondary electrons that are subsequently captured by the sensors to create pictures. The wavelength of an electron beam is shorter than that of visible light, allowing for the creation of high-resolution images of nanoparticles [39, 44]. In this investigation, silver nanoparticles were characterized using SEM equipment JIOL JSM-6390 LV (Jeol, Japan), which has supplemental software that can determine particle average size. The nanoparticles synthesized using aqueous leaf extract of *Punica granatum* was analyzed for surface morphology such as size (using accessory software) and shape using machine mentioned above and electron micrograph was obtained.

2.5.3 Fourier transform infrared spectroscopy (FT-IR) analysis

Nanoparticles' chemical surfaces are extremely sensitive to FT-IR. FT-IR spectra are collected to identify functional groups on the surface of nanoparticles. During the creation of metallic nanoparticles, negatively charged molecules cap the metallic ions. The FT-IR measurement can help identify (only) functional groups in compounds that cap the metallic ions [45]. Shimadzu IR-prestige-21 (Shimadzu Corp., Japan) FT-IR equipment was utilized in this investigation in the diffuse reflectance mode with a resolution of 4 cm^{-1} in the range of $400\text{--}4000\text{ cm}^{-1}$ to analyze the functional groups that could be involved in nanoparticle production.

2.5.4 Dynamic light scattering (DLS) and zeta-potential (ZP) analysis

Dynamic light scattering (also known as static, Rayleigh, or multi-angle light scattering) is a method for directly measuring particle size [46]. When light collides with a tiny object (such as a particle or molecule), it changes direction. A DLS equipment operates by shining a laser beam on a sample. A fast photon detector detects fluctuations in scattered light from a sample, and using known scattering angles, the whole particle size distribution inside a sample may be calculated. DLS devices used in a wide range of applications, including emulsions, micelles, polymers, proteins, colloids, and nanoparticle analysis [30, 44].

Light scattering analysis of nanoparticles is an essential method for determining nanoparticle size in solution. It quantifies the light scattered from a laser traveling through a colloidal solution by analyzing the variation of scattered light intensity as a function of time. The results of the light scattering study show a size distribution by number, intensity, and volume [44]. In the present study the light scattering and ZP analysis of nanoparticles were carried on Malvern Nano ZS (U.K.).

2.6 Animal model

In the investigation, Albino Wistar rats (175–200 g) were utilized. The rats were kept under typical laboratory conditions, with an ambient temperature of $25 \pm 2^\circ\text{C}$ and a relative humidity of $50 \pm 15\%$, and a 12-hour dark-light cycle. The animals were fed a commercial pellet diet and were given free access to water [30]. The experiment was carried out after taking approval from Ranchi University's Animal Ethics Committee (Proceeding no. 46, page no. 137).

2.7 Acute toxicity studies

Following OECD standards 423 [47], the acute oral toxicity of both aqueous extract and synthesized nanoparticles was assessed. The test's guiding idea is to employ the fewest number of animals feasible. The aqueous extract and nanoparticles were administered to separate groups of animals orally at one of the predetermined dosages. The material was examined using a step-by-step approach, with each stage including three animals of a single sex, usually females, because female rats are more sensitive to chemicals than their male counterparts, making it easier to identify any lingering toxicities. The female rats chosen were nulliparous and not pregnant. The next phase was chosen by the lack of substance-related mortality in the animals dosed in one step. The study employed 15 healthy adult albino rats weighing between 175 and 200 g and aged 8–12 weeks. Prior to treatment, the rats were fasted overnight. Food was not allowed for 3–4 hours after medication. Since nothing was known about the lethal level of the drug in rats, three animals were utilized in each phase; the beginning dose was chosen as a minimum (5 mg/kg body weight (BW)). The chemicals were given in a single dosage by oral gavage. Individual animals were watched after dosing at least once in the first 30 minutes, occasionally throughout the first hour, with special attention during the first 4 hours and daily thereafter, for a total of 14 days. However, the period of observation was not set, i.e., dosed animals were caged separately after 14 days of observation and monitored for another 14 days. No mortality was reported up to a dosage of 2000 mg/kg body weight of rats [48].

2.8 Induction of diabetes

Streptozotocin (STZ), dissolved in 0.1 M sodium citrate buffer (pH 4.5), was administered intraperitoneally (i.p.) to rats after they had fasted for 18 hours, at a dosage of 50–60 mg/kg body weight. Following STZ injection, animals were monitored for the first 24 hours to look for any signs of allergic reactions, behavioral abnormalities, or convulsions. To treat the hypoglycemia caused by STZ, animals were given 5% glucose solution. None of the STZ-treated animals showed any abnormal reactions. Blood glucose levels were measured 72 hours after STZ treatment. In order to conduct the study, only animals with glycosuria and blood glucose levels between 200 and 300 mg/dl were chosen. They were then split into groups as described in experimental design (2.9). STZ was not administered to the control group and rest all the groups started with STZ-induced-diabetic rats [48].

2.9 Experimental design

Group 1: normal control group—received 0.5 ml of regular saline orally every day for 21 days. Before the administration of normal saline on day 0 at 10:00 am, then on days 3, 7, 14 and 21 at the same time, blood glucose levels were measured.

Group 2: diabetic control group—received 0.5 ml of ordinary saline orally each day for 21 days. Animals were watched for any signs of altered behavior, hyperglycemia and convulsions. On day 0, before giving this groups normal saline, the blood glucose levels were measured at 10:00 am.

Group 3: extract test group—received daily oral doses of leaf extracts at a dosage of 200 mg/kg (BW) for 21 days. On day 0, before the extract was administered, the blood glucose levels were measured at 10:00 am. The blood glucose levels were then measured 3, 7, 14 and 21 days after medicine was administered. The animals were watched for any signs of hypoglycemia and convulsion.

Group 4: nanoparticle test group—received daily oral doses of leaf extracts at a dosage of 200 mg/kg (BW) for 21 days. On day 0, before the nanoparticles were administered, the blood glucose levels were measured at 10:00 am. The blood glucose levels were then measured 3, 7, 14 and 21 days after medicine was administered. The animals were watched for any signs of hypoglycemia and convulsion.

Group 5: glibenclamide standard group—received daily oral doses of glibenclamide at a dosage of 0.5 mg/kg body weight for 21 days. On day 0, before the medicine was administered, the blood glucose level was measured at 10:00 am. The blood glucose level was then measured 3, 7, 14, and 21 days after medicine was administered. The animals were watched for any signs of hypoglycemia and convulsion.

3. Results

3.1 Phytochemical screening

Preliminary phytochemical screening of *Punica granatum* aqueous leaf extract revealed the presence of alkaloids, tannins, saponins, phenols, and flavonoids. The aqueous extract of *Punica granatum* was subjected to quantitative phytochemical screening after preliminary phytochemical screening. **Figure 1** depicts the findings of quantitative phytochemical screening. Flavonoids

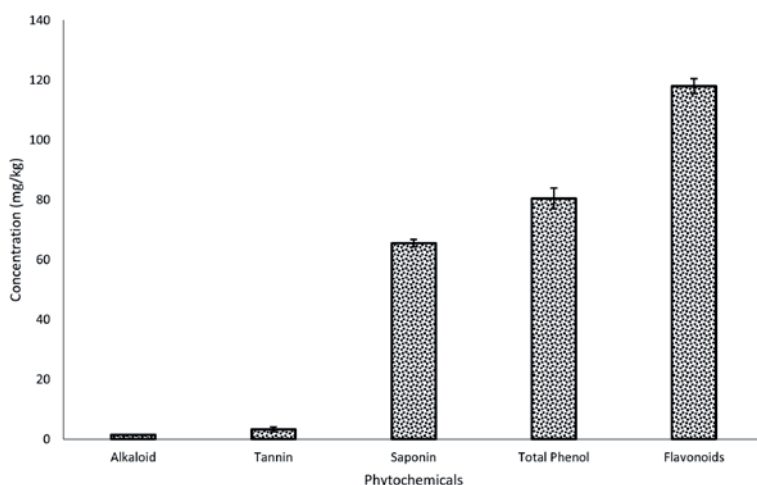


Figure 1.

The concentration of alkaloid, tannin, saponin, total phenol and flavonoids found in the aqueous leaf extract of *Punica granatum*.

were found in the greatest concentration (118 ± 2.5 mg/g), followed by phenol (80.5 ± 3.49 mg/g), saponins (65.5 ± 1.25 mg/g), tannins (3.3 ± 0.8 mg/g), and alkaloids (1.5 ± 0.05 mg/g).

3.2 Synthesis of silver nanoparticles

In the current study, a color change from initial yellow to dark brown was noticed as soon as the *Punica granatum* leaf extract was mixed with aqueous solution of silver nitrate (**Figure 2**).

3.3 Characterization of silver nanoparticles

3.3.1 UV-visible spectrophotometer

The reaction mixture was subjected to UV-visible spectrophotometer (UV-vis) analysis once the preceding step's reaction was finished. **Figure 3** shows the results of UV-vis analysis of AgPg_{aq} . According to several research [22–26], a surface plasmon resonance (SPR) peak with a wavelength between 400 and 490 nm suggests that the particle under investigation is in the nano range (below 100 nm).

3.3.2 Scanning electron microscope (SEM) analysis

Scanning electron microscope analysis can be used to examine the surface morphology of produced nanoparticles. To assess the size and shape of the AgPg_{aq} , SEM analysis was employed. The nanoparticles were spherical, rhomboid, and cubical in shape and had diameter ranging from 88.0 to 120 nm and average size of the particles were 98.93 nm (**Figure 4**).

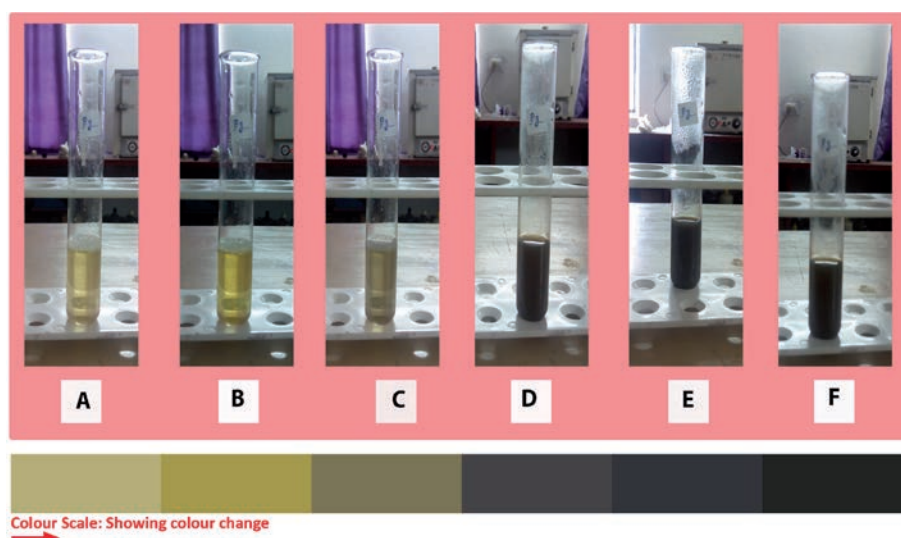


Figure 2.
 Test tubes A to F showing change in colour of mixture of aqueous solution of AgNO_3 and PG_{aq} . (A) Colour (pale yellow) just after mixing of AgNO_3 and PG_{aq} ; B to E, gradual colour change; F, final colour obtained.

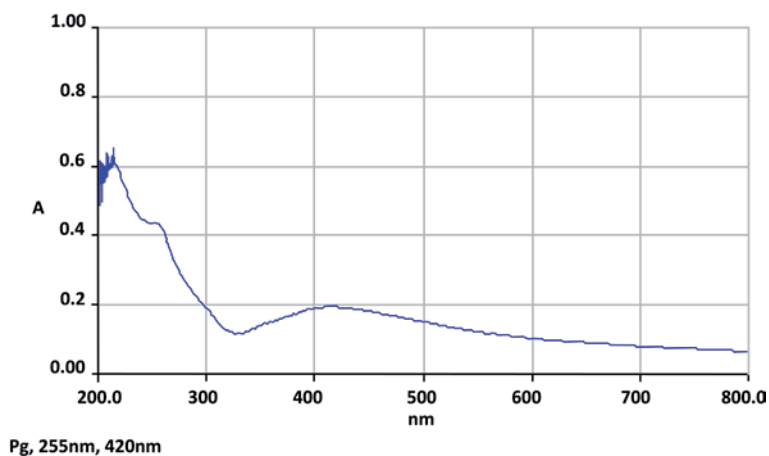


Figure 3.
Graphical result obtained by UV-visible spectrophotometric analysis of AgPg_{aq}

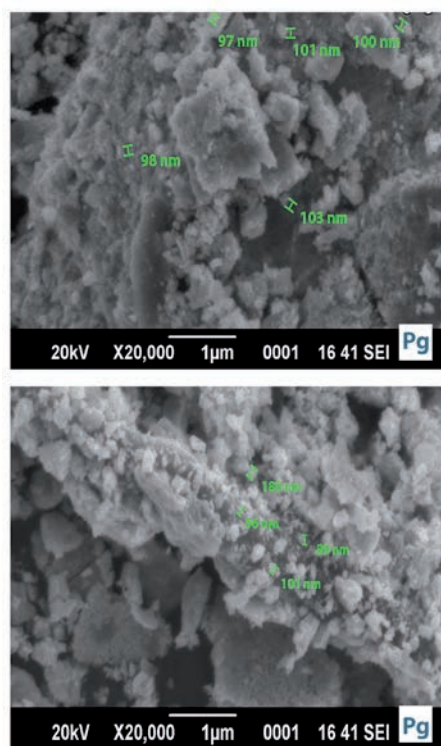


Figure 4.
SEM images of AgPg_{aq}.

3.3.3 Fourier Transform Infrared (FT-IR) spectroscopy

The results of an FT-IR investigation of silver nanoparticles made from aqueous *Punica granatum* leaf extracts are shown graphically in **Figure 5**. The spectra showed

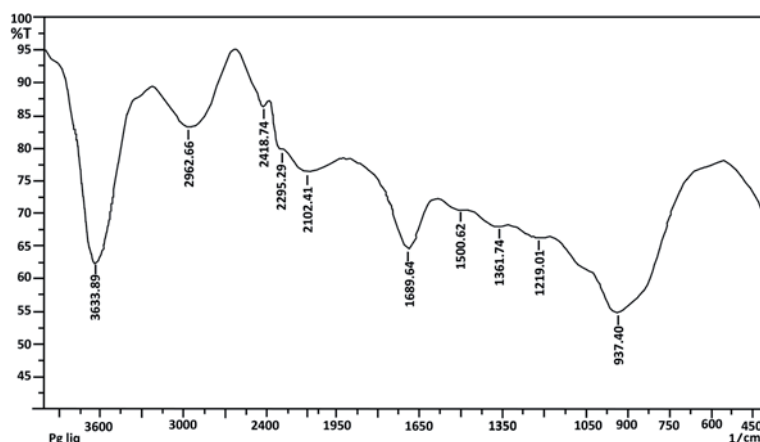


Figure 5.
Result of FT-IR analysis of $AgPg_{aq}$.

broad transmission peaks at 3633.69 cm^{-1} , which corresponds to hydrogen bonded hydroxyl group (O–H and H stretch) of alcohols and phenols. The 2102.41 cm^{-1} peak corresponds to $-\text{SCN}$. The 1500.62 cm^{-1} corresponds to $-\text{C}=\text{C}-$ stretch, which represents alkenes, 1361.74 cm^{-1} corresponds to sulphonates, and 937.40 cm^{-1} corresponds to $\text{C}=\text{N}$ stretch that represents the aliphatic amines.

3.3.4 Dynamic light scattering (DLS) and zeta potential (ZP) analysis

The results of light scattering analysis demonstrate a size distribution by intensity, volume, and number [22, 45] as depicted by the results of DLS analysis of nanoparticles synthesized using aqueous leaf extract of *Punica granatum* in **Figures 6–8**.

Figure 6 shows results of light scattering (intensity distribution) analysis of aqueous leaf extract of *Punica granatum* showing two peaks with intensities of 91.0% and 9.0% at 96.5 nm and 141.6 nm.

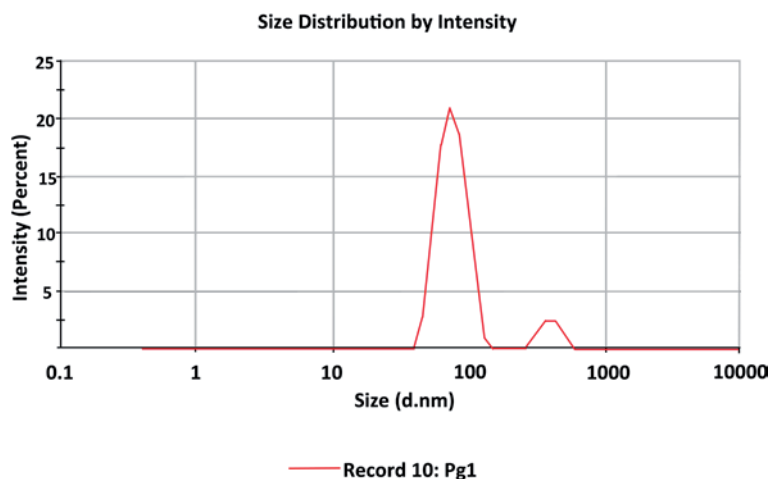


Figure 6.
Result of light scattering analysis of aqueous leaf extract of *Punica granatum* showing size distribution by intensity.

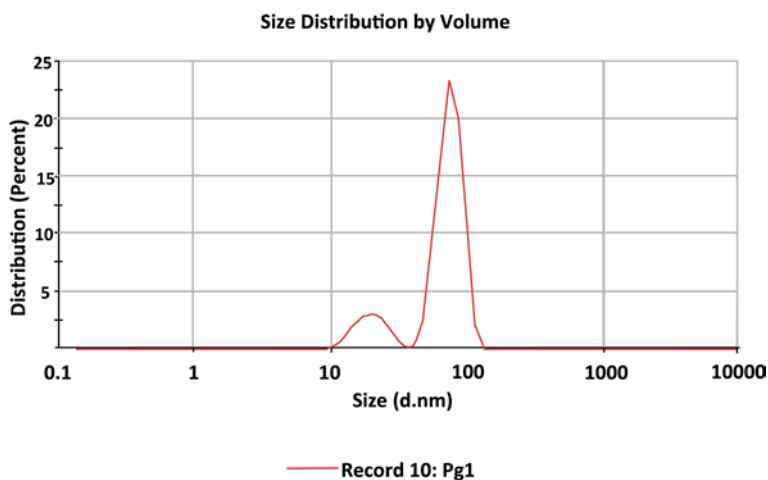


Figure 7.
Result of light scattering analysis of aqueous leaf extract of *Punica granatum* showing size distribution by volume.

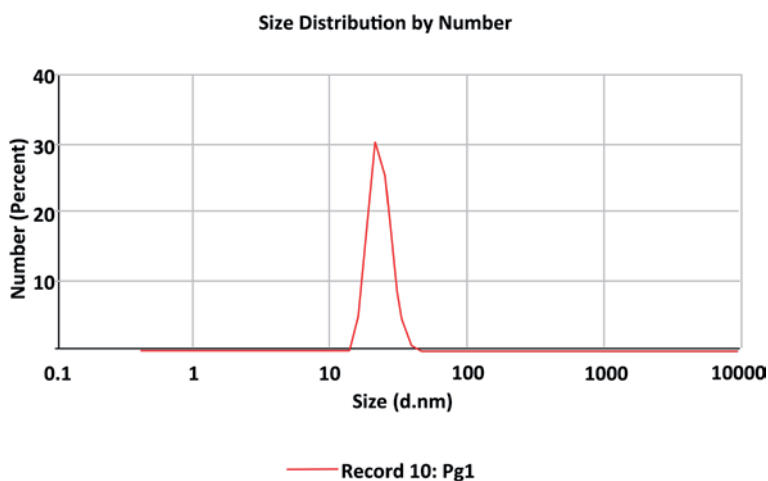


Figure 8.
Result of light scattering analysis of aqueous leaf extract of *Punica granatum* showing size distribution by number.

Two peaks at 85.6 nm and 24.76 nm were seen in the volume distribution graph (**Figure 7**), illustrating the particle volume distribution in different size bins. According to the results, all of the particles produced were nanosized, with 84.2% of them measuring 85.6 ± 14.60 nm and 15.8% measuring 24.76 ± 2.114 nm.

One significant peak with a number distribution of 30% can be seen at 40.32 nm on the number distribution graph (**Figure 8**). This suggests that at least 30% of synthesized nanoparticles were 40.32 ± 4.413 nm in size.

The results of zeta potential analysis of AgP_g_{aq} have been graphically presented as **Figure 9**. The zeta potential value of synthesized silver nanoparticles was -11.6 mV, having a standard deviation of 4.67 mV.

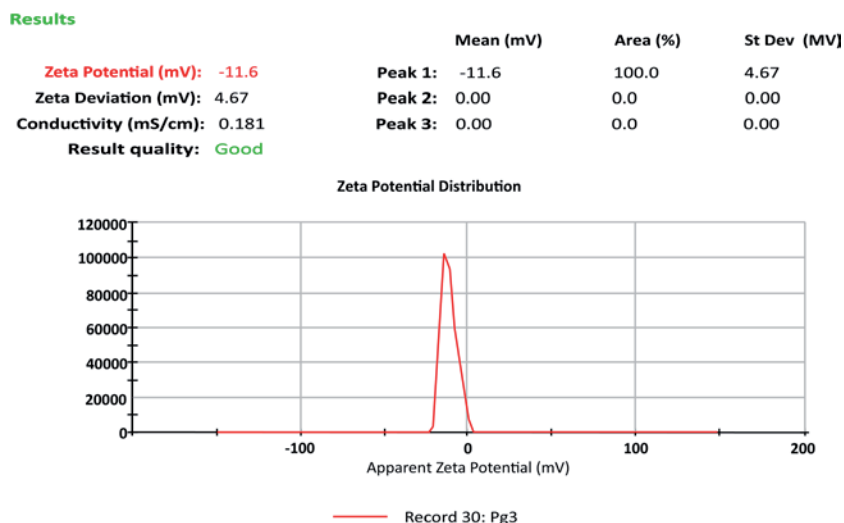


Figure 9.
Result of zeta potential analysis of $AgPg_{aq}$.

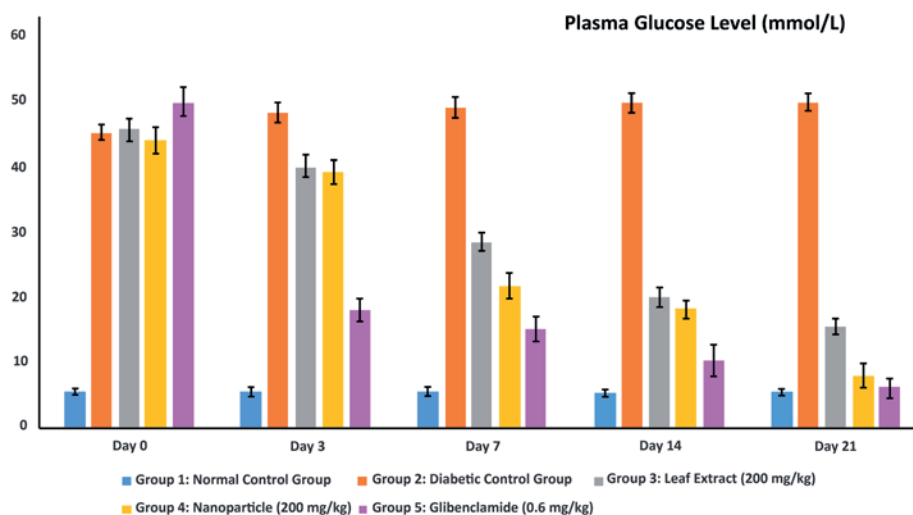
A particle's zeta potential can range from +100 mV to -100 mV. The zeta potential study of *Punica granatum* aqueous leaf extract-produced silver nanoparticles reveal a peak of -11.6 4.7 mV with 100% area dispersion (**Figure 9**).

According to the results obtained from all the various techniques employed in this study to characterize the synthesized silver particles (as described earlier in the text), the size of synthesized silver particle exhibited significant stability with size in nano-range.

3.4 Impact of Pg aqueous leaf extract and silver nanoparticles synthesized using aqueous leaf extract of Pg on mammalian model

The results of impact of PG_{aq} and $AgPg_{aq}$ has been graphically presented as **Figure 10**. The results are expressed as mean $\pm$ SD ($n = 3$). The results clearly confirm the antidiabetic activity of PG_{aq} and $AgPg_{aq}$. They were able to counter the diabetes induced by streptozotocin. The results of study of antidiabetic impact of PG_{aq} and $AgPg_{aq}$ against streptozotocin induced diabetes in rats have been tabulated under **Table 1**. The data obtained was statistically analyzed using t-test and value of $P < 0.05$ was judged as significant. The table clearly shows that the group which received aqueous leaf extract of *Punica granatum* orally showed significant ($P = 0.0002$) decrease in plasma glucose levels from 45.9 ± 1.8 mmol/L (day 0) to 15.68 ± 1.3 mmol/L (day 21); in the other group which received silver nanoparticles loaded with aqueous leaf extracts of *Punica granatum*, the plasma glucose level decreased significantly ($P = 0.0002$) from 44.3 ± 2.0 mmol/L to 10.22 ± 1.8 mmol/L. Both the leaf extracts of *Punica granatum* and nanoparticles synthesized using leaf extract of *Punica granatum* showed significant antidiabetic activity against streptozotocin induced diabetes in rats.

Statistical comparison of the antidiabetic activity of PG_{aq} and $AgPg_{aq}$ showed that on there was non-significant ($P = 0.7659$) difference between plasma glucose levels on day-3 between rats receiving PG_{aq} (40.23 ± 1.75 mmol/L) and $AgPg_{aq}$ (39.43 ± 1.8 mmol/L).

**Figure 10.**

Comparative representation of impact of PG_{aq} and AgP_{gaq} against induced diabetes in rats (mean $\pm$ SD, $n = 3$).

Groups	Plasma glucose levels (mmol/L)				
	Day 0	Day 3	Day 7	Day 14	Day 21
Group 1: normal control group	5.6 $\pm$ 0.52	5.66 $\pm$ 0.62	5.59 $\pm$ 0.66	5.46 $\pm$ 0.45	5.7 $\pm$ 0.51
Group 2: diabetic control group	45.5 $\pm$ 1.22	48.6 $\pm$ 1.5	49.33 $\pm$ 1.6	50.01 $\pm$ 1.52	50.11 $\pm$ 1.3
Group 3: PG_{aq} (200 mg/kg)	45.9 $\pm$ 1.8	40.23 $\pm$ 1.75	28.65 $\pm$ 1.42	20.12 $\pm$ 1.4	15.68 $\pm$ 1.3*
Group 4: AgP_{gaq} (200 mg/kg)	44.3 $\pm$ 2.0	39.43 $\pm$ 1.8 ^A	21.95 $\pm$ 1.9 ^B	18.33 $\pm$ 1.35 ^C	8.22 $\pm$ 1.8 ^{D,#}
Group 5: Glibenclamide (0.6 mg/kg)	50.11 $\pm$ 2.3	18.26 $\pm$ 1.8	15.22 $\pm$ 2.0	10.43 $\pm$ 2.5	6.35 $\pm$ 1.8 ^{##}

*Difference in plasma glucose levels on day-21 as compared to day-0: $P = 0.0002$.
#Difference in plasma glucose levels on day-21 as compared to day-0: $P = 0.0002$.
##Difference in plasma glucose levels on day-21 as compared to day-0: $P = 0.0001$.
^ADifference in plasma glucose level of group 3 and group 4 on day 3: $P = 0.7659$.
^BDifference in plasma glucose level of group 3 and group 4 on day 7: $P = 0.0476$.
^CDifference in plasma glucose level of group 3 and group 4 on day 14: $P = 0.409$.
^DDifference in plasma glucose level of group 3 and group 4 on day 21: $P = 0.0283$.

Table 1.

Showing the antidiabetic impact of PG_{aq} and AgP_{gaq} on diabetes induced by streptozotocin in rats (mean $\pm$ SD, $n = 3$).

On Day 7 there was as significant decrease ($P = 0.0476$) in blood glucose level of group 4 (receiving AgP_{gaq}) (21.95 ± 1.9 mmol/L) as compared to group 3 (receiving PG_{aq}) (28.65 ± 1.42 mmol/L). On day 14 there was non-significant (0.409) difference between the plasma glucose levels of group 3 and group 4. On the 21st day group 4 exhibited significantly lower ($P = 0.0283$) plasma glucose level (8.22 ± 1.8 mmol/L) as compared to group 3 (15.68 ± 1.3 mmol/L). Group 5 which received the drug Glibenclamide always showed significant decrease in the plasma glucose level as compared to group 3 and group 4.

4. Discussion

4.1 Phytochemical screening

The amount and kind of phytochemical in each class are crucial to their effects. Flavonoids and phenols are substances found in broad variety of vascular plants, with over 8000 identified unique compounds [49]. They serve as antioxidants, antimicrobials, photoreceptors, visual attractants, feeding repellants, and light screens in plants [50]. Many studies have shown that both flavonoids and phenols have biological properties such as antioxidant, antiallergic, antiviral, and anti-inflammatory activity. However, the antioxidant activity of flavonoids and phenols have received the greatest attention due to its capacity to inhibit free radical production and scavenge free radicals [51]. The flavonoid content was found to be highest followed by phenols in the aqueous leaf extract of *Punica granatum* in the current investigation, indicating the extract to have significant antioxidant property. Dehshahri et al. reported the leaf extract of *Moringa peregrina* to possess substantial antioxidant activities, which they attributed to the flavonoids and phenols present in the extract [52]. Shang-Tse et al. investigated the antioxidant activities of 18 indigenous tree species in Taiwan and discovered that flavonoid as well as phenols were key elements in the majority of the plants that had high antioxidant activity [53]. Wairata et al. in their correlation study found that there is a positive relationship of total phenolic and flavonoid contents with antidiabetic activity [54]. Praparatana et al. [55] and Iqbal et al. [56] in their studies on extracts of different plants have found straight correlation between the antidiabetic activities and the phenol and flavonoid contents of the studied plants.

Saponins cut blood lipids, reduce cancer risks, and improve blood glucose responsiveness. A high saponin diet can be used to prevent dental cavities and platelet aggregation, cure hypercalciuria in humans, and act as an antidote to acute lead poisoning [57]. Saponins, according to Barky et al. [58] and Metwally et al. [59] have been proven to lower blood glucose levels by rejuvenating insulin function, increasing plasma insulin levels, and releasing insulin from the pancreas. Saponin can also reduce glucose synthesis in the circulation [60]. Various other workers such as Singh et al. [61], Elekofehinti [62], and Jiang et al. [63] have reported that the saponins possess anti-diabetic properties.

Tannins have also been shown to have a variety of physiological effects, including anti-diabetic property and ability to speed up blood clotting, lower blood pressure, and lower serum cholesterol levels [64]. In their review, Laddha and Kulkarni noted that tannins play a role in the prevention and therapy of different diabetes problems [65]. Mohammed et al. [66] in their review found that the plant extracts having tannin as a constituent exhibited antidiabetic properties. Saratale et al. [67] studied the tannin extracted from grape pomace and reported it to possess antimicrobial, antioxidant and antidiabetic property as well.

Alkaloids are basic forms of salts with acids. They are nitrogen containing heterocyclic organic compounds of plant origin with different pharmacological activities [68]. Muhammad et al. in their review noted that alkaloids have antidiabetic activities [69]. Rasouli et al. concluded that the alkaloids have potentials to be used as alternative additives for treatment of hyperglycemia, suggesting need of further studies and researches [70]. Agrawal et al. studied the impact of alkaloids of *Aerva lanata* roots on streptozotocin-nicotinamide induced diabetes in rats and concluded that the alkaloids showed significant effects on streptozotocin-nicotinamide induced diabetes in rats [71].

Since all the phytochemicals (flavonoids, saponins, tannins, alkaloids) reported in the aqueous leaf extracts of *Punica granatum* have previously been reported to possess anti diabetic property, the leaf extract is expected to possess antidiabetic properties.

4.2 Synthesis of silver nanoparticles

Biomolecules found in plant extracts can convert metallic ions to nanoparticles in a single step. This method is rather simple and may be performed at ambient temperature and pressure. It is ecologically benign to employ plant extracts in the production of metallic nanoparticles [44]. In 2017, we introduced the name green nanoparticles for nanoparticles synthesized utilizing extracts from green plants' leaves [22]. The term 'green' used here has a dual connotation. On the one hand, the phrase 'green' refers to a safer, cleaner, and more environmentally friendly approach of nanoparticle production utilizing plant extracts; on the other hand, the term 'green' refers to the presence of the green pigment chlorophyll in leaf extracts. As a result, any nanoparticle synthesized using extracts from chlorophyllous extracts may be referred to as "green nanoparticles," whereas nanoparticles synthesized using extracts from achlorophyllous extracts may be referred to as "white nanoparticles" [44].

This is widely accepted confirmation for the reduction of silver ions by constituents of various phytochemicals present in the plant extract [19, 20, 22–25, 44]. After 90 minutes, no color change was noticed, indicating that the reduction of silver ions by the phytochemicals included in the leaf extract was complete.

4.3 Characterization of silver nanoparticles

4.3.1 UV–visible spectrophotometer analysis

UV–visible spectrophotometry is a method for calculating extinction, which is total of light that has been absorbed and scattered by a sample [45]. Extinction is a measurement of how much light has been absorbed and scattered by a material.

In this study, silver nanoparticles synthesized from *Punica granatum* aqueous leaf extracts showed an SPR peak (**Figure 3**) at 420 nm (and 255 nm). Several investigations making use of *Tridax procumbens* [72], *Argemone mexicana* [73], Papaya fruit extract [74], and *Trianthema decandra* [75] have observed absorbance peaks from 450 to 500 nm for silver nanoparticle formation.

4.3.2 Scanning electron microscope analysis

The size and shape of synthesized nanoparticles varies with the plant and metal characteristics. Alkammash [76] performed synthesis and SEM characterization of silver nanoparticles synthesized using *Calotropis procera* plant extracts, they reported the silver nanoparticles were spherical (size 8–20 nm). Our results are similar to those reported by Sharma et al. [77], Liaqat et al. [78], and Ali et al. [79].

4.3.3 Fourier transform infrared (FT-IR) spectroscopy

FT-IR analysis is highly sensitive to the chemical surface of nanoparticles. FT-IR spectra are obtained to detect the functional groups present on the surface of nanoparticles. During the creation of metallic nanoparticles, negatively charged

molecules cap the metallic ions. Only the functional groups of substances that cap the metallic ion, may be identified via FT-IR analysis [45]. The FT-IR analysis's measured spectra were compared to Coates's previously reported reference values [80].

Multiple peaks were found by Rautela et al. [81] illustrating the complexity of *Tectona grandis* aqueous seed extracts. They noticed a change in the silver nanoparticle peak shapes and hypothesized that the functional groups in the seed extract were involved in the creation of the silver nanoparticles in their research [81]. Our results are in accordance with that of Rautela et al. [81].

4.3.4 Dynamic light scattering (DLS) and zeta potential (ZP) analysis

Particle size is directly measured by dynamic light scattering [46]. When light strikes a microscopic object or molecule, light scattering takes place. Applications for DLS devices include the study of emulsions, micelles, polymers, proteins, colloids, and nanoparticles [45]. A crucial method for figuring out the size of nanoparticles in a solution is DLS. To measure the light scattered from a laser passing through the colloidal solution, it studies the variation of scattered light intensity as a function of time.

The intensity data illustrates how light is scattered by a certain particle size bin; in this case, the data reveals that particles with a size of 96.5 ± 4.843 nm dispersed 91% of light. Particles that were 141.6 ± 32.40 nm in size dispersed 9% of the light. Thus, the graphs support the existence of silver particles synthesized at the nanoscale. Our results are in accordance with those of Ranjan et al. [23], Kumar et al. [44], and Kumar et al. [45].

Figure 7 represents the volume distribution graph of DLS analysis. The volume distribution graph shows the total volume of particles in different size bins. Our results were in accordance with the studies of Dandapat et al. [24], Rautela et al. [81].

Figure 8 represents graph showing size distribution of synthesized nanoparticles by number. The graph depicts that at least 30% of synthesized nanoparticles had size of 40.32 ± 4.413 nm. Our results are in accordance with Carvalho et al. [82].

4.3.5 Zeta potential analysis

A method for figuring out the surface charge of nanoparticles in a colloid (solution) is zeta potential analysis. A thin layer of ions with an opposing charge are drawn to the surface of nanoparticles due to their surface charge. As the nanoparticle diffuses through the fluid, a double layer of ions follow. The zeta potential of the particle refers to the electric potential at the double layer's border. Most zeta potentials with values larger than +25 mV and less than -25 mV are highly stable. Our results are in accordance with previously published works [22, 45, 48].

4.4 Impact of *Pg* aqueous leaf extract and silver nanoparticles synthesized using aqueous leaf extract of *Pg* on mammalian model

Punica granatum is a fruit-bearing tree from Punicaceae family. It has healing qualities of quite an interest, which is due to the presence of phytochemicals alkaloids, tannins, saponins, phenols, and flavonoids [83]. Traditional systems of medicine, such as Ayurveda, Traditional Chinese Medicine, and Unani have cited various medicinal uses of leaves of *Punica granatum* [84, 85]. Fakudze et al. in their review discovered that *Punica granatum* has anticancer, antioxidant, anti-inflammatory,

antiviral properties [83]. Das and Barman in their study reported that ethanolic leaf extract of *Punica granatum* showed antidiabetic property against rats with induced diabetes [86]. Streptozotocin is widely used to induce diabetes in animal models, such as rats and mice. It causes necrosis of β cells followed by β -cell loss and atrophy of islets [59].

Scientists have been more interested in metallic nanoparticles because of the induction of green synthetic processes in the synthesis of these particles, which are mediated by plant extracts, have decreased cost consequences, and repeatability and rapid reproducibility [45]. The metallic nanoparticles due to their small size, high surface area to volume ratio and other characteristics have attracted great interest of the scientific community. Silver nanoparticle synthesized from plant extract has been reported by many workers to have enhanced medicinal impact as compared to the extract alone [30, 44, 45]. Silver nanoparticles synthesized from plant extracts are known to show antimicrobial and anti-inflammatory activity, antibacterial, antiviral, and antifungal activities [45].

To the best of our knowledge, no studies on the effects of silver nanoparticles and their comparison with extract have been previously documented.

Owing to the above apprehensions both the aqueous leaf extract of *Punica granatum* (PG_{aq}) and nanoparticles synthesized using aqueous leaf extract of *Punica granatum* (AgPg_{aq}) were administered in rats (as described in the 'materials and methods' section), in which diabetes was induced using streptozotocin. The results revealed that the aqueous leaf extract of *Punica granatum* and the synthesized nanoparticles have significantly reduced the increased blood sugar induced by streptozotocin toxicity. Statistical analysis revealed that the silver nanoparticles were more effective as an anti-diabetic agent as compared to the extract alone. Similar observations were noted in many experimental diabetes researches [71–74]. Our results are in accordance with the findings of Balan et al. [87], Wahab et al. [88], and Bhagyalakshmi et al. [89].

Thus, on the basis of results of present study it can be concluded that the aqueous leaf extract of *Punica granatum* and silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum* showed significant antidiabetic activity on streptozotocin diabetic rats. Further it was found that the AgPg_{aq} had significantly higher alleviating effect against streptozotocin diabetes in rats as compared to the impact of the aqueous extract alone.

5. Conclusion

Thus, based on results of present study it can be concluded that the aqueous leaf extract of *Punica granatum* and silver nanoparticles synthesized using aqueous leaf extract of *Punica granatum* showed significant antidiabetic activity on streptozotocin diabetic rats. The aqueous leaf extract loaded silver nanoparticles had enhanced antidiabetic impact as compared to the extract alone which is a novel finding.

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Conflict of interest

The authors declare no conflict of interest.

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Oxytocin as a Positive Allosteric Modulator for Particular Opioid Receptors

Hideaki Fujii, Takaaki Mizuguchi and Yasuhito Uezono

Abstract

Oxytocin plays important roles in parturition, lactation, social bonding, and social behavior. It is also related to pain modulation, exhibiting analgesic effects through both oxytocin receptor-dependent and oxytocin receptor-independent mechanisms. Although evidence suggests that these analgesic effects are related to endogenous opioid peptides, the detailed mechanism of action remains unclear. It was recently elucidated that oxytocin is a positive allosteric modulator (PAM) for the μ and κ opioid receptors. Interestingly, the oxytocin structure is distinct from that of any other known PAMs for the opioid receptors. The synthesis of several oxytocin analogs led to the identification of the cyclic structure containing a disulfide bond, and the C-terminal three amino acid residues, as essential for the PAM activity toward the μ opioid receptor. Based on the accumulated evidence that oxytocin induces analgesic effects in several experimental animals, some clinical trials have tested oxytocin as an analgesic. However, the available results do not indicate that oxytocin has significant analgesic effects in humans compared to placebo. Here, we will discuss the possible use of oxytocin as an analgesic and/or analgesic adjuvant.

Keywords: opioid, opioid receptor, positive allosteric modulator (PAM), structure–activity relationship, analgesic, analgesic adjuvant

1. Introduction

Oxytocin is a nine-amino acid hormone that is synthesized in the hypothalamic paraventricular and supraoptic nuclei in the central nervous system and is well-known for its roles in parturition, lactation, social bonding, and social behavior [1–3]. Evidence accumulated over the last decade also highlights the involvement of oxytocin in nociceptive processing and pain modulation. The findings reported to date suggest that oxytocin exerts analgesic effects by manipulating the pain-sensing ability of central and/or peripheral sensory neurons [4–8].

Recent studies demonstrate that oxytocin contributes to pain regulation through both oxytocin receptor (OTR)-dependent and OTR-independent mechanisms [8, 9]. Oxytocin is released from descending hypothalamic projections in the spinal cord

and then activates OTRs located on superficial dorsal horn laminae I and II [6, 10]. Activated OTRs reportedly enhance the activity of inhibitory GABAergic interneurons, resulting in presynaptic inhibition of nociceptive A δ - and C-fiber transmission and thereby causing antinociception [6, 10]. Moreover, oxytocin may exert analgesic effects *via* OTR-independent mechanisms, such as through modulation of the endogenous opioid system. Notably, oxytocin-induced antinociception is partially reversed by opioid receptor (OR) antagonists, such as naloxone, which suggests that the antinociceptive effects of oxytocin may be related to OR-mediated pathways [11, 12].

Several investigations have demonstrated that oxytocin can act as a positive allosteric modulator (PAM)—that is, a compound that enhances the activities of orthosteric ligands—for the μ OR (MOR), which is activated by morphine or endomorphin-1 (EM-1). Although oxytocin alone cannot activate the MOR, it significantly enhances the responses induced by endogenous or exogenous MOR agonists—including EM-1, β -endorphin, morphine, fentanyl, and the MOR specific agonist DAMGO—in experiments using human embryonic kidney (HEK)293 cells expressing human MOR [8]. Moreover, it has been demonstrated that oxytocin also behaves as a PAM for the κ OR (KOR), while it does not act as a PAM for the δ OR (DOR) [9].

In the present review, we will describe the evidence that oxytocin has PAM activity for particular types of ORs, as well as the research investigating which structural components of oxytocin are essential for its PAM activity toward the MOR. Furthermore, we will discuss the potential clinical therapeutic use of oxytocin as a pain modulator, in light of the findings that oxytocin has PAM activity for some types of ORs.

2. Oxytocin acts as a PAM for certain types of ORs

2.1 Oxytocin is a PAM for both MORs and KORs, but not DORs

In 2018, Meguro et al. provided the first direct evidence that oxytocin functions as a PAM for the MOR [8]. Using HEK293 cells that stably express human MOR, they demonstrated that while oxytocin alone did not activate MORs, oxytocin significantly enhanced MOR responses to several opioid agonists—including the endogenous opioids EM-1 and β -endorphin, and the exogenous opioids morphine, fentanyl, and DAMGO (**Figure 1A and B**). Importantly, oxytocin did not alter the binding affinities of these agonists to ORs, indicating that it acts as a true PAM rather than an orthosteric ligand [8].

Functionally, the modulation by oxytocin was evident through increased signal transduction in electrical impedance assays of cellular responses (the CellKey™ assay) and of adenylate cyclase activity (a cAMP assay) [13], confirming that oxytocin boosts the efficacy of MORs [8]. These findings suggest that oxytocin could potentiate opioid analgesia, while potentially minimizing the necessary opioid dosage and related adverse effects.

Building on the findings of oxytocin's PAM activity toward MORs, in 2021, Miyano et al. further explored whether oxytocin could also modulate other OR subtypes, such as DOR and KOR [9]. They demonstrated that oxytocin enhanced KOR signaling in response to the KOR agonists U50488 and dynorphin A, but did not affect DOR signaling (**Figure 1C–F**).

The activation of G protein-coupled receptors (GPCRs), such as ORs, induces two major GPCR signaling pathways: G protein-dependent pathways and β -arrestin signaling pathways [9, 14]. G protein-dependent signaling pathways involve the

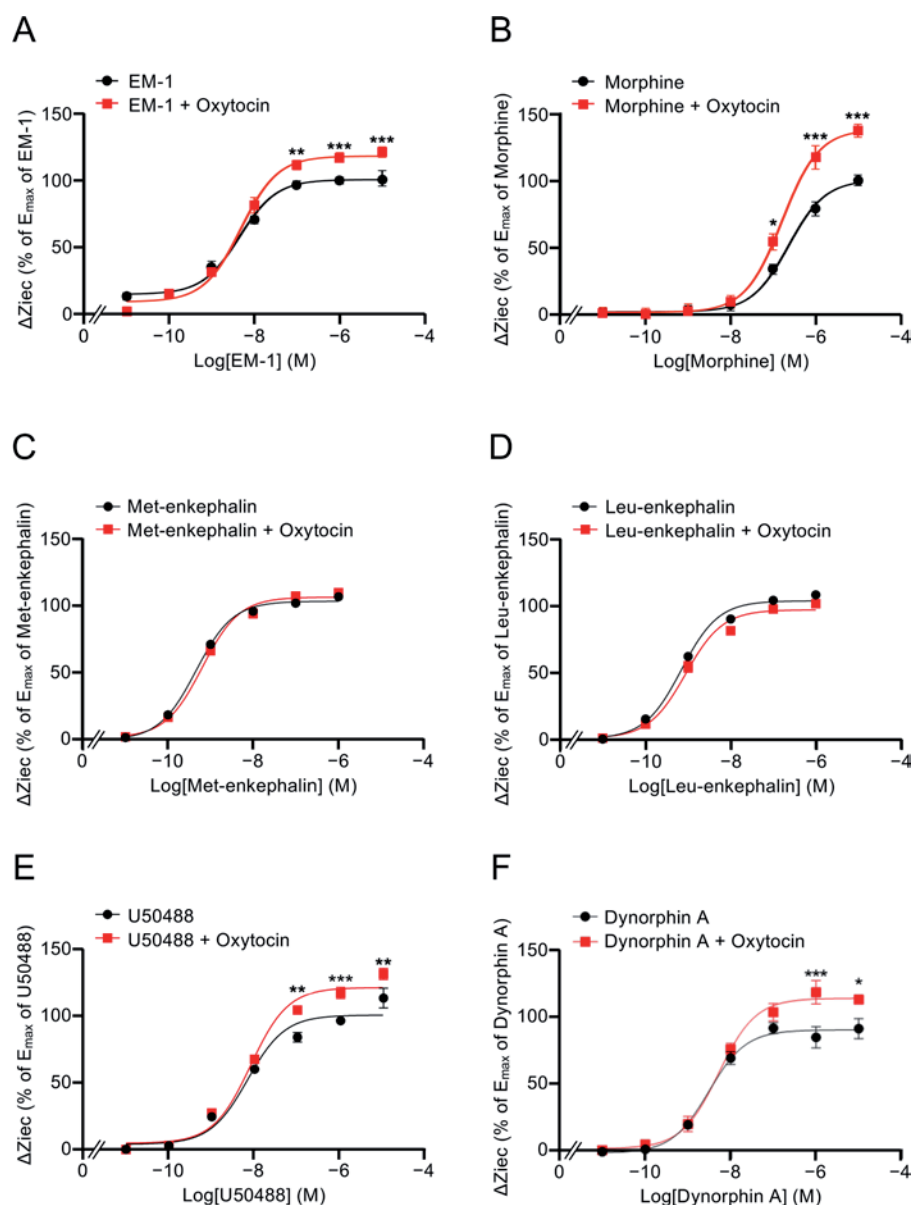


Figure 1.
 Oxytocin enhances the MOR- and KOR-induced, but not DOR-induced responses, as determined using the CellKey™ assay. (A and B) Concentration-response curves for ΔZ_{iec} caused by EM-1 (A) and morphine (B) in cells expressing MOR. (C and D) Concentration-response curves for ΔZ_{iec} caused by Met-enkephalin (C) and Leu-enkephalin (D) in cells expressing DOR. (E and F) Concentration-response curves for ΔZ_{iec} caused by U50488 (E) and dynorphin A (F) in cells expressing KOR. Black lines indicate the absence of oxytocin. Red lines indicate the presence of oxytocin (10^{-6} M). (A and B) $n = 9-21$. (C-F) $n = 3$. Two-way ANOVA was performed, followed by Bonferroni's test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to each agonist treatment alone.

inhibition of adenylyl cyclase and activation of G protein-coupled inwardly rectifying K^+ channels, resulting in pain relief. On the other hand, β -arrestin-dependent signaling pathways entail OR internalization *via* β -arrestin recruitment to ORs and are related to adverse effects, such as respiratory depression and tolerance [9]. In

their study, Miyano et al. also demonstrated that oxytocin selectively amplifies G protein-mediated cAMP inhibition, without affecting β -arrestin-mediated internalization [9]. This suggests that oxytocin may act as a G-protein-biased PAM of both MORs and KORs and thus might confer analgesic benefits without triggering adverse

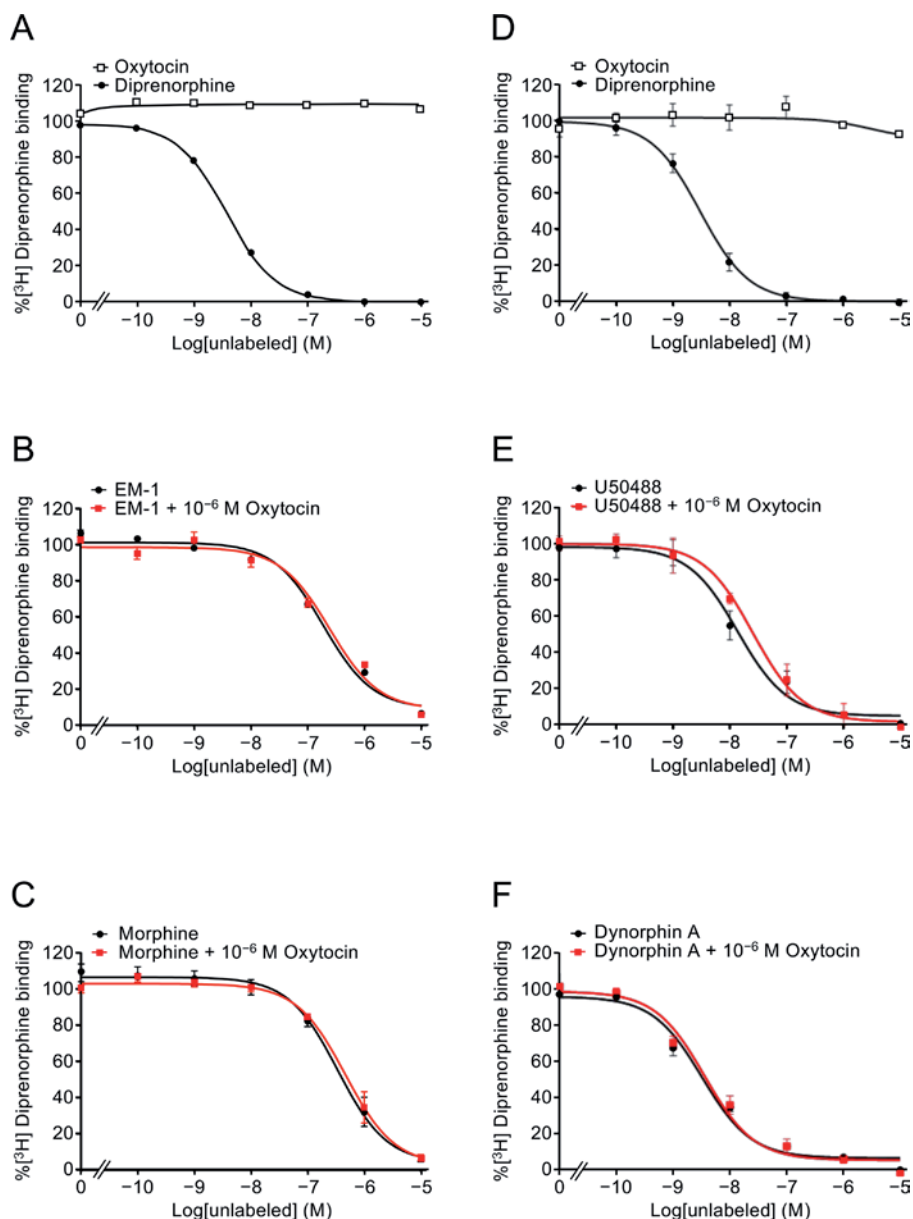


Figure 2.

Oxytocin does not affect the capability of MOR or KOR ligands to bind their respective ORs. Competitive OR binding assays for MOR and KOR were conducted using membranes prepared from Chinese hamster ovary cells expressing MOR (A–C) and KOR (D–F). (A and D) White squares indicate trials with oxytocin, and black circles indicate trials with diprenorphine. (B, C, E, and F) Black circles indicate trials without oxytocin, and red squares indicate trials with the presence of oxytocin (10^{-6} M). In all presented assays, [^{3} H]diprenorphine was used for the binding assay. Oxytocin did not bind to orthosteric binding sites or enhance the binding affinity for MOR and KOR ligands. (A–C) $n = 6$. (D–F) $n = 3$.

β -arrestin-dependent effects, like respiratory depression, constipation, and tolerance [8, 9]. Based on the accumulating evidence, oxytocin is emerging as a novel neuro-modulatory agent in pain biology, capable of acting as a G-protein-biased PAM for MORs and KORs. Through this unique mechanism, oxytocin enhances OR signaling in a ligand-dependent, β -arrestin-sparing manner, potentially offering safer and more effective analgesia.

2.2 Oxytocin does not influence opioid agonist binding properties to MOR and KOR

A study investigated whether oxytocin modulates the affinity of EM-1 or morphine for MOR, and revealed that oxytocin alone did not bind to the orthosteric site of MOR (**Figure 2A**), and the presence of oxytocin did not alter the affinities with which the ligands bound to the MOR (**Figure 2B and C**) [8]. Similarly, oxytocin did not bind to the orthosteric site of KOR (**Figure 2D**) and did not affect the binding affinities of the KOR-selective ligands U50488 and dynorphin A (**Figure 2E and F**) [9]. Conn et al. proposed a model for allosteric modulation [15] in which allosteric ligands bind to distinct sites (allosteric sites) but not to ligand-binding sites (orthosteric sites). According to the results in **Figure 2**, oxytocin specifically modulates the efficacy of both MORs and KORs, without affecting their orthosteric binding profiles, which supports the proposal by Conn [8, 9, 15]. With growing interest in non-traditional pain therapeutics, oxytocin and its analogs represent promising candidates for the next generation of opioid-sparing analgesics.

2.3 Oxytocin could exert analgesic effects partly *via* enhancement of opioid signaling through MORs and KORs

Several reports show that when oxytocin was administered into the spinal cord, periaqueductal gray, and nucleus accumbens, its analgesic effects were inhibited by both the MOR antagonist β -funaltrexamine and the KOR antagonist norbinaltorphimine [4, 16, 17]. Based on the findings that oxytocin is a PAM for both MORs and KORs [8, 9], the enhancement of analgesic effects by endogenously released or exogenously administered oxytocin, *via* influences on MOR and KOR activity, could be attributed to analgesic regulation in the body. Overall, the available evidence suggests that oxytocin could be an analgesic agent that behaves as a PAM for MORs and KORs, but not DORs; in other words, oxytocin might act as a protective natural ligand in painful situations by modulating OR signaling [8, 9, 18, 19].

3. Structure–activity relationship study of oxytocin as a PAM for the MOR

Oxytocin is a novel PAM for the MOR, which is structurally unlike other low-molecular-weight PAMs (BMS-986122, MS1, etc.) [20–22]. To identify structural elements of oxytocin that are key for the MOR-PAM activity, a structure–activity relationship study was carried out using several oxytocin analogs [23]. In this study, oxytocin analog **1** was a linear peptide that included two *S*-carbamoylmethylcysteines (**Figure 3**). Modification of the tool groups of bioactive cyclic peptides is often performed to examine whether the cyclic structure with an intramolecular disulfide bond is necessary for a molecule's function [24]. However, unlike oxytocin, linear analog **1** did not enhance the agonistic activity of EM-1 for the MOR; in other words,

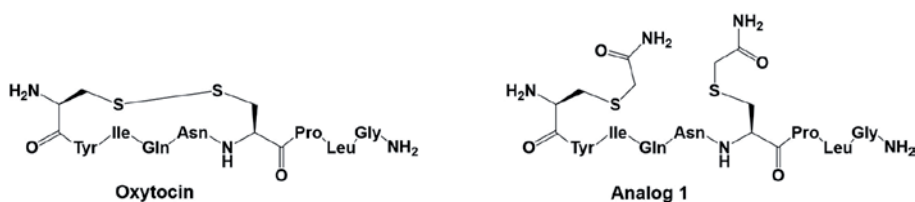


Figure 3.
Structures of oxytocin and analog 1 with S-carbamoylmethylcysteines.

analog 1 did not function as a PAM for the MOR. This finding indicates that the MOR-PAM activity does not rely on the amino acid sequence but rather on the cyclic structure of oxytocin.

Analog 2 included an intramolecular ethylene bridge ($-\text{CH}_2-\text{CH}_2-$) that formed the same 20-membered ring as in oxytocin (**Figure 4**). It was expected to exhibit levels of flexibility and hydrophobicity similar to a disulfide linkage in a cyclic peptide. Analog 3 was another linear peptide, which was related to analog 2. Like analog 1, analogs 2 and 3 did not enhance the MOR agonistic activity of EM-1 at all. These findings with analog 3 reconfirmed that linear oxytocin analogs did not exert MOR-PAM effects. Moreover, the results with analog 2 demonstrated that a cyclic structure containing the disulfide linkage, as found in oxytocin, would be more useful for developing new analogs with MOR-PAM activity.

Analog 4 included an intramolecular amide bridge instead of the disulfide bond found in oxytocin (**Figure 5**). The ring size in analog 4 was nearly the same as that of oxytocin, with both comprising 20-membered rings. This conversion is often used to examine the necessity of a disulfide structure in bioactive peptides [25–27]. The more stable oxytocin analog carbetocin also has an intramolecular amide linkage [28, 29]. Based on their retention times in RP-HPLC analyses, analog 4, oxytocin, and analog 2 each exhibited similar hydrophobicity [23]. However, the assay results indicated that analog 4 did not significantly function as an MOR-PAM (em-oh-ar). A likely explanation is that the amide tether with partial double-bond character might make the cyclic structure of analog 4 more rigid than that of oxytocin. Notably, the results with analogs 2 and 4 suggested that the flexible disulfide bridge of oxytocin may play an

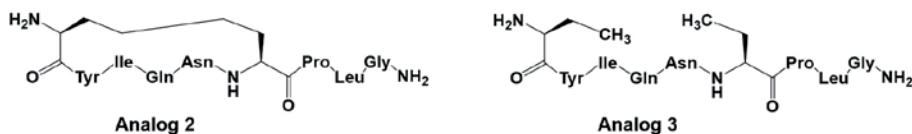


Figure 4.
Structures of analogs 2 and 3.

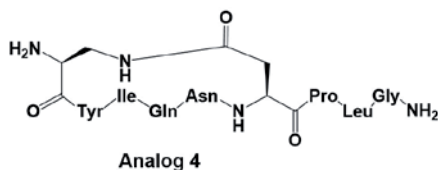


Figure 5.
Structure of analog 4.

important role in the MOR-PAM activity. In particular, the sulfur atoms of the cyclic structure in oxytocin are likely crucial for this function.

Analogs 5–10 (Figure 6) were prepared to examine how MOR-PAM activity was affected by the configuration at the Cys residues and the steric effect around the disulfide bridge of oxytocin. These analogs had two d-Cys or penicillamine (Pen) residues at positions 1 and 6, instead of the cysteine residues found on oxytocin. Pen has geminal dimethyl groups at the β -position, and is a cysteinyl analog that can be used to investigate the effects of the side-chain on biological activities [30, 31]. Analogs 5–10 did not exhibit any PAM activities at the MOR on intact cells. These findings for analogs 5–7 indicated that the two l-cysteine residues in oxytocin likely affect the MOR-PAM activity. The results for analogs 8–10 revealed that the sterically unhindered linker containing a disulfide bond between two l-cysteines of oxytocin is likely a critical structure enabling the PAM activity.

In analogs 11–13 (Figure 7), an amino acid was truncated one by one from the C-terminus of oxytocin, with the aim of examining whether the Pro, Leu, and Gly residues outside the cyclic structure were involved in the MOR-PAM activity. Analogs 11–13 did not enhance the agonistic activity of EM-1, indicating that these three

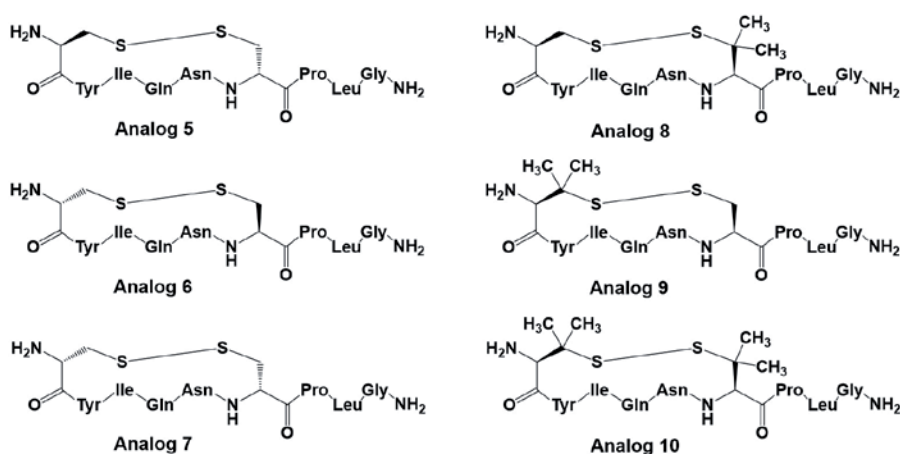


Figure 6.
 Structures of analogs 5–10.

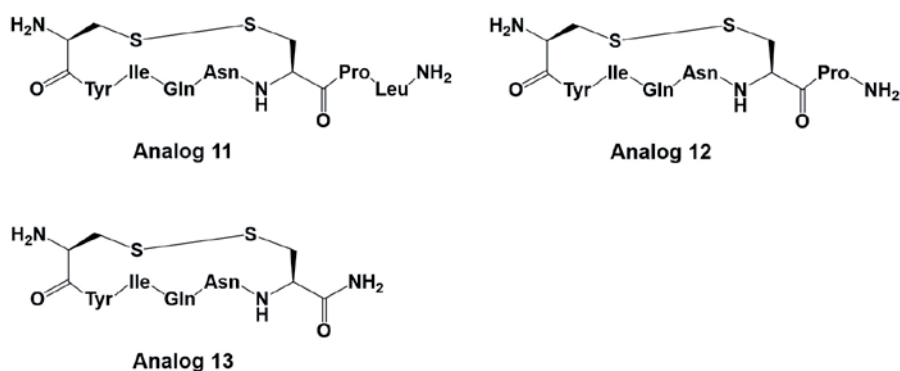


Figure 7.
 Structures of analogs 11–13.

c-terminal residues were likely necessary for the PAM activity. In particular, it can be presumed that the small Gly residue is a key moiety involved in oxytocin's PAM activity for the MOR. This finding is unusual and interesting.

The analyses of analogs 1–13 revealed that oxytocin's cyclic structure with an intramolecular disulfide bridge is a crucial component of the PAM activity. It was also found that three c-terminal residues (Pro, Leu, and Gly) of oxytocin were necessary for PAM activity. Among these residues, the small Gly residue appears to be a particularly critical structure for oxytocin's function as an MOR-PAM (em-oh-ar). There remains a need for a future structure–activity relationship study of oxytocin as a PAM for KOR.

4. Potential use of oxytocin as a pain reliever

Opioid analgesics are the mainstay of medication for severe pain, but they are associated with serious side effects, including constipation, respiratory depression, tolerance for analgesia, dependence, and others [32, 33]. Therefore, formidable efforts have been made to find safer analgesics. Oxytocin would be a promising candidate for safer analgesics. Many reports show that oxytocin exerts antinociceptive effects in experimental animals. Rash et al. performed a database search and systematic review of the articles describing the analgesic effects of oxytocin, which were published between 1950 and 2012 [7]. Their systematic review discussed the oxytocin-induced antinociceptive effects reported in rodents and dogs. In one study, oxytocin was intrathecally administered to patients with low back pain and exhibited analgesic effects [34]. In another study, continuous infusion of oxytocin among patients with irritable bowel syndrome increased their thresholds for visceral perception [35]. Neither clinical trial reported any side effects from oxytocin use. However, Rash et al. concluded that “additional research is needed to determine the stability and reliability of the oxytocin-pain relationship in humans.”

It has been reported that oxytocin can induce antinociception *via* both peripheral and central mechanisms [4–8]. Clinical trials have investigated various routes of administration, including intravenous [34–36], subcutaneous [37, 38], intrathecal [34, 39, 40], and intranasal [41–43] administrations. Some reports show that the central administration of oxytocin produces more profound analgesic effects than systemic administration [34, 44]. Since it is a peptide compound, oxytocin exhibits low permeability through the blood–brain barrier. Thus, it may be preferable to use administration methods that deliver directly to the central nervous system—such as intrathecal administration—but such methods are highly invasive. An alternative option could be intranasal administration. In 2023, Lopes et al. conducted a meta-analysis of 15 clinical trials in which oxytocin was intranasally administered and concluded that intranasal oxytocin administration did not yield significant analgesic effects compared with placebo [42]. However, their analysis has some limitations. Notably, Lopes et al. pointed out that most clinical studies administered only a single dose of intranasal oxytocin. Another limitation is that many trials evaluated the effectiveness against pain induced by experimental stimuli, rather than against pain in pathological conditions.

To overcome the first limitation, Lopes et al. proposed that future studies should investigate multiple doses or continuous administration of oxytocin, rather than a single dose, considering that the effects of oxytocin are short-lasting. Another promising strategy may be to develop more stable and longer-lasting oxytocin derivatives.

Carbetocin is a clinically used oxytocin analog that is longer-lasting than oxytocin itself. In human studies, the half-lives of intravenously injected carbetocin and oxytocin are reported to be 41 minutes [45] and about 10–15 minutes [46–48], respectively. Although no information is available about the half-lives following intranasal administration, carbetocin would be more stable than oxytocin in the central nervous system. Carbetocin has exhibited analgesic effects in clinical trials, albeit such reports are limited [49]. Studies have also examined other oxytocin analogs with enhanced stability that include modifications of the labile disulfide bond moiety in the cyclic structure of oxytocin [50, 51]. Some such analogs have exhibited potent antinociception in mice tail-flick tests following intravenous administration [51].

Compared to nonpeptidic low-molecular-weight compounds, peptide compounds are generally more hydrophilic and more vulnerable to enzymatic hydrolysis and have thus been considered non-druggable compounds. With regards to low-molecular-weight compounds, various rules for drug-likeness have been proposed [52–58], since Lipinsky's Rule of 5 was reported [59]; however, research on the drug-likeness of peptide compounds has not progressed much. The following rules for the drug-likeness of cyclic peptides were recently proposed: (1) comprising 9–11 amino acid residues, (2) exhibiting high hydrophobicity ($\text{CLogP} \geq 12.9$, when 11 residues [60]), (3) possessing $\geq$ six *N*-methyl amino acids, and (4) it is preferable to have fewer polar/charged amino acid residues (up to two hydroxy groups can be tolerated to gain sufficient membrane permeability) [61, 62]. Oxytocin only includes a hydroxy group on the tyrosine residue, has no *N*-methyl amino acid, and has high hydrophilicity ($\text{LogD}_{7.4} = -1.38$ [63]). As oxytocin comprises nine amino acid residues, it appears to meet the criterion of including between 9 and 11 residues. However, this rule was proposed based on the physicochemical properties of cyclic peptides without side chains. Therefore, oxytocin should not be considered to meet the criterion because it comprises two parts: a cyclic structure with six amino acids and a side chain with three amino acids. By considering the rules for the drug-likeness of cyclic peptides, it may be possible to modify the fragile disulfide bond and thereby create more stable oxytocin analogs that exhibit robust analgesic effects.

To adjust the second limitation, it would be important to pay attention to oxytocin's PAM activity for the MOR and KOR. Oxytocin enhances the activity of endogenous opioid peptides, as shown above. Thus, it may be necessary to clinically evaluate the effects of oxytocin on pain under pathological conditions because endogenous opioid peptide levels are elevated in states of stress or pain [64]. Additionally, when planning clinical trials, it may be important to carefully select what types of pain to evaluate.

Although it is an attractive idea to use oxytocin itself or its more stable analogs as an analgesic, the results of the meta-analysis by Lopes et al. strongly suggest that while oxytocin may exhibit analgesic effects, the effects are not very potent. Considering that oxytocin has PAM activity for the MOR and KOR, it may be more useful to apply oxytocin as an analgesic adjuvant. BMS-986122 has been reported to be a PMA for the MOR, which enhances the [^{35}S]GTP γ S binding activities of morphine and DAMGO [20], and the intracerebroventricular administration of BMS-986122 in mice enhanced the antinociception of methadone and morphine in hot-plate tests [65]. Another MOR-PAM, MS1, and its derivative, Compd5, are also reportedly useful as analgesic adjuvants. In mice, MS1 boosted the antinociception of methadone in hot-plate tests and a model of post-operative pain. In a model of inflammatory pain induced by complete Freund's adjuvant, MS1 potentiated the analgesia of a low dose of oxycodone, which did not exhibit an analgesic effect on its

own [22]. Compd5 showed similar effects to MS1, although it was tested with different co-administered opioid agonists [22]. Notably, BMS-986122, MS1, and Compd5 did not potentiate the adverse effects induced by MOR agonists [22, 66].

Oxytocin reportedly exhibits antinociceptive effects *via* both OTR-dependent and OTR-independent mechanisms [8, 9]. Therefore, oxytocin is expected to produce profound analgesia as an analgesic adjuvant, through an additive or synergistic effect of the mechanism of action mediated by the OTR plus the PAM effect for the MOR and KOR. However, even if oxytocin is used as an analgesic adjuvant, its lack of stability would remain an issue that must be tackled. When developing stable oxytocin derivatives for use as analgesic adjuvants, it will be necessary to maintain not only oxytocin activity but also PAM activity. As described above, both the cyclic structure with a disulfide bond and the tripeptide moiety in the C-terminal of oxytocin are indispensable for the MOR-PAM activity. Thus, although the disulfide bond in the cyclic structure is labile, modification of this moiety is hardly permissible. It is presently unclear whether the rules for drug-likeness of cyclic peptides [61, 62] are applicable to cyclic peptides with an oligopeptide side chain. Overall, despite the potential benefits of developing compounds with both oxytocin and PAM activities for use as analgesic adjuvants, it may be challenging to create compounds with such profiles by improving oxytocin.

It is well-known that the chronic treatment of oxytocin induces the downregulation and/or desensitization of OTR [67–70]. Although it is unclear whether the chronic treatment of oxytocin leads to antinociceptive tolerance, developing new oxytocin derivatives with the following properties may overcome concerns about analgesic tolerance. The development of partial agonists may be effective because partial agonists with low efficacy generally induce less desensitization of receptors compared to full agonists [71]. Another method would be the search for G-protein-biased agonists with no or weak β -arrestin recruitment activity, which is closely related to receptor internalization [72–74]. On the other hand, regarding the analgesic effects of oxytocin through its MOR and KOR-PAM activities, analgesic tolerance may be less of a concern because there are reports that PAMs enabled the delay of desensitization of certain types of receptors induced by their (cognate) orthosteric agonist [75, 76].

5. Conclusion

Oxytocin has reportedly produced analgesic effects in several experimental animals, and its mechanisms of action appear to be related not only to the OTR but also to endogenous opioid peptides. However, details regarding the mechanism of action involving endogenous opioid peptides remain unclear. A recent study demonstrated that oxytocin does not directly bind to the orthosteric sites of the MOR and KOR but rather functions as a PAM for the MOR and KOR (but not the DOR). Moreover, the oxytocin-induced analgesic effects were attenuated by both the MOR antagonist β -funaltrexamine and the KOR antagonist norbinaltorphimine but not by the DOR antagonist naltrindole. Taken together, these findings strongly suggest that oxytocin's PAM activities for the MOR and KOR play an important role in its analgesic effect.

Since oxytocin is a G-protein-biased PAM for MOR and KOR, oxytocin itself is a promising candidate for the next generation of analgesics. However, oxytocin has a short half-life and short-lasting effects. Therefore, it would be preferable to develop more stable and long-lasting oxytocin analogs with PAM activity. As oxytocin is

structurally different from other known MOR-PAMs, it is unclear which structural parts are essential for its PAM activity. A recent investigation revealed that the cyclic structure and the C-terminal three amino acid residues are crucial for oxytocin's MOR-PAM activity. Specifically concerning the cyclic structure in oxytocin, the disulfide bridge and the configuration of the cysteine residue (1-Cys) are essential. This information will be very important for designing new oxytocin analogs with PAM activity.

Although the stability of oxytocin remains an issue, since oxytocin is already in clinical use, developing oxytocin itself as an analgesic has advantages from the perspective of drug repositioning. Indeed, several clinical trials have investigated oxytocin as an analgesic, but unfortunately, the results have not demonstrated that oxytocin has significant analgesic effects compared to placebo. As mentioned by Lopes et al., it may be important to identify the optimal method of oxytocin administration. To take full advantage of oxytocin's properties—for example, its MOR-PAM and KOR-PAM activities—it would also be useful to examine oxytocin's effects on pain under pathological conditions.

Another potential clinical use of oxytocin is as an analgesic adjuvant. PAMs for the ORs, especially the MOR, are believed to be able to potentiate the antinociceptive effects of opioid analgesics, as well as reduce side effects, such as constipation, respiratory depression, dependence, and so on. Furthermore, because oxytocin enables the induction of analgesic effects *via* the OTR, it may be expected to yield additive or synergistic effects. Hopefully, the new finding that oxytocin has G-protein-biased PAM activity for the MOR and KOR will lead to the development of new pain treatments, as well as further investigation of oxytocin's physiological roles.

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Section 2

Advances in Drug
Formulation and
Pharmacological Strategies

Lipid-Based Oral Formulation Strategies for Enhancing Bioavailability of Aromatase Inhibitors

*Chowdhury Mobaswar Hossain, Sabyasachi Choudhuri,
Rituparna Das, Suravi Saha and Anusmita Banerjee*

Abstract

Aromatase inhibitors (AIs) are frontline agents in the hormonal treatment of estrogen receptor-positive breast cancer. However, their poor aqueous solubility, variable gastrointestinal absorption, and extensive first-pass metabolism significantly limit their oral bioavailability and therapeutic efficacy. Lipid-based drug delivery systems—including self-emulsifying drug delivery systems (SED DS), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and liposomes—offer promising strategies to overcome these limitations by enhancing solubility, permeability, lymphatic transport, and cellular uptake. This chapter presents a mechanistic and comparative review of lipid-based oral formulations designed to improve the pharmacokinetics, pharmacodynamics, and therapeutic outcomes of AIs. We critically evaluate the physicochemical properties, biopharmaceutical mechanisms, in vitro and in vivo performance, and translational potential of these delivery systems. Emphasis is placed on P-glycoprotein (P-gp) inhibition, CYP450 metabolism modulation, and lymphatic uptake pathways. Furthermore, we discuss ADMET considerations, IC₅₀ values, pharmacokinetic parameters, and the limitations of current preclinical models. By integrating formulation science with breast cancer pharmacotherapy, this chapter aims to provide a coherent framework for the development of next-generation oral AI therapies.

Keywords: aromatase inhibitors, lipid-based drug delivery, bioavailability, SED DS, SLNs, nanostructured lipid carriers, pharmacokinetics, breast cancer

1. Introduction

Breast cancer is the most frequently diagnosed malignancy among women worldwide, with estrogen receptor-positive (ER+) subtypes accounting for a significant majority of cases. Aromatase inhibitors (AIs), such as letrozole,

anastrozole, and exemestane, are key agents in endocrine therapy, functioning by inhibiting the aromatase enzyme responsible for estrogen biosynthesis from androgens. By reducing systemic and intratumoral estrogen levels, AIs help suppress tumor proliferation and recurrence. However, despite their clinical efficacy, AIs suffer from critical pharmacokinetic drawbacks—including poor aqueous solubility, low permeability across the gastrointestinal (GI) barrier, extensive first-pass metabolism, and significant interindividual variability in absorption.

Addressing these biopharmaceutical limitations is an essential step for optimizing AI therapy. Lipid-based oral drug delivery systems have emerged as a transformative platform in this context. These formulations—ranging from SEDDS and SNEDDS to SLNs, NLCs, and liposomes—offer multiple mechanistic advantages: enhancing drug solubilization, bypassing hepatic metabolism via lymphatic transport, inhibiting efflux transporters like P-glycoprotein (P-gp), and improving permeability through epithelial tight junction modulation [1]. Moreover, these systems provide opportunities for site-specific delivery, controlled release, and improved patient compliance.

The aim of this chapter is to provide a comprehensive, mechanism-driven review of lipid-based formulation strategies for enhancing the oral bioavailability of AIs. Rather than listing individual formulations, we categorize delivery systems by their molecular targets (e.g., P-gp, CYP450 enzymes), pharmacokinetic behavior, and formulation class. We integrate evidence from *in vitro*, *in vivo*, and clinical studies, with an emphasis on pharmacokinetic parameters (e.g., $C_{\max}$, $T_{\max}$, and AUC), IC_{50} values, and ADMET profiles. Additionally, we critique the methodological rigor of the studies reviewed and identify translational gaps and safety concerns that must be addressed in future clinical development. This chapter seeks to guide researchers and formulators in designing next-generation delivery systems that can maximize therapeutic efficacy while minimizing systemic toxicity and variability.

2. Challenges faced in oral delivery of aromatase inhibitors

Oral delivery of AIs has become the standard of care in the treatment of hormone receptor-positive breast cancer due to its convenience, improved patient compliance, and quality of life [2]. The therapeutic efficacy of AIs is, however, adversely affected by a few significant difficulties associated with this mode of administration [3]. To improve the clinical efficacy of AI therapy, it is crucial to recognize and address these issues as follows.

2.1 Poor aqueous solubility

Aromatase inhibitors, such as anastrozole, letrozole, and exemestane, are characterized by their poor aqueous solubility [4, 5, 6]. The reduced solubility caused by this property in the gastrointestinal fluid ultimately inhibits medication absorption. Poor solubility is a major barrier as it affects the quantity of the medicine that is accessible for systemic circulation, thereby restricting the therapeutic impact.

2.2 Low permeability

Even if an AI is dissolved, the rate at which it can pass through the intestinal epithelial barrier remains a significant challenge [7]. The low permeability of AIs

can result in incomplete absorption, which is further compounded by their poor solubility. Low permeability hinders the drug's ability to reach its target site of action, reducing its effectiveness.

2.3 Variable absorption

The absorption of AIs can vary significantly between individuals due to various factors such as the presence of food in the stomach, gastric pH, and the activity of efflux transporters. These variations make it challenging to achieve consistent therapeutic drug levels. They also run the danger of giving patients an inadequate or excessive dosage, which would compromise both safety and effectiveness.

2.4 First-pass metabolism

AIs must traverse to the liver upon absorption where they undergo significant first-pass metabolism. The metabolism of some AIs can be extensive, leading to the formation of active and inactive metabolites. This first-pass effect can further reduce the amount of active drug available for therapeutic action [8].

2.5 Drug-drug interactions

AIs are frequently used with other drugs, including cancer medicines or selective estrogen receptor modulators (SERMs). The potential for drug-drug interactions poses a challenge in achieving optimal AI levels [9]. Some concomitant medications impair with AI's metabolism, potentially leading to suboptimal drug levels or adverse effects.

2.6 Side effects

Oral administration of AIs comes with gastrointestinal side effects, such as nausea, diarrhea, or abdominal pain. These side effects can reduce patient compliance and potentially impact treatment efficacy. Furthermore, patients may become reluctant to continue AI therapy if they continue to experience severe discomfort. There are also other side effects such as long-term use of tamoxifen increases the risk of endometrium cancer and anastrozole decreases bone mineral density and increases risk of fracture [10, 11].

2.7 Patient compliance

Patient compliance is a crucial factor in the success of AI therapy. The need for long-term, often 5–10 years administration of AIs makes it challenging for some patients to adhere to the treatment regimen. There are several factors such as side effects, pill burden, and the duration of therapy that can all contribute to poor compliance [12].

2.8 Resistance development

Prolonged oral AI therapy may lead to the development of resistance in some patients. This resistance can be attributed to various factors, including changes in the tumor's genetic profile and clonal evolution. Sometimes, AI treatment regimen and drug needs to be changed as per patients' need. The risk of resistance highlights the need for alternative delivery strategies to optimize treatment outcomes.

2.9 Pediatric and geriatric populations

AIs are also used in special populations, including pediatric and geriatric patients. Dosing, solubility, and administration can be especially challenging in these groups, and the need for tailored approaches to address these challenges is evident.

2.10 Regulatory considerations

The development and approval of new drug delivery systems for AIs can be a complex and lengthy process, involving rigorous regulatory requirements. These requirements include demonstrating the safety and efficacy of the proposed delivery system, making it imperative for manufacturers to meet these standards while addressing the unique challenges of AI oral delivery [8].

The oral delivery of aromatase inhibitors faces several challenges that impact their bioavailability, efficacy, and patient compliance. These challenges include poor aqueous solubility, low permeability, variable absorption, first-pass metabolism, drug-drug interactions, gastrointestinal side effects, and the development of resistance. Addressing these issues is essential to optimize the therapeutic benefits of AI therapy and improve the quality of life for patients undergoing treatment. Innovations in drug delivery technologies, personalized medicine approaches, and ongoing research are likely to play a pivotal role in overcoming these challenges and improving the clinical outcomes of AI therapy.

3. Lipid-based formulations for enhancing bioavailability of aromatase inhibitors

To address the bioavailability challenges of AIs, lipid-based formulations offer several mechanistically distinct and pharmaceutically viable platforms. These systems enhance the solubilization of poorly water-soluble drugs, improve intestinal permeability, reduce P-gp mediated efflux, and facilitate lymphatic absorption, thereby bypassing hepatic first-pass metabolism. This section outlines the fundamental physicochemical attributes and solubilization mechanisms that enable lipid-based carriers to optimize AI delivery.

3.1 Physicochemical properties of lipid-based formulations for oral drug delivery

Lipid-based formulations vary significantly in composition, structure, and interaction with biological membranes. Key formulation attributes influencing AI bioavailability include:

3.1.1 Type of lipids

Lipids serve as solvents and structural matrices. Long-chain triglycerides (LCTs) and medium-chain triglycerides (MCTs) enhance solubilization and lymphatic transport [13]. Phospholipids form bilayer vesicles like liposomes and micelles, providing amphiphilic environments for hydrophobic drug encapsulation.

3.1.2 Surfactants and co-surfactants

Surfactants like Tween 80, Cremophor EL, and Labrasol reduce interfacial tension, stabilizing emulsions [14]. Co-surfactants (e.g., ethanol, PEG 400) further enhance self-emulsification and facilitate micro-/nano-emulsion formation. These excipients can also inhibit P-gp and modulate tight junctions [15].

3.1.3 Droplet size and surface charge

Formulations with droplet sizes <200 nm increase surface area and drug dissolution rates. Zeta potential influences particle stability and mucosal interactions. Nanometric emulsions can facilitate enhanced permeation through intestinal epithelia [16].

3.1.4 Phase behavior and stability

Lipid-surfactant systems exhibit varying phase behavior (e.g., liquid crystals, lamellar phases), affecting dispersion and digestion [17]. Stability under gastrointestinal pH and enzymatic conditions is essential for preserving formulation integrity.

3.2 Mechanisms of enhanced bioavailability

3.2.1 Improved solubilization

Lipophilic AIs like letrozole and exemestane exhibit higher solubility in lipidic matrices compared to aqueous media. Solubilization in lipid carriers ensures better dissolution in the gastrointestinal tract, critical for absorption.

3.2.2 Permeability enhancement

Surfactants can transiently open tight junctions and disrupt membrane lipids, increasing transcellular and paracellular transport [18]. Some lipids may also interact with membrane transporters to facilitate uptake.

3.2.3 Lymphatic transport

Highly lipophilic drugs ($\log P > 5$, high triglyceride solubility) can associate with chylomicrons and bypass hepatic first-pass metabolism via the intestinal lymphatic system [19]. This pathway significantly enhances systemic bioavailability.

3.2.4 P-gp inhibition

Excipients like Cremophor EL and Pluronic block copolymers inhibit P-gp-mediated efflux. This allows intracellular accumulation of AIs, especially relevant for agents subject to efflux transporters in the gut [20].

3.2.5 Protection from degradation

Encapsulation within lipid matrices can shield AIs from acid and enzymatic degradation in the stomach and intestines, preserving drug potency.

Drug	Lipid system	Size (nm)	IC ₅₀ (MCF-7)	Mechanism	Reference
Letrozole	SLN	~150	9.1 μ M	Lymphatic transport	[21]
Anastrozole	SNEDDS	<100	12.5 μ M	P-gp inhibition	[22]
Exemestane	NLC	~120	8.6 μ M	CYP3A4 metabolism evasion	[6]

Table 1.
AI formulation with pharmacodynamic and pharmacokinetic enhancements.

3.3 Formulation examples and performance

This table summarizes representative AI formulations with characterized pharmacodynamic and pharmacokinetic enhancements (**Table 1**). However, further preclinical and clinical validation is essential for translational progress.

4. Self-emulsifying drug delivery systems (SEDDS) and their applications in AI therapy

SEDDS represent an advanced lipid-based oral formulation technology specifically designed to improve the solubility and absorption of poorly water-soluble drugs such as AIs. These systems spontaneously form fine oil-in-water emulsions upon mild agitation in the GI tract, enabling increased surface area, enhanced dissolution, and superior absorption. The mechanism of action is shown in **Figure 1**.

4.1 Composition and classification

SEDDS formulations typically consist of oils (e.g., medium-chain triglycerides) [23], surfactants (e.g., Tween 80, Cremophor EL) [14], and co-surfactants (e.g., ethanol, PEG 400) [15]. They are classified based on droplet size:

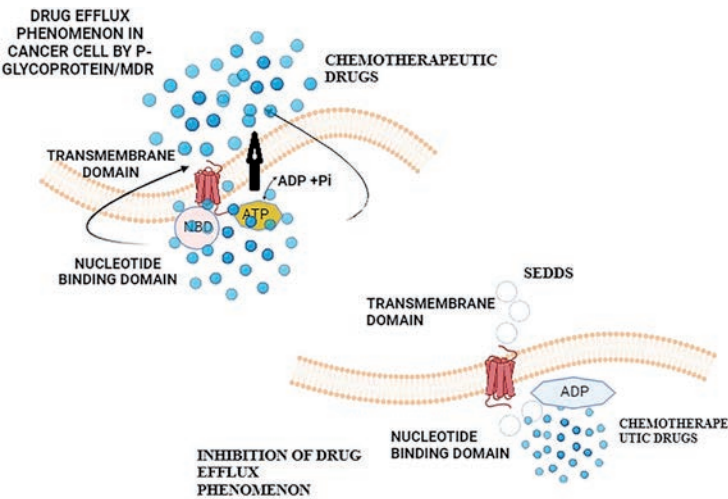


Figure 1.
Probable mode of action of SEDDS by blocking the efflux pump in cancer cells.

- SEDDS: emulsion size >250 nm
- SMEDDS (self-microemulsifying): 100–250 nm
- SNEDDS (self-nanoemulsifying): <100 nm

The small droplet sizes of SNEDDS facilitate closer contact with the intestinal epithelium and improved drug transport. The classification of lipid formulation is shown in **Table 2**.

4.2 Mechanisms of enhanced AI absorption

4.2.1 Increased solubilization surface area

SNEDDS systems generate nanoscale droplets, increasing surface area for drug release and facilitating improved dissolution kinetics in the intestinal lumen.

4.2.2 Inhibition of P-glycoprotein

Several surfactants in SEDDS, including Labrasol and Cremophor EL, inhibit P-gp efflux transporters. This enhances intracellular accumulation of AIs, which are often substrates of these transporters [15].

4.2.3 Enhanced lymphatic uptake

By incorporating long-chain triglycerides, SNEDDS promote chylomicron formation and lymphatic transport. This bypasses hepatic first-pass metabolism, improving systemic exposure of AIs.

4.2.4 Transient tight junction modulation

Surfactants can transiently open tight junctions in epithelial cells, enhancing paracellular permeability of AIs.

Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils without surfactants	Oil and water insoluble surfactants	Oil, surfactants, co-solvents (both water-insoluble and water-soluble excipients)	Oil, surfactants, co-solvents (both water-insoluble and water-soluble excipients)	Water-soluble surfactants, cosolvents (no oils)
Non-dispersible	Emulsion (SEDDS)	SEDDS/SMEDDS formed with water soluble surfactants	SEDDS/SMEDDS formed with water soluble surfactants and low oil contents	Disperses typically to form a micellar solution
Requires digestion	Will be digested	Digestion may not be necessary	Digestion may not be necessary	Limited digestion

Table 2.
Lipid formulation classification system.

Formulation	Drug	Droplet size	IC ₅₀ (MCF-7)	Absorption mechanism	Reference
SNEDDS	Letrozole	85 nm	8.4 μ M	Lymphatic + P-gp inhibition	[22]
SMEDDS	Exemestane	120 nm	10.2 μ M	Enhanced solubilization	[6]
SEDDS (Type IIIB)	Anastrozole	~250 nm	11.5 μ M	Tight junction modulation	[24]

Table 3.

Formulation case studies and its mechanism of action.

There are multiple case studies to prove the above-mentioned mechanisms. A brief study is mentioned in **Table 3** shows a brief case study of few of them.

4.3 Different types of SEDDS and advantages and disadvantages of SNEDDS over SMEDDS

In this context, the demonstration in **Table 4** which depicts the different types of SEDDS based on composition and stability, the probable clinical advantages and disadvantages are pointed below:

- SNEDDS are nano sized isotropic mixtures rapidly dispersed into fine globules upon gastric motility and fine droplets of drug dispersed in oil interface enhances the surface area for more distribution in the gastrointestinal tract.
- The nano sized SNEDDS could by-pass the rate limiting steps of dissolution and reduces the first pass metabolism, thereby increasing the lymphatic absorption with better therapeutic response [25, 26].
- SNEDDS comes with improved penetration capability for poorly permeable drugs falling under BCS class III, thus enhancing their permeability and penetration through intestinal area. In an investigation, it was mentioned that SNEDDS

Properties	SEDDS	SMEDDS	SNEDDS
Size	>300 nm	<250 nm	<100 nm
Appearance	Turbid	Optically clear	Optically clear
Classification as per the lipid formulation classification system	Type II	Type IIIB	Type IIIB
Concentration of oil	40–80%	>20%	>20%
Concentration of surfactants	30–40%	40–80%	40–80%
Stability	Thermodynamically unstable	Thermodynamically unstable Kinetically stable dispersion	Thermodynamically stable Kinetically unstable dispersion

Table 4.

Types of SEDDS and their physiological properties.

can result into rapid onset of action of drugs by improving permeability and oral absorption of drugs [27, 28]. The study reported that SNEDDS formulation $t_{\max}$ was 6 h which was reduced by two-folds (12 h) compared to a conventional formulation indicating a quicker onset of action.

- Inactivation of P-gp efflux pump is one of the most significant criteria observed with SNEDDS formulation. Some formulations consisting of lipids, namely, glyceryl monooleate, akoline MCM (diglyceride of caprylic acid), monoglyceride of caprylic acid, and some surfactants like labrasol, cremophore EL, and polysorbate 80 are capable of increasing the absorption of drugs through the intestinal wall by inhibiting the P-gp efflux pump and improving the bioavailability of the drugs [26].

4.4 Physiochemical and biological barriers causing low therapeutic efficacy of anticancer drugs and aromatase inhibitors

4.4.1 Poor aqueous solubility

Therapeutic response of a drug can only be achieved if rate and distribution of the drug in systemic circulation is optimum. Decreased aqueous solubility of a drug has impeded therapeutic efficacy, and BCS (Biopharmaceutical Classification System) classifies this class of drugs as not being soluble in 250 mL or less aqueous media over pH 1–7.5 range upon oral administration [29]. Anticancer drugs like etoposide, paclitaxel, and docetaxel are chemically weak acid or base and hence possess poor aqueous solubility which causes high investment of lattice energy to dissolve them.

4.4.2 Low membrane permeability

Higher or lower log P values are responsible for poor permeability of drug molecules through intestinal epithelium. Drug molecules are often transported through intestinal membrane by three different transport system, namely, carrier-mediated active transport system, concentration-dependent passive transport system, and specialized routes such as paracellular transport and endocytosis. Biological membrane permeability and oral bioavailability of anticancer drugs such as letrozole, anastrozole, doxorubicin, cyclophosphamide, and methotrexate are classified as BCS class III drugs [29, 30].

4.4.3 Transmembrane efflux pump

P-gp is one of the important members of superfamily of ABC efflux transporters that are expressed in different glands, hepatocytes, kidney, intestinal epithelial membrane, and capillary endothelial cells responsible to excrete drugs back into intestinal epithelium lumen [31, 32]. Oral entry of amphiphilic drug molecules like etoposide, docetaxel, irinotecan, topotecan, and paclitaxel are limited due to the expression of P-gp expression in the apical membrane of the intestinal lumen. MRP2 and MRP4 are members of MRP family (multidrug resistance-associated protein family) usually overexpressed in the apical membrane of hepatocytes, placenta, and intestines which enhances the efflux of uncharged hydrophobic molecules like cisplatin, vincristine, vinblastine, docetaxel, topotecan and irinotecan. Another important multidrug-resistance efflux protein transporter named BCRP (breast cancer-resistance protein)

is expressed in the liver, small intestine, mammary gland, colon, bladder, and kidney due to cross-resistance to diverse class of chemotherapeutic agents [33].

4.4.4 Presystemic metabolism

The amount of drug absorbed from the intestinal lumen and the extent of first-pass metabolism are the cumulative function of oral bioavailability of a drug. Both liver and intestinal enzymes metabolize a significant amount of orally administered drugs. Anticancer drugs significantly face the presystemic metabolism issues and reduction in therapeutic efficacy when delivered orally, thereby reducing the proper clinical outcome. CYP450 family of microsomal enzymes are secreted from the endoplasmic reticulum of liver hepatocytes responsible for metabolizing the drugs by Phase I metabolism reactions [34].

4.5 Clinical and translational considerations

While SEDDS demonstrate significant improvements in preclinical bioavailability and pharmacokinetics, challenges remain in clinical translation. These include the following:

- Variability due to fed vs. fasted state
- Long-term stability and scalability
- Limited human pharmacokinetic data
- Regulatory hurdles regarding excipient safety

4.6 Future directions

Emerging strategies involve hybrid systems such as SEDDS-in-capsules or SEDDS adsorbed onto solid carriers for improved dose precision and patient compliance. Further studies are needed to assess long-term safety, optimize formulations for lymphatic targeting, and evaluate efficacy in clinical breast cancer models.

5. Solid lipid nanoparticles (SLNs) for aromatase inhibitor delivery

Solid lipid nanoparticles (SLNs) are submicron colloidal carriers composed of physiological lipids that remain solid at both room and body temperatures [35]. As promising alternatives to traditional carriers, SLNs exhibit high biocompatibility, protect encapsulated drugs from degradation, and provide controlled release properties. Their lipidic matrix enables efficient loading of lipophilic AIs, enhancing systemic availability and therapeutic efficacy.

5.1 Composition and advantages

SLNs are typically formulated from triglycerides, fatty acids, and waxes, stabilized by surfactants such as poloxamers or lecithin [35, 36]. Advantages include the following:

- Protection from enzymatic and chemical degradation
- Enhanced stability compared to liposomes or emulsions
- Reduced risk of systemic toxicity due to controlled release
- Improved patient compliance through oral administration

5.2 Mechanisms of enhanced bioavailability

SLNs enhance AI delivery through multiple mechanisms:

- *Sustained release*: Matrix structure allows gradual drug diffusion.
- *Mucosal adhesion*: Prolongs gastrointestinal residence time.
- *Lymphatic uptake*: Promoted by lipophilic matrix components.
- *Efflux transporter modulation*: Certain SLN excipients inhibit P-gp activity.

The mechanism of actions mentioned has various outcomes based on the cell line used as shown in **Table 5**.

5.3 Limitations and challenges

Despite promising data, SLNs face several formulation and translational challenges also shown in **Table 6**.

Drug	SLN type	Cell line/model	IC ₅₀	Key outcome	Reference
Letrozole	Drug-enriched core	MCF-7 (in vitro)	8.9 μ M	Apoptosis induction, uptake $\uparrow$	[37]
Anastrozole	Solid solution	Wistar rats (oral)	—	$\uparrow$ Bioavailability $\times 3$	[38]
Exemestane	Core-shell	MDA-MB-231	9.6 μ M	Tumor inhibition, P-gp inhibition	[39]

Table 5.
Preclinical evidence of SLNs.

SLN type	Study type	Advantages	Disadvantages	Remarks
Solid solution model	<i>In vitro</i>	• Long-term drug release capacity • Correlation with lipids	Drug stability issues	Efficient when combined with lipophilic drugs
Drug enriched-shell model	<i>In vitro</i>	• Effective burst release • Risen drug solubility	Inappropriate for extended release	Necessary for control burst release
Drug enriched-core model	<i>In vitro</i>	Regulated drug release	Lack of loading capacity	Applicable to highly soluble drugs

Table 6.
Advantages and disadvantages of various types of SLNs.

Name of formulation	Pharmacological effect	Drug loaded	Study type	Reference
RES-SLN	• Inhibit cell proliferation in dose-dependent manner in MDA-MB-231 cell lines • Cell cycle arrest at G0/G1 phase through cyclin D-dependent pathway	Resveratrol	<i>In vitro</i>	[35]
RGD-DOX-SLN	• Suppress tumor growth • Anti-tumor effect by i.v. administration	Doxorubicin	<i>In vitro, in vivo</i>	[36]
SLN-DTX	• Arrest cell growth at G2/M phase • Arrest cell proliferation and breast cancer metastasis	Docetaxel	<i>In vitro, in vivo</i>	[36]
FA-DATS-SLNs	• Cytotoxicity at aggressive TNBC • Upregulate pro-apoptotic caspase-9 but downregulate anti-apoptotic Bcl2	Folic acid conjugated diallyl trisulfide	<i>In vitro</i>	[40]

Table 7.

Clinical applications of drug loaded SLN/LNP in breast cancer therapy.

- *Polymorphic transitions* affecting drug release kinetics
- *Low drug loading capacity* for some APIs
- *Potential aggregation during storage*
- *Need for precise particle size control* for reproducibility

5.4 Translational outlook

To improve clinical translation, future strategies include the following:

- Surface functionalization for *targeted delivery* (e.g., HER2, ER α antibodies) [40]
- Co-delivery of *multiple agents* (e.g., AI + chemotherapeutic) [41]
- Integration with *bioresponsive coatings* for GI-triggered release

Overall, SLNs represent a versatile and evolving platform for oral delivery of AIs with strong potential for improving breast cancer treatment outcomes. There are multiple clinical applications of SLN/LNP as shown in **Table 7**.

6. Nanostructured lipid carriers (NLCs) and their role in AI bioavailability enhancement

Nanostructured lipid carriers (NLCs) are the second-generation lipid nanoparticles designed to overcome the limitations of solid lipid nanoparticles (SLNs), such

as low drug-loading capacity and potential drug expulsion during storage [35]. NLCs incorporate both solid and liquid lipids, leading to an imperfect crystalline structure that allows higher drug solubilization and sustained release [42]. Their tunable physicochemical properties and superior encapsulation efficiency make them attractive candidates for delivering aromatase inhibitors (AIs).

6.1 Composition and structural advantages

NLCs are formulated using a blend of solid lipids (e.g., glyceryl monostearate, stearic acid) [43] and liquid lipids (e.g., oleic acid, medium-chain triglycerides) [44], stabilized with surfactants like poloxamer 188 or Tween 80. The various excipients used in the formulation of NLCs are shown in **Table 8**. This lipid mixture produces a less-ordered matrix that:

- Enhances drug loading and entrapment efficiency
- Minimizes drug crystallization and expulsion
- Enables controlled and sustained drug release
- Maintains long-term physical stability

6.2 Types of NLCs

NLC type I (imperfect crystal model): Imperfect kind of crystal NLC is made up of a very disorganized matrix with plenty of holes and spaces that can hold plenty of drug molecules in amorphous clusters. These crystal order flaws are brought about by combining sufficient amounts of liquid and solid lipids (oils) [35, 45].

NLC type II (multiple type): Water, lipid, and oil types make up multiple type NLC. Drugs that are lipophilic dissolve more readily in liquid fats than in solid fats. This concept results in the creation of several types of NLC with a high liquid lipid content [44, 46].

NLC type III (amorphous type): The formulation of amorphous-type NLC involves meticulously combining lipids to reduce medication leakage resulting from the crystallization process [47, 48].

The structure of different types of NLCs is shown in **Figure 2**.

Ingredients	Examples
Solid lipid	Bee Wax, Carnauba Wax 2442, stearic acid, cetyl palmitate, cholesterol, palmitic acid, tristearin.
Liquid lipids (oils)	Castor oil, Davana oil, Palm oil, oleic acid, olive, oil, argan oil, linolenic acid
Emulsifying agents	Pluronic F68 (Poloxamer 188), pluronic F127 (poloxamer 107), sodium deoxycholate, sodium glycocholate,
Counter ions	Sodium hexadecyl sulfate, monodecyl phosphate, dextran sulfate sodium (DSS), soyabean oil.

Table 8.
Excipients used in formulating NLCs.

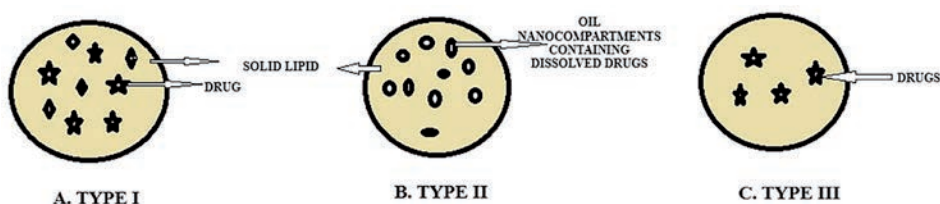


Figure 2.
Types of NLC.

6.3 Hindrances associated with lipophilic drugs upon oral delivery

According to the biopharmaceutical classification system (BCS), these medications are typically classified as Class II or IV. Their low water solubility is the reason for their low oral bioavailability. In addition, various variables include intra-enterocyte metabolism, drug efflux by P-gp [49, 50], hepatic first pass metabolism, and chemical and enzymatic degradation may be responsible for the limited oral bioavailability of these medications.

6.3.1 Intestinal barrier

The primary hydrophilic barrier preventing the absorption of hydrophobic medications is the unstirred water layer (UWL), which divides the small intestine lumen's bulk fluid phase from the brush border (apical) membrane of enterocytes [51]. The UWL must be crossed by drug molecules in the bulk phase of the intestinal lumen in order to reach the brush border membrane [52]. Given the extreme poor solubility of lipids and lipophilic compounds in aqueous fluids, this translates to a significant diffusional barrier for these molecules [53].

6.3.2 Enterocyte CYP3A4 enzyme

Notwithstanding the aforementioned obstacles, a medication molecule faces metabolic barriers upon entering the enterocyte that impact its absorption. The majority of drug metabolism in the intestinal wall is carried out by the enterocyte CYP 3A4 (CYP3A4) enzymes, which are found in the enterocyte's endoplasmic reticulum. It is a significant obstacle to the absorption of lipophilic medicines, as evidenced by numerous studies [54, 55].

6.3.3 P-glycoprotein associated drug efflux

Drug efflux transporters, including P-gp, are also responsible for the low oral bioavailability of a number of medications, including atorvastatin, digoxin, paclitaxel, and doxorubicin [22]. While some of the enterocyte's apical wall's transporters aid in absorption, others serve as efflux transporters. The apical P-gp efflux pumps, which reduce the amount of drug absorption by returning the drug from the enterocyte to the intestinal lumen, are the most thoroughly researched transporters [20]. The P-gp system and the activity of the metabolic CYP3A4 enzymes are related in that they cooperate to stop lipophilic medications from entering the systemic circulation [56].

6.4 Significance of lipid-based formulation to surmount the inadequacies of lipophilic drugs

Nowadays, the usage of formulations including natural lipids in mixtures with surfactants, cosurfactants, and cosolvents has received a lot of attention. Future developments in these lipid-based systems show promise for the administration of medications with low water solubility. These lipidic formulations fall into two general categories: SLNs and LEs. The self-emulsifying drug delivery system, the micro-emulsifying drug delivery system, lipid solutions, emulsions, and micellar systems are examples of LE systems [57]. **Table 8** describes the advantages and disadvantages of NLCs in formulating lipophilic drugs (**Table 9**).

6.4.1 NLCs assistances in improving the *in vivo* drug disposition

Poor solubilization is a significant barrier to the absorption of lipophilic drugs, so it would be advantageous to improve the biliary secretion course by incorporating lipid excipients into the formulation [53]. This would support the upper part of the GIT's emulsification-dependent absorption cascade. Because of the way NLC is made up, both liquid and solid lipids resemble a diet high in fat, which causes the small intestine to secrete bile and combine with it to produce mixed micelles. These micelles would expedite the lipid digestion products' transit across the UWL and increase their luminal solubility, creating a concentration gradient for absorption [58].

6.4.2 Modulation of drug efflux transporters like *P-glycoprotein*

The drug-integrated NLC formulations with particular lipid excipients are significant because they have the ability to significantly change the pharmacokinetics of the delivered substance and influence P-gp mediated efflux activity. Extensive research has demonstrated that certain lipids and surfactants used in NLC can prevent the gut wall's P-gp-mediated drug efflux [22]. Surfactants that have the ability to alter efflux pump activity include Solutol HS 15 and Cremophor EL, Pluronic P85 block copolymer [59]. There are several theories that propose potential mechanisms for inhibiting

Advantages	Disadvantages
Higer drug loading and entrapment competence	Cytotoxicity depends upon the nature of the lipid matrix used for formulating
Variation in drug release	Stability of lipid are questionable
Higher drug loading capacity with minutest surfactant	Delivery of gene, proteins and peptides are still not explored
Probability in loading lipophilic and hydrophilic drugs	Irritable and sensitizing action of surfactants
Long-term stability upon storage	
Ease in penetrating corneal layers of skin	

Table 9.
Advantages and disadvantages of NLCs.

P-gp efflux, including changes to the cell membrane's integrity, competitive, noncompetitive, or allosteric blocking of binding sites, disruption of ATP hydrolysis, and the creation of an ineffective cycle of ATP hydrolysis [60, 61].

6.4.3 Promoting absorption

The liver is not involved in the transport of NLC as it goes through the thoracic lymph duct, the intestinal lymphatic system, and the junction of the left subclavian vein and the jugular vein. It was found that, though medium chain lipids included in NLC are weak lymphatic transport stimulators, long chain lipids used in NLC promote lymphatic transport. It has been shown that the uptake and lymphatic transport of intact colloidal nanosized NLC significantly contributed to the promotion of drug absorption, particularly for liver-metabolized pharmaceuticals including lovastatin, vinpocetine, tamoxifen, and simvastatin [62].

6.4.4 Lymphatic uptake system

When included in NLC, highly lipophilic medications tend to enter the systemic blood circulation through the intestine lymphatic system as opposed to entering the portal blood directly. Since intestinal lymph passes through the liver and enters the systemic circulation directly, the presence of solid and liquid lipids in the formulation modifies the medication's transport pathway to the systemic circulation rather than the portal vein, reducing the likelihood of first-pass drug metabolism. As a result, many lipophilic medications with low solubility can be effectively targeted via the lymphatic system using NLC formulations that have higher bioavailability. Consequently, lymphatic transport can save medications that undergo severe hepatic metabolism during their initial passage via the liver and greatly improve their oral bioavailability [63, 64].

6.5 Bioavailability of lipophilic drugs

The BDDCS classification is used to choose drug candidates for the oral NLC system. Thus, in addition to drug permeability and solubility, other parameters that need to be taken into account are drug metabolism, disposition, and affinity toward efflux transporters, particularly P-gp. Research teams throughout the world have created NLC of extremely lipophilic and weakly water-soluble medications such as vinpocetine, lovastatin, simvastatin, and etoposide utilizing appropriate liquid and solid lipid excipients. When drug-loaded NLC was tested for its anticancer potential in cell cultures, the results showed a significant reduction in the growth of malignant cells (HeLa human cervical cancer cells for CMT-3 integrated in NLC, and MCF-7 and ZR-75-1 for tamoxifen-integrated NLC) [65]. Numerous *in vivo* studies have demonstrated that when medications are manufactured as NLC, they improve the absorption of highly lipophilic and weakly water-soluble medicines. The NLC formulation of hydrophobic medications has significantly increased the oral bioavailability of many medications, including etoposide, SQV, lovastatin, simvastatin, vinpocetine, and fenofibrate [46, 66]. Additionally, the NLC formulations have incredibly tiny particle sizes, which increase the NLC's surface area. The high dispersibility of NLC is another aspect that makes absorption easier in the intestinal environment. In addition, the NLC has the ability to stick to the intestinal wall, extending the duration of residence and, as a result, the absorption [67]. The review provides an interesting explanation

Drug	NLC composition	Size (nm)	IC ₅₀ (MCF-7)	Key outcome	Reference
Letrozole	Stearic acid + OA	135	7.8 μ M	↑ Cellular uptake, ↓ IC ₅₀	[37]
Anastrozole	GMS + MCT	110	10.5 μ M	Sustained release $\times 24$ h	[38]
Exemestane	Precirol + capmul	125	8.1 μ M	Enhanced oral bioavailability $\times 2.8$	[39]

Table 10.
Preclinical evidence and case studies of NLC.

of how NLC interacts with several oral absorption barriers for lipophilic medicines, such as intra-enterocyte, P-gp efflux, UWL, and hepatic metabolism (**Table 10**).

6.6 Application of NLC in chemotherapeutic drug delivery

NLCs' well-established safety and biocompatibility characteristics prompted extensive research on them as delivery vehicles for a range of medical and cosmetic uses. In example, NLCs greatly improved the encapsulation efficiency for labile hydrophilic and hydrophobic pharmaceuticals, shielding them from the body's degradation process, boosting their bioavailability, and regulating their release, demonstrating a rising potential as drug carriers. The use of NLCs as carriers for chemotherapeutic agents has received particular attention because of their capacity to improve the incorporated drugs' physical and chemical stability and to greatly increase the potential of therapeutically effective agents that were withdrawn because of their subpar pharmacokinetic qualities [68]. These studies' usage of NLCs as carriers for various chemotherapeutic medicines shows how impressive it is to increase the medications' therapeutic effects across a wide range of cancers. Research was done to see whether NLCs could address the well-known issue of multiple drug resistance brought on by the prolonged use of chemotherapy drugs. Several solutions to this issue have been made feasible by the high loading capacity of NLC's formulation, which allowed for the mixing of several chemotherapeutic drugs in a single NLC particle. To combat drug resistance following chemotherapy and the risk of lymph cancer relapses, Dong et al. created NLCs containing doxorubicin (DOX) and vincristine (VCR) in combination. Both medications were able to maintain their release for up to 16 and 48 hours, respectively, after being included into NLCs [69]. Wang and colleagues employed a comparable methodology to generate non-lipid cannulas (NLCs) that were loaded with both doximab and PTX, potentially yielding synergistic effects during lung cancer treatment. The NLCs loaded with both anticancer drugs at a PTX/DOX weight ratio of 1/1 demonstrated an IC₅₀ that was three times lower than that of the free drug formula and single drug delivery PTX-NLC and DOX-NLC, according to in vitro cytotoxicity experiments conducted on NCL-H460 large cell lung cancer cells. The co-administration of both chemotherapeutic medicines boosted their ability to target tumors and improved their inhibitory effects, according to an in vivo study using a model of non-small cell lung cancer in mice [70]. Results imply that increased anticancer activity in vivo may not necessarily follow NLC improvement of physiochemical and pharmacokinetic characteristics. For example, non-targeted PTX-loaded NLCs showed an unexpected increase in cell viability, and transferrin-targeted PTX-NLCs showed an observable decrease in U-87 brain cancer cell growth that was exclusively ascribed to the conjugation with transferrin [71].

6.7 Application of NLCs in treatment of breast cancer

One selective estrogen receptor modulator used to reduce postmenopausal women's risk of breast cancer is raloxifene hydrochloride (RLX). Because of its significant first pass metabolism and limited water solubility, RLX has a low bioavailability following oral administration. After being prepared, RLX-loaded NLCs consisting of glyceryl monostearate as SL and glyceryl caprylate as LL (15:85 w:w) with 1% w/v polyvinyl alcohol (PVA) as stabilizer were given orally to healthy female Wistar rats weighing between 200 and 250 g. The control group received RLX oral suspension. The outcomes demonstrated that preparing RLX-loaded NLCs with glyceryl caprylate and glyceryl monostearate in that particular ratio increased the drug's oral bioavailability and pharmacokinetic characteristics [72]. Due to DOX's non-specific distribution to healthy normal tissue, its hazardous side effects, which include cardiotoxicity, myelosuppression, mucositis, and baldness, restrict its therapeutic applicability. By creating co-delivery methods, DOX's anticancer effectiveness must be enhanced while its systemic toxicity must be decreased. One of the most effective platinum drugs, cisplatin (CDDP), is effective against breast cancer. When two medications are used in vitro on tumor cells, D-C-NLCs exhibit the greatest cytotoxicity and synergistic impact. In a breast cancer model, the in vivo research shows the most anti-tumor effectiveness of all the formulations. For the purpose of treating breast cancer, the built NLCs may be utilized to co-deliver CDDP and DOX. One potential targeted and combinational therapeutic nanomedicine is D-C-NLCs [73]. Nanostructured lipid carriers increased LTZ's effectiveness in breast cancer cells MCF-7. These nano-systems changed the course of the cell cycle and improved the induction of apoptosis in cancer cells. In summary, this approach may be thought of as an alternate protocol to traditional techniques.

6.8 Upcoming guidelines

Low molecular weight medications have been the subject of the majority of research on employing NLCs as drug delivery systems for chemotherapeutic medicines. As a result, their range of uses has to be expanded to encompass high molecular weight medicines including peptides, proteins, and nucleic acids that are utilized to treat cancer. This could provide a better chance to treat a larger range of cancers. Indeed, it has been documented that NLCs can function as an innovative vector in cancer gene therapy. Formulating anti-cancer medications into a safe and efficient delivery system that may selectively target tumor cells with fewer systemic adverse effects is one of the major goals of cancer research. NLC is a novel class of lipid-based nanoparticles that is now being used in cancer treatment plans. Lastly, additional research on the absorption, distribution, metabolism, and excretion of NLCs, as well as strategies for scaling up their production, can further explore their potential. In the near future, NLCs may be used in clinical trials, and the results could offer a safer and more effective way to deliver chemotherapeutic agents.

7. Liposomes and lipid-based micelles for aromatase inhibitor delivery

Liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core, making them versatile carriers for both hydrophilic and lipophilic drugs. In the context of aromatase inhibitor (AI) therapy,

liposomal systems have been explored to enhance solubility, control drug release, and enable targeted delivery to tumor tissues via the enhanced permeability and retention (EPR) effect [74].

7.1 Composition and formulation parameters

Liposomes typically include the following:

- Phospholipids (e.g., phosphatidylcholine, phosphatidylserine)
- *Cholesterol* (for membrane rigidity and stability)
- *Surface modifiers* (e.g., PEG for stealth properties)

Formulations may vary by:

- *Lamellarity*: Unilamellar (ULVs), multilamellar (MLVs), multivesicular (MVVs)
- *Size*: Typically, 50–200 nm for systemic applications
- *Charge*: Cationic liposomes may enhance cellular uptake

7.2 Mechanisms of therapeutic enhancement

Liposomes improve AI delivery through several mechanisms (**Table 11**) [75]:

- *Improved solubility*: AIs can be solubilized within the bilayer (lipophilic) or core (hydrophilic)
- *Prolonged circulation*: PEGylation confers stealth characteristics, reducing RES clearance
- *Passive tumor targeting*: EPR effect allows accumulation in tumor interstitium
- *Controlled drug release*: Lipid composition can modulate release kinetics

The difference between liposomes versus other lipid systems is given in **Table 12**.

Drug	Liposome type	Size (nm)	IC ₅₀ (MCF-7)	Key findings	Reference
Letrozole	PEGylated liposome	95	7.2 μM	Tumor targeting, ↑ circulation t _{1/2}	[37]
Anastrozole	Cationic liposome	105	10.8 μM	Enhanced uptake, sustained release	[38]
Exemestane	Neutral liposome	130	8.7 μM	Improved solubility, ↓ systemic toxicity	[39]

Table 11.
Preclinical evidence and case studies of liposome.

Parameter	Liposomes	SLNs/NLCs	SEDDS/SNEDDS
Structure	Bilayer vesicles	Solid/lipidic matrix	Oil-in-water emulsions
Drug location	Bilayer or aqueous core	Lipid matrix	Emulsion droplet
Release profile	Controlled/sustained	Sustained	Fast/immediate
Targeting	Possible (via PEG, ligands)	Limited (passive)	No

Table 12.
Liposomes vs. other lipid systems.

Although most AI liposome formulations are still in preclinical stages, liposomal platforms are FDA-approved for other oncology drugs (e.g., Doxil®), indicating a promising regulatory precedent.

8. Comparative evaluation of lipid-based systems for aromatase inhibitor delivery

A comparative analysis of lipid-based drug delivery systems is essential for identifying the most suitable platform for enhancing the oral bioavailability and therapeutic efficacy of aromatase inhibitors (AIs). Each formulation class—SEDDS, SLNs, NLCs, and liposomes—has distinct physicochemical properties, mechanisms of absorption enhancement, and clinical translation potential. The comparative summary table is shown in **Table 13**.

8.1 Key findings from comparative studies

- *Bioavailability*: SNEDDS and NLCs showed superior enhancement (2–4× increase) over SLNs and conventional formulations in preclinical models [76].
- *IC₅₀ reduction*: Liposomal and NLC formulations reduced IC₅₀ values for letrozole and exemestane by up to 30–40%, indicating improved intracellular delivery [77].
- *Lymphatic transport*: SNEDDS and NLCs demonstrated the most efficient bypass of hepatic first-pass metabolism [78].
- *Drug retention and release*: SLNs and NLCs offered sustained plasma concentrations over 24–48 hours, favorable for once-daily dosing.

Formulation design should be guided by a balance between physicochemical compatibility, desired pharmacokinetic profile, and clinical development pathway.

9. Challenges, safety, and future directions in lipid-based AI delivery

Despite the promising potential of lipid-based delivery systems for enhancing the oral bioavailability of aromatase inhibitors (AIs), several challenges remain that must be addressed to ensure safe, effective, and scalable translation to clinical settings.

System	Structure	Drug loading	Stability	Release profile	Lymphatic transport	Targeting potential	Scale-up feasibility	Key limitations
SEDDS	Isotropic emulsion	Moderate	Good	Immediate/fast	High (with LCTs)	Low	High	Risk of precipitation post-dispersion
SLNs	Solid lipid matrix	Low-Moderate	Good	Sustained	Moderate	Limited	Moderate	Polymorphic transitions
NLCs	Mixed solid-liquid lipid	High	Excellent	Sustained/controlled	High	Moderate	Moderate	Lipid ratio optimization
Liposomes	Bilayer vesicle	Variable	Moderate	Controlled/sustained	Low	High (with PEG/ligands)	Low-Moderate	Short shelf-life, RES clearance

Table 13.
 Comparative summary table.

9.1 Formulation and manufacturing challenges

- *Stability*: Lipid nanoparticles are prone to aggregation, polymorphic transitions, or drug expulsion over time.
- *Encapsulation efficiency*: Especially for lipophilic AIs with poor aqueous solubility, achieving high entrapment without compromising stability is difficult.
- *Scale-up*: Techniques such as high-pressure homogenization, ultrasonication, or micro fluidization require careful optimization for industrial batch sizes.
- *Batch-to-batch reproducibility*: Maintaining consistency in droplet size, zeta potential, and drug release profiles can be challenging.

9.2 Safety and toxicological considerations

- *Excipient toxicity*: Surfactants like Cremophor EL and ethanol may cause GI irritation or hypersensitivity reactions in sensitive individuals.
- *Lymphatic overload*: Excessive use of lipid excipients may alter lipid metabolism or lymphatic function.
- *Immunogenicity*: Particularly relevant to PEGylated or ligand-targeted liposomes, which may induce immune responses.
- *Long-term exposure*: Limited data exist on chronic administration of lipid-based systems in humans.

9.3 Regulatory and clinical barriers

- *Undefined guidelines*: Regulatory agencies still lack standardized evaluation protocols for complex lipid formulations.
- *Limited human data*: Most AI lipid-based systems are supported only by preclinical data, with few clinical trials underway.
- *Cost and accessibility*: Advanced formulations like liposomes or functionalized NLCs may be cost-prohibitive for large-scale use [79].

9.4 Future research directions

- *Smart delivery systems*: pH-sensitive, redox-sensitive, or enzyme-responsive lipid carriers tailored to the tumor microenvironment.
- *Co-delivery approaches*: Combining AIs with siRNA, immune modulators, or other chemotherapeutics within the same lipid vehicle.
- *Personalized formulation design*: AI delivery systems customized to patient-specific pharmacogenomic profiles.

- **ADMET profiling:** Systematic investigation of absorption, distribution, metabolism, excretion, and toxicity for AI-loaded lipid carriers.

To fully leverage the benefits of lipid-based formulations in AI therapy, multidisciplinary collaboration is needed across formulation science, oncology, pharmacology, and regulatory affairs. Future studies should prioritize clinically relevant models, long-term toxicity assessments, and real-world pharmacokinetic validation.

10. Conclusion

Lipid-based oral delivery systems—including SEDDS, SLNs, NLCs, and liposomes—offer powerful platforms for overcoming the biopharmaceutical limitations of aromatase inhibitors. By enhancing solubility, permeability, lymphatic absorption, and cellular uptake, these systems promise to improve therapeutic outcomes in estrogen receptor-positive breast cancer. Continued innovation, combined with rigorous preclinical and clinical validation, will be essential to ensure their successful translation from bench to bedside.

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Chapter 5

The Pharmacotherapy of Corticosteroids

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Abstract

Corticotherapy, also known as corticosteroid therapy, involves the use of corticosteroid medications to treat various inflammatory and autoimmune conditions. Corticosteroids are synthetic drugs that mimic the effects of hormones produced by the adrenal glands. They are powerful anti-inflammatory and immunosuppressive agents. Common corticosteroids include prednisone, dexamethasone, and hydrocortisone. Common indications for corticosteroids, by field, include the following: (1) Allergy and pulmonology: asthma exacerbation, COPD exacerbation, anaphylaxis, and urticaria (2) Dermatology: contact dermatitis, pemphigus vulgaris (3) Endocrinology: adrenal insufficiency, congenital adrenal hyperplasia (4) Gastroenterology: inflammatory bowel disease, autoimmune hepatitis (5) Hematology: hemolytic anemia, leukemia, lymphoma, idiopathic thrombocytopenic purpura (6) Rheumatology: rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, (7) Ophthalmology: uveitis, keratoconjunctivitis (8) Other: organ transplantation, nephrotic syndrome, cerebral edema, multiple sclerosis. While effective, corticosteroids can have side effects, especially with long-term use. These may include weight gain, high blood pressure, diabetes, osteoporosis, increased risk of infections, and mood changes. It is important to use corticosteroids under the guidance of a healthcare professional to manage these risks.

Keywords: corticosteroid therapy, anti-inflammatory agents, immunosuppressive agents, mechanisms, pathogenesis, genetic factors, medicinal uses

1. Introduction

Corticotherapy, also known as corticosteroid therapy, involves the use of corticosteroids, which are synthetic analogs of hormones naturally produced by the adrenal cortex. These medications mimic the effects of cortisol and other corticosteroids, which play a crucial role in regulating inflammation, immune response, metabolism, and stress responses in the body [1].

Corticosteroids are broadly classified into two main categories:

1. *Glucocorticoids* (e.g., prednisone, dexamethasone, hydrocortisone): Primarily involved in regulating metabolism and immune response, glucocorticoids are commonly used for their potent anti-inflammatory and immunosuppressive effects. They are widely prescribed for conditions such as rheumatoid arthritis,

systemic lupus erythematosus, asthma, chronic obstructive pulmonary disease (COPD), and various allergic reactions [1].

2. *Mineralocorticoids* (e.g., fludrocortisone): Primarily involved in maintaining fluid and electrolyte balance, mineralocorticoids are used less frequently and are primarily indicated for conditions like adrenal insufficiency (e.g., Addison's disease) to replace deficient hormones [1].

Mechanism of action: Corticosteroids exert their effects by binding to specific intracellular receptors, which then modulate the transcription of target genes involved in inflammation and immune responses. Glucocorticoids, in particular, reduce inflammation by inhibiting the synthesis of pro-inflammatory cytokines, chemokines, and other mediators, while promoting the production of anti-inflammatory proteins. This dual effect makes them effective in managing autoimmune diseases, allergic reactions, and other inflammatory conditions [2].

Clinical applications: Corticotherapy is used in a wide range of medical conditions due to its ability to modulate inflammation and immune responses. These conditions include [2]:

- *Autoimmune diseases:* Such as rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease [3].
- *Allergic conditions:* Including asthma, allergic rhinitis, and anaphylaxis [3].
- *Endocrine disorders:* Such as adrenal insufficiency and congenital adrenal hyperplasia [3].
- *Oncology:* As part of chemotherapy regimens or to manage certain hematological malignancies [3].
- *Organ transplantation:* To prevent and treat graft rejection by suppressing the immune response [4].

Considerations and risks: While corticosteroids are highly effective, their use is associated with a range of potential side effects, especially with long-term therapy. These may include osteoporosis, hypertension, hyperglycemia, increased susceptibility to infections, weight gain, and adrenal suppression. Therefore, the benefits of corticotherapy must be carefully weighed against its risks, and the lowest effective dose should be used for the shortest duration necessary [5].

Corticotherapy remains a cornerstone in the management of many inflammatory and autoimmune conditions, offering potent therapeutic benefits when used judiciously under medical supervision [6].

2. Pathogenesis of asthma exacerbation

- *Immune response:* Asthma is primarily an inflammatory disease of the airways. In exacerbations, the immune system responds excessively to triggers like allergens, infections, or irritants. This response involves Th2 cells that release cytokines (IL-4, IL-5, IL-13) leading to inflammation and hyperresponsiveness of the airways.

- *Airway inflammation:* Eosinophils, mast cells, and other inflammatory cells are recruited to the airways, leading to mucus production, airway edema, and bronchoconstriction. This narrows the airways and makes breathing difficult.
- *Smooth muscle constriction:* In response to the inflammation, the smooth muscles surrounding the airways constrict, further narrowing the airways and leading to symptoms like wheezing and shortness of breath.
- *Mucus hypersecretion:* Goblet cells in the airway lining produce excessive mucus, which can obstruct the airways and worsen airflow limitation [7, 8].

3. Pathogenesis of COPD exacerbation

- *Chronic inflammation:* COPD is characterized by chronic inflammation of the airways, primarily involving neutrophils, macrophages, and CD8+ T-lymphocytes. Exacerbations occur due to an acute increase in this inflammation, often triggered by infections (bacterial or viral) or environmental pollutants.
- *Airway and alveolar damage:* The chronic inflammatory response leads to structural changes in the lungs, including destruction of alveolar walls (emphysema) and thickening of airway walls (chronic bronchitis). During exacerbations, these changes become more pronounced, causing increased airflow obstruction.
- *Oxidative stress:* Exacerbations are associated with increased oxidative stress, which further damages lung tissues and exacerbates inflammation.
- *Mucus hypersecretion and airway edema:* Similar to asthma, COPD exacerbations involve increased mucus production and airway edema, leading to further airway obstruction [9, 10].

4. Pathogenesis of anaphylaxis

- *IgE-mediated reaction:* Anaphylaxis is typically triggered by the binding of an allergen to IgE antibodies attached to the surface of mast cells and basophils. This cross-linking leads to the rapid release of histamine and other inflammatory mediators (like leukotrienes and prostaglandins) from these cells.
- *Systemic vasodilation and increased vascular permeability:* Histamine release causes widespread vasodilation and increased vascular permeability, leading to a drop in blood pressure (hypotension), swelling (angioedema), and fluid leakage into tissues.
- *Smooth muscle contraction:* The mediators released also cause smooth muscle contraction in the respiratory and gastrointestinal tracts, leading to bronchoconstriction (difficulty breathing), gastrointestinal symptoms, and cardiovascular collapse in severe cases.

- *Late-phase reaction:* Some patients may experience a biphasic reaction, where a secondary wave of symptoms occurs hours after the initial anaphylactic response due to the continued release of mediators [11, 12].

5. Pathogenesis of urticaria (hives)

- *Histamine release:* Urticaria is primarily caused by the release of histamine and other vasoactive substances from mast cells in the skin. This release can be triggered by allergens, stress, physical stimuli, or sometimes idiopathically.
- *Vascular changes:* Histamine causes dilation of blood vessels and increased permeability, leading to the characteristic red, swollen welts (hives). The fluid leakage from vessels into the surrounding tissues causes edema.
- *Immune-mediated mechanisms:* Urticaria can be immune-mediated (IgE-dependent, as in allergic reactions) or non-immune-mediated (triggered by direct mast cell activation through physical factors, medications, or other stimuli).
- *Autoimmune mechanisms:* In chronic urticaria, autoantibodies against IgE receptors on mast cells or IgE itself may play a role, leading to persistent or recurrent episodes of hives [13, 14].

These pathways explain how the body's immune system and various cells respond to triggers, resulting in the symptoms observed during exacerbations or acute episodes.

6. Pathogenesis of contact dermatitis

Contact dermatitis is an inflammatory skin condition that occurs due to direct contact with irritants or allergens. It is categorized into two main types: irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) [15].

6.1 Irritant contact dermatitis (ICD)

- *Direct damage to skin barrier:* ICD results from direct chemical or physical damage to the skin barrier. Irritants such as soaps, solvents, or friction disrupt the stratum corneum (outermost skin layer), leading to the loss of moisture and lipid content.
- *Inflammatory response:* The damage triggers an inflammatory response mediated by keratinocytes (skin cells) and innate immune cells like macrophages and neutrophils. Pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α are released, leading to redness, swelling, and sometimes blistering.
- *Non-immune-mediated:* Unlike allergic contact dermatitis, ICD does not involve an adaptive immune response or sensitization. The severity of ICD depends on the concentration and duration of exposure to the irritant, as well as individual skin susceptibility [16].

6.2 Allergic contact dermatitis (ACD)

- *Sensitization phase:* ACD is an immune-mediated hypersensitivity reaction. During the initial exposure to an allergen (e.g., nickel, poison ivy, or certain chemicals in cosmetics), the skin's Langerhans cells (a type of dendritic cell) capture the allergen and present it to T-cells in the lymph nodes, leading to the activation and proliferation of allergen-specific T-cells.
- *Elicitation phase:* Upon subsequent exposure to the same allergen, these memory T-cells are reactivated, migrating to the skin and releasing cytokines and chemokines. This leads to an inflammatory cascade involving additional immune cells (such as macrophages and more T-cells), resulting in the characteristic redness, itching, and vesicles (blisters) of ACD.
- *Delayed-type hypersensitivity:* ACD is a type IV hypersensitivity reaction, meaning that it typically develops 24 to 72 hours after exposure to the allergen [17].

7. Pathogenesis of pemphigus vulgaris

Pemphigus vulgaris (PV) is an autoimmune blistering disorder that affects the skin and mucous membranes. It is characterized by the formation of intraepidermal blisters due to the loss of cell-to-cell adhesion in the epidermis [18].

- *Autoantibodies against desmogleins:* The hallmark of PV is the production of autoantibodies against desmogleins 1 and 3, which are cadherin-type adhesion molecules found in desmosomes. Desmosomes are structures that hold epidermal cells (keratinocytes) together.
 - *Desmoglein 3:* Predominantly expressed in the lower layers of the epidermis (basal and suprabasal layers), its targeting by autoantibodies leads to mucosal involvement, which is common in PV.
 - *Desmoglein 1:* Found in the upper layers of the epidermis, autoantibodies against this molecule lead to skin blistering.
- *Loss of cell adhesion (acantholysis):* The binding of autoantibodies to desmogleins disrupts desmosomal adhesion, leading to a loss of cohesion between keratinocytes. This process is known as acantholysis and results in the formation of intraepidermal blisters, which are fragile and easily rupture, leading to erosions and ulcers.
- *Inflammatory cascade:* The disruption of desmosomes and the resulting acantholysis trigger a local inflammatory response, which further exacerbate tissue damage. Cytokines and proteases are released, contributing to the breakdown of the epidermis.
- *Clinical presentation:* PV typically presents with flaccid blisters and erosions on the skin and mucous membranes, often starting in the oral cavity. Nikolsky's sign, where the skin shears off easily with slight pressure, is a diagnostic feature of the disease [19, 20].

Both contact dermatitis and pemphigus vulgaris involve complex immune mechanisms leading to skin damage, but they differ significantly in their triggers and underlying processes.

8. Pathogenesis of adrenal insufficiency

Adrenal insufficiency results from the inability of the adrenal glands to produce sufficient levels of cortisol and, in some cases, aldosterone. It is categorized into primary, secondary, and tertiary forms, each with distinct underlying mechanisms [21].

8.1 Primary adrenal insufficiency (Addison's disease)

- *Autoimmune adrenalitis:*
 - *Mechanism:* The most common cause of primary adrenal insufficiency is autoimmune destruction of the adrenal cortex. In this condition, the immune system produces autoantibodies against adrenal antigens, leading to the gradual destruction of adrenal cortical cells.
 - *Immune mediators:* T-lymphocytes, particularly CD8+ cytotoxic T-cells, infiltrate the adrenal glands and contribute to cellular damage. Autoantibodies against 21-hydroxylase (an enzyme involved in cortisol synthesis) are often present and serve as a marker of the disease.
 - *Hormonal consequences:* The destruction of the adrenal cortex leads to decreased production of glucocorticoids (cortisol), mineralocorticoids (aldosterone), and adrenal androgens. This results in symptoms such as fatigue, hypotension, hyponatremia, hyperkalemia, and skin hyperpigmentation due to elevated ACTH levels [22].
- *Infectious causes:*
 - *Tuberculosis:* Historically, tuberculosis was a major cause of adrenal insufficiency, leading to granulomatous destruction of the adrenal glands. Other infections, such as fungal infections (e.g., histoplasmosis) or HIV, can also damage the adrenal cortex.
 - *Adrenal hemorrhage:* Conditions like Waterhouse-Friderichsen syndrome (caused by severe bacterial infections, often *Neisseria meningitidis*) can lead to bilateral adrenal hemorrhage and acute adrenal insufficiency [23].
- *Other causes:* Genetic disorders (e.g., adrenoleukodystrophy), metastatic cancer, and certain medications (e.g., ketoconazole, which inhibits adrenal steroid synthesis) can also cause primary adrenal insufficiency [23].

8.2 Secondary and tertiary adrenal insufficiency

- *Secondary adrenal insufficiency:*

- *Mechanism:* This occurs due to insufficient production of ACTH by the pituitary gland, often caused by pituitary tumors, surgery, radiation, or long-term use of exogenous glucocorticoids that suppress ACTH secretion.
- *Hormonal impact:* The adrenal glands remain intact but are under-stimulated due to low ACTH levels, leading to reduced cortisol production. Aldosterone production is typically unaffected because it is primarily regulated by the renin-angiotensin-aldosterone system.
- *Tertiary adrenal insufficiency:*
 - *Mechanism:* This results from impaired hypothalamic release of corticotropin-releasing hormone (CRH), often due to chronic glucocorticoid therapy. When exogenous steroids are abruptly discontinued, the hypothalamic-pituitary-adrenal (HPA) axis may not recover quickly, leading to adrenal insufficiency.
 - *Hormonal impact:* Similar to secondary adrenal insufficiency, cortisol production is impaired, while aldosterone levels remain normal [21–23].

9. Pathogenesis of congenital adrenal hyperplasia (CAH)

Congenital adrenal hyperplasia (CAH) is a group of genetic disorders caused by enzyme deficiencies that disrupt adrenal steroidogenesis, leading to a spectrum of hormonal imbalances [24].

9.1 21-hydroxylase deficiency

- *Enzyme deficiency:*
 - *Mechanism:* The most common form of CAH is 21-hydroxylase deficiency, caused by mutations in the CYP21A2 gene. This enzyme is essential for converting 17-hydroxyprogesterone to 11-deoxycortisol (a cortisol precursor) and progesterone to 11-deoxycorticosterone (an aldosterone precursor).
 - *Hormonal consequences:* The block in cortisol synthesis results in reduced negative feedback on the hypothalamus and pituitary, leading to increased secretion of CRH and ACTH. The chronic elevation of ACTH causes adrenal hyperplasia (enlargement of the adrenal glands) and overproduction of adrenal androgens due to the shunting of steroid precursors into the androgen synthesis pathway.
 - *Androgen excess:* The excess androgens cause virilization in females, leading to ambiguous genitalia at birth and, in both sexes, early onset of puberty. In severe forms, aldosterone deficiency also leads to salt-wasting crises, characterized by hyponatremia, hyperkalemia, dehydration, and hypotension [24].

9.2 Other forms of CAH

- *11 β -Hydroxylase deficiency:*

- *Mechanism:* This deficiency is caused by mutations in the CYP11B1 gene, leading to a block in the conversion of 11-deoxycortisol to cortisol. The accumulation of 11-deoxycorticosterone, a potent mineralocorticoid, causes hypertension. Additionally, excess androgen production leads to virilization.
- *17 α -Hydroxylase deficiency:*
 - *Mechanism:* Caused by mutations in the CYP17A1 gene, this deficiency impairs the production of both cortisol and sex steroids, leading to hypertension (due to excess mineralocorticoids) and ambiguous genitalia or delayed puberty [25].

9.3 Adrenal hyperplasia

- *Mechanism:* The chronic elevation of ACTH due to impaired cortisol synthesis leads to the hypertrophy and hyperplasia of the adrenal cortex. Despite the enlarged glands, the adrenal cortex cannot produce sufficient cortisol or aldosterone, but it can produce excess androgens, leading to the clinical manifestations of CAH [26].

Both adrenal insufficiency and congenital adrenal hyperplasia involve complex disruptions in the HPA axis and adrenal steroidogenesis, leading to distinct but potentially severe clinical consequences.

10. Pathogenesis of inflammatory bowel disease (IBD)

Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders of the gastrointestinal (GI) tract, primarily including Crohn's disease (CD) and ulcerative colitis (UC). The exact cause of IBD is not fully understood, but it is believed to result from a complex interplay of genetic, environmental, immunological, and microbial factors [27].

10.1 Genetic susceptibility

- *Genetic predisposition:* Multiple genes have been implicated in IBD, with NOD2 (nucleotide-binding oligomerization domain-containing protein 2) being a well-known susceptibility gene for Crohn's disease. Other genes related to immune function, epithelial barrier integrity, and microbial recognition also contribute to disease risk.
- *Immune dysregulation:* Genetic factors may predispose individuals to an inappropriate immune response to intestinal microbes, leading to chronic inflammation [28].

10.2 Immune system dysregulation

- *Innate immune response:* In IBD, the innate immune system may respond abnormally to gut microbiota. This can involve overactivation of pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) and NOD-like receptors (NLRs), leading to the production of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and IL-12.

- *Adaptive immune response:*
 - *Crohn's disease:* Th1 and Th17 cells are predominant in CD, with the production of cytokines like IL-12, IL-23, IFN- γ , and IL-17, driving chronic inflammation and granuloma formation.
 - *Ulcerative colitis:* UC is associated with a Th2-like response, where cytokines such as IL-5 and IL-13 play a role. The immune response in UC is generally more localized to the mucosal layer, while in CD, inflammation can penetrate deeper into the bowel wall [29].

10.3 Epithelial barrier dysfunction

- *Barrier integrity:* The intestinal epithelium acts as a barrier to protect the body from pathogens and antigens in the gut lumen. In IBD, this barrier is compromised due to genetic and immune factors, leading to increased intestinal permeability (“leaky gut”).
- *Microbial translocation:* The compromised barrier allows gut microbes and their products to penetrate the epithelial layer, further activating the immune system and perpetuating inflammation [27].

10.4 Microbial factors

- *Dysbiosis:* Patients with IBD often have an altered gut microbiome (dysbiosis), characterized by a reduction in beneficial bacteria and an increase in pathogenic or pro-inflammatory microbes. This imbalance can trigger or exacerbate the inflammatory response [28].
- *Loss of tolerance:* The immune system in IBD patients may lose tolerance to commensal (normally harmless) bacteria, treating them as pathogens and mounting an inflammatory response against them [29].

10.5 Environmental factors

- *Diet:* High-fat, low-fiber diets, along with specific food additives, may contribute to gut inflammation.
- *Smoking:* Smoking has opposite effects on the two main types of IBD—worsening Crohn's disease while seeming to be protective against ulcerative colitis.
- *Infections:* Previous infections or antibiotic use can disrupt the gut microbiome, possibly triggering IBD in genetically susceptible individuals [27].

11. Pathogenesis of autoimmune hepatitis (AIH)

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease characterized by an immune-mediated attack on hepatocytes (liver cells). The exact cause is

unknown, but it involves a combination of genetic predisposition, environmental triggers, and immune system dysregulation [30].

11.1 Genetic susceptibility

- *HLA genes:* The strongest genetic association in AIH is with certain human leukocyte antigen (HLA) alleles, particularly HLA-DR3 and HLA-DR4. These alleles encode for specific types of MHC class II molecules that present antigens to T cells, potentially triggering an autoimmune response.
- *Non-HLA genes:* Other genes involved in immune regulation and tolerance, such as those related to cytokines and co-stimulatory molecules, may also contribute to the risk of AIH [29].

11.2 Loss of immune tolerance

- *T-cell activation:* In AIH, the loss of immune tolerance leads to the activation of autoreactive CD4+ T cells against liver-specific antigens. These T cells produce pro-inflammatory cytokines such as IFN- γ , TNF- α , and IL-17, which recruit and activate other immune cells, such as CD8+ cytotoxic T cells, macrophages, and B cells [31].
- *Autoantibodies:* The immune response in AIH also involves the production of autoantibodies, such as anti-smooth muscle antibodies (SMA), anti-liver/kidney microsomal antibodies (anti-LKM), and anti-nuclear antibodies (ANA). These autoantibodies are markers of the disease and may contribute to liver injury [31].

11.3 Liver inflammation and damage

- *Interface hepatitis:* The hallmark of AIH is interface hepatitis, characterized by the infiltration of lymphocytes and plasma cells into the liver parenchyma, particularly at the junction between the portal tract and liver lobules (the interface). This results in hepatocyte necrosis and apoptosis (cell death).
- *Fibrosis and cirrhosis:* Chronic inflammation can lead to progressive liver damage, fibrosis (scarring), and eventually cirrhosis. This can impair liver function and increase the risk of liver failure and hepatocellular carcinoma [31].

11.4 Environmental triggers

- *Infections:* Viral infections (e.g., hepatitis viruses, Epstein-Barr virus) or other infections may act as triggers for AIH by causing molecular mimicry, where the immune system mistakes liver antigens for viral antigens.
- *Medications:* Certain drugs, such as minocycline or nitrofurantoin, have been implicated in triggering AIH, likely by modifying liver proteins and making them appear foreign to the immune system.
- *Hormonal factors:* AIH is more common in females, suggesting that sex hormones may influence the disease's pathogenesis. Estrogens may modulate immune responses and contribute to the higher prevalence of AIH in women [29].

Both inflammatory bowel disease and autoimmune hepatitis involve complex interactions between genetic, immunological, and environmental factors leading to chronic inflammation and tissue damage.

12. Pathogenesis of hemolytic anemia

Hemolytic anemia is characterized by the premature destruction of red blood cells (RBCs), leading to anemia. The causes can be broadly categorized as intrinsic (due to defects within the RBCs) or extrinsic (due to external factors) [32].

12.1 Intrinsic causes

- *Membrane defects:*
 - *Hereditary spherocytosis:* A genetic disorder where mutations in genes encoding proteins like ankyrin, spectrin, or band 3 lead to defects in the RBC membrane. This causes the RBCs to become spherical and less flexible, making them prone to being trapped and destroyed in the spleen [32].
- *Enzyme deficiencies:*
 - *G6PD deficiency:* Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that protects RBCs from oxidative damage. Deficiency leads to the buildup of oxidative stress within RBCs, causing hemolysis when exposed to certain triggers (e.g., certain drugs, infections, fava beans) [32].
- *Hemoglobinopathies:*
 - *Sickle cell disease:* A mutation in the hemoglobin gene leads to the production of abnormal hemoglobin S. Under low oxygen conditions, hemoglobin S polymerizes, causing RBCs to become rigid and sickle-shaped. These sickled cells are prone to hemolysis and can cause blockages in small blood vessels [32].

12.2 Extrinsic causes

- *Autoimmune hemolytic anemia (AIHA):*
 - *Warm AIHA:* Autoantibodies (usually IgG) bind to RBCs at body temperature, marking them for destruction by splenic macrophages (extravascular hemolysis). This can be idiopathic or secondary to other conditions like lupus.
 - *Cold AIHA:* Autoantibodies (usually IgM) bind to RBCs at cooler temperatures (e.g., in the extremities), leading to complement activation and intravascular hemolysis. It can be associated with infections like *Mycoplasma pneumonia* [33].
- *Alloimmune hemolysis:*
 - *Hemolytic disease of the newborn:* Occurs when maternal antibodies against fetal RBC antigens (e.g., Rh incompatibility) cross the placenta and destroy fetal RBCs.
- *Mechanical hemolysis:*

- *Microangiopathic hemolytic anemia (MAHA)*: RBCs are physically damaged as they pass through small blood vessels obstructed by fibrin strands, often seen in conditions like thrombotic thrombocytopenic purpura (TTP) or disseminated intravascular coagulation (DIC) [34].

13. Pathogenesis of leukemia

Leukemia is a group of cancers that originate in the bone marrow and result in the uncontrolled proliferation of abnormal white blood cells (WBCs). The pathogenesis of leukemia involves genetic mutations, chromosomal abnormalities, and disruptions in normal hematopoiesis [35].

13.1 Genetic and chromosomal abnormalities

- *Oncogene activation*:
 - *Translocations*: Chromosomal translocations can activate oncogenes or create fusion genes that drive leukemia. For example, the Philadelphia chromosome, resulting from a translocation between chromosomes 9 and 22, creates the BCR-ABL fusion gene in chronic myeloid leukemia (CML), leading to uncontrolled cell proliferation [35].
- *Tumor suppressor gene inactivation*:
 - *Mutations*: Loss of function mutations in tumor suppressor genes (e.g., TP53, Rb) can prevent the normal regulation of cell cycle and apoptosis, contributing to the development of leukemia.
- *Epigenetic modifications*:
 - *DNA methylation*: Abnormal epigenetic changes, such as hypermethylation of tumor suppressor gene promoters, can silence these genes and contribute to leukemogenesis [35].

13.2 Disruption of normal hematopoiesis

- *Clonal expansion*: Leukemia begins with a single hematopoietic stem cell or progenitor cell acquiring genetic mutations that give it a growth advantage. This leads to the clonal expansion of malignant cells.
- *Impaired differentiation*: In acute leukemias (e.g., acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL)), the malignant cells fail to differentiate into mature blood cells. Instead, they accumulate as blasts in the bone marrow and blood.
- *Bone marrow failure*: The overproduction of malignant cells in the bone marrow suppresses normal hematopoiesis, leading to anemia, thrombocytopenia, and neutropenia [36].

13.3 Types of leukemia

- *Chronic leukemias:* CML and chronic lymphocytic leukemia (CLL) typically involve the gradual accumulation of mature but functionally abnormal cells. CML is driven by the BCR-ABL fusion gene, while CLL involves the clonal proliferation of B cells, often associated with deletions or mutations in genes like TP53.
- *Acute leukemias:* AML and ALL involve the rapid proliferation of immature blasts that fail to differentiate. These aggressive forms of leukemia require immediate treatment [37].

14. Pathogenesis of lymphoma

Lymphomas are cancers of the lymphatic system, originating from lymphocytes (B cells, T cells, or natural killer (NK) cells). The pathogenesis involves genetic mutations, chromosomal translocations, and dysregulation of normal immune function [38].

14.1 Genetic and molecular abnormalities

- *Chromosomal translocations:*
 - *B-cell lymphomas:* Many B-cell lymphomas, such as Burkitt lymphoma, are driven by chromosomal translocations involving the MYC gene, leading to its overexpression and uncontrolled cell proliferation. In follicular lymphoma, the t(14;18) translocation leads to the overexpression of the BCL2 gene, which prevents apoptosis.
 - *T-cell lymphomas:* T-cell lymphomas can involve translocations or mutations in genes regulating T-cell receptor signaling, such as ALK in anaplastic large cell lymphoma (ALCL) [38].
- *Oncogene activation and tumor suppressor gene inactivation:*
 - *Oncogenes:* Activation of oncogenes such as MYC, BCL2, and BCL6 drives cell proliferation and survival.
 - *Tumor suppressors:* Inactivation of tumor suppressor genes such as TP53 and CDKN2A allows malignant lymphocytes to evade cell cycle control and apoptosis [38].

14.2 Immune dysregulation

- *Immune escape:* Lymphomas often develop mechanisms to evade the immune system, such as downregulating major histocompatibility complex (MHC) molecules or secreting immunosuppressive cytokines like IL-10.
- *Chronic immune stimulation:* Chronic infections (e.g., Epstein-Barr virus in Burkitt lymphoma) or autoimmune diseases can provide prolonged immune stimulation, increasing the risk of lymphomagenesis [39].

14.3 Types of lymphoma

- *Hodgkin lymphoma (HL)*: Characterized by the presence of Reed-Sternberg cells, which are large, abnormal B cells. The pathogenesis involves dysregulation of NF- κ B signaling and immune evasion mechanisms.
- *Non-Hodgkin lymphoma (NHL)*: A diverse group of lymphomas, including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, and mantle cell lymphoma, each with distinct genetic and molecular features driving malignancy [40].

15. Pathogenesis of idiopathic thrombocytopenic purpura (ITP)

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by the immune-mediated destruction of platelets, leading to thrombocytopenia (low platelet count) and an increased risk of bleeding [41].

15.1 Autoimmune mechanisms

- *Autoantibody production*:
 - *Platelet-associated antibodies*: In ITP, the immune system produces autoantibodies (typically IgG) that target platelet surface glycoproteins, such as GPIIb/IIIa and GPIb/IX. These antibodies mark platelets for destruction by macrophages in the spleen and liver.
 - *Impaired platelet production*: In addition to increased destruction, autoantibodies may impair platelet production by targeting megakaryocytes (the precursor cells in the bone marrow that produce platelets).
- *Phagocytosis and destruction*:
 - *Splenic macrophages*: Once coated with autoantibodies, platelets are recognized and engulfed by macrophages in the spleen, leading to their destruction. This process is mediated by the Fc receptors on macrophages binding to the Fc region of the autoantibodies [41].

15.2 T-cell dysregulation

- *T-helper cells*: In ITP, there is an imbalance between regulatory T cells (Tregs) and effector T-helper cells (Th1 and Th17). The overactivation of Th1 and Th17 cells contributes to the production of pro-inflammatory cytokines such as IFN- γ and IL-17, which support autoantibody production.
- *Cytotoxic T cells*: In some cases, cytotoxic CD8⁺ T cells directly target and destroy platelets [42].

15.3 Chronic vs. acute ITP

- *Acute ITP*: Often seen in children, acute ITP can be triggered by viral infections and typically resolves spontaneously within weeks to months.

- *Chronic ITP*: More common in adults, chronic ITP is a persistent condition that may require long-term management. The exact triggers are often unclear, and the autoimmune response may be more complex and resistant to resolution [43].

The pathogenesis of these conditions involves a variety of mechanisms, including genetic mutations, immune dysregulation, and autoimmunity, leading to significant clinical manifestations.

16. Pathogenesis of rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of the synovial joints, leading to joint damage and systemic effects [44].

16.1 Genetic factors

- *HLA-DRB1 gene*: The strongest genetic association is with the HLA-DRB1 gene, which encodes for MHC class II molecules. Certain alleles, such as the shared epitope, increase the risk of RA by affecting antigen presentation and T-cell activation.
- *Other genes*: Variants in genes related to immune regulation, such as PTPN22 (a protein tyrosine phosphatase) and STAT4 (a transcription factor), are also associated with RA susceptibility [44].

16.2 Immune system dysregulation

- *Autoantibody production*:
 - *Rheumatoid factor (RF)*: RF is an autoantibody (typically IgM) against the Fc region of IgG. While not specific to RA, it is found in many RA patients and contributes to immune complex formation.
 - *Anti-citrullinated protein antibodies (ACPAs)*: ACPAs are highly specific for RA and target proteins with citrullinated residues. These antibodies are involved in the pathogenesis by promoting inflammation and joint damage.
- *T-cell activation*:
 - *Th17 cells*: CD4⁺ T-helper 17 (Th17) cells are prominent in RA. They produce pro-inflammatory cytokines such as IL-17 and IL-22, which recruit and activate other immune cells and promote synovial inflammation.
 - *Autoimmune response*: T cells recognize self-antigens presented by antigen-presenting cells, leading to chronic inflammation in the synovium [45].

16.3 Synovial inflammation

- *Synovitis*: The synovial membrane becomes inflamed and thickened, leading to the formation of a pannus (an abnormal granulation tissue). This pannus invades and erodes cartilage and bone.

- *Cytokine release:* Pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 are produced in excess, driving inflammation and joint damage [46].

16.4 Joint damage

- *Cartilage destruction:* Enzymes like matrix metalloproteinases (MMPs) are released by activated synovial cells and contribute to cartilage degradation.
- *Bone erosion:* Osteoclast activation leads to bone resorption and erosion of the subchondral bone beneath the cartilage [46].

17. Pathogenesis of systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by widespread inflammation and tissue damage affecting multiple organs [47].

17.1 Genetic factors

- *HLA genes:* Variants in HLA class II genes, such as HLA-DR2 and HLA-DR3, are associated with increased susceptibility to SLE.
- *Other genetic factors:* Mutations or polymorphisms in genes related to immune regulation (e.g., complement system genes like C1q, C2, and C4) and DNA repair (e.g., TREX1) also contribute to SLE risk [47].

17.2 Autoimmune mechanisms

- *Autoantibody production:*
 - *Antinuclear antibodies (ANA):* ANA, including anti-double-stranded DNA (anti-dsDNA) and anti-Smith (anti-Sm) antibodies, are commonly found in SLE. These antibodies target nuclear components and form immune complexes.
 - *Immune complexes:* The formation and deposition of immune complexes in tissues lead to inflammation and damage through complement activation and recruitment of inflammatory cells.
- *T-cell dysregulation:*
 - *Hyperactivation:* T cells in SLE patients are hyperactive and can recognize self-antigens, leading to an overactive immune response.
 - *Defective regulatory T Cells:* There is a reduction in regulatory T cells (Tregs), which normally suppress autoimmunity and maintain tolerance to self-antigens [48].

17.3 Organ damage

- *Kidney involvement (lupus nephritis):* Immune complex deposition in the kidneys leads to glomerulonephritis, characterized by inflammation and damage to the glomeruli.

- *Skin and mucosal manifestations:* Characteristic rashes (e.g., butterfly rash) and mucosal ulcers are common due to immune complex deposition and inflammation in the skin and mucous membranes.
- *Systemic effects:* SLE can affect virtually any organ system, including the cardiovascular system (e.g., pericarditis, endocarditis), nervous system (e.g., neuropsychiatric symptoms), and joints (e.g., arthritis) [49].

18. Pathogenesis of dermatomyositis

Dermatomyositis is an idiopathic inflammatory myopathy characterized by muscle weakness and skin manifestations [50].

18.1 Autoimmune mechanisms

- *Autoantibody production:*
 - *Specific autoantibodies:* Several autoantibodies are associated with dermatomyositis, including anti-Jo-1 (anti-histidyl-tRNA synthetase), anti-Mi-2, and anti-MDA5 (anti-cytoplasmic helicase). These antibodies target components of muscle fibers and contribute to inflammation.
 - *Immune complexes:* The deposition of immune complexes in the muscle and skin can trigger local inflammation and damage.
- *T-cell mediated muscle damage:*
 - *CD4+ and CD8+ T cells:* Muscle inflammation is driven by the infiltration of CD4+ and CD8+ T cells into muscle tissue. These cells release cytokines that promote muscle damage and weakness.
 - *Endothelial activation:* Inflammation also affects the endothelial cells of small blood vessels in muscle tissues, contributing to ischemia and muscle damage [50].

18.2 Skin manifestations

- *Dermatologic features:* Characteristic skin findings include heliotrope rash (violaceous rash on the eyelids) and Gottron's papules (erythematous papules on the dorsal surfaces of the joints). These manifestations are due to immune-mediated inflammation in the skin [51].

18.3 Muscle involvement

- *Muscle fiber damage:* Inflammatory cells infiltrate muscle tissue, leading to muscle fiber necrosis and atrophy. The destruction of muscle fibers results in weakness and functional impairment.
- *Muscle enzyme elevation:* Elevated levels of muscle enzymes, such as creatine kinase (CK) and aldolase, are commonly found in dermatomyositis due to muscle cell damage [52].

These autoimmune conditions involve complex interactions between genetic, immunological, and environmental factors leading to chronic inflammation and tissue damage.

19. Pathogenesis of uveitis

Uveitis is inflammation of the uvea, the middle layer of the eye, which includes the iris, ciliary body, and choroid. It can be classified into anterior uveitis (iritis), intermediate uveitis, posterior uveitis, and panuveitis. The pathogenesis of uveitis involves various mechanisms depending on the underlying cause [53].

19.1 Infectious causes

- *Bacterial infections:* Pathogens such as *Mycobacterium tuberculosis*, *Toxoplasma gondii*, and *Syphilis* can cause uveitis through direct infection of the uvea or by causing systemic infection that leads to secondary uveitis.
- *Viral infections:* Viruses such as herpes simplex virus (HSV), cytomegalovirus (CMV), and varicella-zoster virus (VZV) can directly invade the uveal tissues or cause systemic infections that lead to uveitis [53].

19.2 Autoimmune and systemic diseases

- *Behçet's disease:* A systemic vasculitis that can cause recurrent uveitis along with oral and genital ulcers. The immune system attacks blood vessels, including those in the uvea.
- *Sarcoidosis:* A systemic granulomatous disease that often involves the uvea, leading to chronic inflammation and granuloma formation.
- *Juvenile idiopathic arthritis (JIA):* In children with JIA, chronic anterior uveitis is associated with an autoimmune response targeting the eye [54].

19.3 Immune system dysregulation

- *T-cell mediated inflammation:* Activated T cells, particularly CD4⁺ Th1 and Th17 cells, release cytokines such as TNF- α , IL-1, and IL-6 that drive inflammation in the uvea. This can be due to an autoimmune response or a reaction to infectious agents.
- *Autoantibodies:* In some cases, autoantibodies target components of the uveal tissues, leading to chronic inflammation [54].

19.4 Genetic factors

- *HLA associations:* Certain HLA alleles are associated with an increased risk of uveitis. For example, HLA-B27 is associated with anterior uveitis in conditions like ankylosing spondylitis [53].

19.5 Trauma and surgery

- *Post-traumatic uveitis*: Physical injury to the eye can lead to inflammation of the uvea as part of the body's response to trauma.
- *Post-surgical uveitis*: Surgery, particularly intraocular surgery, can trigger uveitis due to irritation and the body's inflammatory response [54].

20. Pathogenesis of keratoconjunctivitis

Keratoconjunctivitis refers to inflammation of both the cornea (keratitis) and conjunctiva (conjunctivitis). It can be caused by infectious agents, environmental factors, or autoimmune conditions [54].

20.1 Infectious causes

- *Viral keratoconjunctivitis*:
 - *Adenoviral infection*: Common cause of viral conjunctivitis, leading to inflammation of both the conjunctiva and cornea. Symptoms include redness, watering, and discomfort.
 - *Herpes simplex virus (HSV)*: Can cause epithelial keratitis and dendritic ulcers on the cornea, as well as conjunctival inflammation.
- *Bacterial keratoconjunctivitis*:
 - *Common bacteria*: *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* are common pathogens. Bacterial infections can lead to purulent discharge, corneal ulcers, and conjunctival redness.
 - *Fungal and parasitic infections*: Less common but can cause severe keratoconjunctivitis. *Fusarium* and *Acanthamoeba* are examples of pathogens that can affect the cornea [54].

20.2 Allergic reactions

- *Allergic conjunctivitis*: Caused by allergens such as pollen, dust mites, or pet dander. It leads to itching, redness, and watery discharge. Often associated with atopic conditions like hay fever.
- *Vernal keratoconjunctivitis*: A chronic allergic condition typically seen in children and young adults, characterized by intense itching, photophobia, and cobblestone-like papillae on the conjunctiva [55].

20.3 Autoimmune conditions

- *Sjogren's syndrome*: An autoimmune disorder characterized by dry eyes and mouth. It can cause keratoconjunctivitis sicca (dry eye syndrome) due to decreased tear production and inflammation of the corneal and conjunctival tissues.

- *Rheumatoid arthritis*: Can be associated with dry eyes and keratoconjunctivitis as part of a systemic autoimmune response [54].

20.4 Environmental and mechanical factors

- *Dry eye disease*: Resulting from insufficient tear production or poor tear quality, leading to inflammation of the cornea and conjunctiva. It can be exacerbated by environmental factors such as low humidity or prolonged screen use.
- *Contact lens use*: Poor hygiene or prolonged wear of contact lenses can lead to keratoconjunctivitis, including corneal ulcers and conjunctival irritation [55].

20.5 Genetic and environmental factors

- *Genetic predisposition*: Some individuals may be genetically predisposed to allergic reactions or autoimmune conditions that contribute to keratoconjunctivitis.
- *Environmental exposure*: Exposure to irritants like smoke, chemicals, or dust can exacerbate inflammation of the cornea and conjunctiva [55].

Both uveitis and keratoconjunctivitis involve inflammation of ocular tissues, but they differ in their causes and the specific tissues affected. Treatment strategies depend on the underlying cause and may include anti-inflammatory medications, antibiotics, antivirals, or immunosuppressive therapies.

21. Pathogenesis of organ transplantation

Organ transplantation involves replacing a diseased or non-functional organ with a healthy one from a donor. The primary challenge is to prevent rejection of the transplanted organ and to manage complications [56].

21.1 Immune rejection

- *Hyperacute rejection*: Occurs within minutes to hours after transplantation due to pre-existing antibodies against donor antigens (e.g., blood group antigens). This leads to rapid complement activation and thrombosis of the transplanted organ's blood vessels, causing organ failure.
- *Acute rejection*: Develops within days to months and is mediated by T cells recognizing donor antigens as foreign.
 - *Cell-mediated (type I) acute rejection*: CD8+ cytotoxic T cells target donor cells, causing cellular infiltration, inflammation, and tissue damage.
 - *Humoral (type II) acute rejection*: Anti-donor antibodies (often against donor HLA antigens) form immune complexes that activate the complement cascade, leading to inflammation and tissue damage.

- *Chronic rejection*: Occurs over months to years, characterized by progressive damage to the transplanted organ. It involves a combination of immune mechanisms, including chronic low-level T-cell activation and antibody-mediated damage. This results in fibrosis and irreversible loss of organ function [56].

21.2 Immunosuppressive therapy

- *Prevention of rejection*: Patients receive immunosuppressive medications (e.g., calcineurin inhibitors like tacrolimus, corticosteroids, and anti-proliferative agents like mycophenolate mofetil) to prevent rejection by suppressing T-cell activation and proliferation [57].

21.3 Infections and complications

- *Increased susceptibility*: Immunosuppressive therapy increases susceptibility to infections, which can complicate the post-transplant course.
- *Post-transplant lymphoproliferative disorder (PTLD)*: A complication related to Epstein-Barr virus (EBV) infection, where abnormal lymphocyte proliferation occurs due to immunosuppression [56].

22. Pathogenesis of nephrotic syndrome

Nephrotic syndrome is characterized by significant proteinuria, hypoalbuminemia, edema, and hyperlipidemia. The pathogenesis involves glomerular damage leading to increased permeability of the glomerular filtration barrier [58].

22.1 Primary causes

- *Minimal change disease (MCD)*: The most common cause in children, characterized by diffuse foot process effacement seen on electron microscopy. The exact etiology is unclear, but it may involve T-cell dysregulation and release of factors that alter glomerular permeability.
- *Focal segmental glomerulosclerosis (FSGS)*: Characterized by segmental scarring of glomeruli. It can be primary (idiopathic) or secondary to conditions like HIV or drug toxicity. The pathogenesis involves injury to podocytes and glomerular endothelial cells, leading to proteinuria.
- *Membranous nephropathy*: Characterized by diffuse thickening of the glomerular basement membrane due to subepithelial immune complex deposition. This condition can be primary or secondary to infections, drugs, or malignancies [58].

22.2 Secondary causes

- *Diabetic nephropathy*: In diabetes, hyperglycemia leads to the formation of advanced glycation end-products (AGEs) and activation of the renin-angiotensin system, causing glomerular damage and proteinuria.

- *Systemic lupus erythematosus (SLE)*: Lupus nephritis can cause nephrotic syndrome due to immune complex deposition and inflammation in the glomeruli [59].

22.3 Mechanisms of proteinuria

- *Increased glomerular permeability*: Damage to the glomerular filtration barrier, particularly the podocytes and the basement membrane, leads to leakage of large amounts of protein into the urine.
- *Reduced protein reabsorption*: In some cases, the renal tubules may also be involved, reducing their capacity to reabsorb filtered protein [58].

23. Pathogenesis of cerebral edema

Cerebral edema refers to the accumulation of excess fluid in the brain, which can result from various conditions and lead to increased intracranial pressure [60].

23.1 Types of cerebral edema

- *Vasogenic edema*: Caused by breakdown of the blood-brain barrier (BBB), leading to leakage of plasma proteins and fluid into the extracellular space. This type of edema is commonly seen in tumors, infections, and trauma.
- *Cytotoxic edema*: Occurs due to cellular injury and failure of cellular ion pumps (e.g., Na⁺/K⁺ ATPase), leading to intracellular accumulation of sodium and water. It is seen in conditions like ischemic stroke and certain types of encephalopathy.
- *Interstitial edema*: Results from the accumulation of cerebrospinal fluid (CSF) in the interstitial spaces, often seen in conditions such as hydrocephalus [60].

23.2 Mechanisms

- *Inflammatory response*: Inflammation can disrupt the BBB, leading to vasogenic edema.
- *Ischemia*: Reduced blood flow leads to cytotoxic edema due to failure of cellular ion pumps and accumulation of intracellular fluid.
- *Trauma*: Mechanical injury can damage the BBB and cause both vasogenic and cytotoxic edema [61].

23.3 Clinical consequences

- *Increased intracranial pressure*: Edema leads to compression of brain structures, which can result in symptoms such as headache, nausea, vomiting, and altered consciousness.

- *Herniation*: Severe edema can cause brain herniation, where parts of the brain are displaced, potentially leading to life-threatening complications [61].

24. Pathogenesis of multiple sclerosis (MS)

Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system (CNS), characterized by demyelination and neurodegeneration [62].

24.1 Immune system dysregulation

- *Autoimmune attack*: MS is thought to be an autoimmune disease where the immune system mistakenly attacks myelin, the protective sheath around nerve fibers. This leads to inflammation and damage to the myelin and underlying axons.
- *T-cell activation*: Activated CD4⁺ T-helper 1 (Th1) and Th17 cells release pro-inflammatory cytokines (e.g., IFN- γ , IL-17) that recruit other immune cells, such as macrophages and B cells, to the CNS.
- *B cells and autoantibodies*: B cells produce autoantibodies and contribute to inflammation. Oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) are indicative of B cell involvement [62].

24.2 Demyelination and neurodegeneration

- *Demyelination*: The attack on myelin leads to the formation of sclerotic plaques or lesions in the CNS. The loss of myelin impairs the conduction of electrical impulses along nerves, resulting in neurological symptoms.
- *Axonal damage*: Chronic inflammation and demyelination lead to secondary axonal damage and neurodegeneration. This contributes to the progressive loss of function seen in some forms of MS [62].

24.3 Genetic and environmental factors

- *Genetic susceptibility*: Certain genetic factors, such as variations in HLA-DRB1, are associated with an increased risk of MS. These genes may influence immune responses and susceptibility to autoimmune attacks.
- *Environmental triggers*: Factors such as viral infections (e.g., Epstein-Barr virus), vitamin D deficiency, and smoking may act as environmental triggers that precipitate or exacerbate MS in genetically predisposed individuals [63].

24.4 Disease course

- *Relapsing-remitting MS (RRMS)*: Characterized by episodic exacerbations followed by periods of partial or complete recovery.

- *Primary progressive MS (PPMS)*: Involves a gradual progression of symptoms without distinct relapses.
- *Secondary progressive MS (SPMS)*: Follows an initial relapsing-remitting course, eventually transitioning to a progressive form with continuous worsening.

Understanding these mechanisms helps guide treatment strategies and manage complications associated with these conditions [63].

25. General indications for corticosteroids by field are as follows

25.1 Allergy and pulmonology

25.1.1 Asthma

The main treatments for asthma are steroids and other anti-inflammatory medications. All asthma medications help control asthma and prevent asthma attacks. As a result, the airways are less inflamed and less prone to asthma attacks, allowing people with asthma to better manage their condition. Steroids are used for rapid relief of moderate asthma.

The 2023 Global Initiative for Asthma (GINA) recommended the use of SABA and inhaled corticosteroids as rapid treatment for asthma. This method treats symptoms and inflammation of the airways.

Inhaled corticosteroids are the main controllers' medications to prevent airway inflammation, thereby controlling symptoms, preventing exacerbations, and reducing respiratory distress [64].

25.1.2 Choice of controller medications

The 2023 Turkish Asthma Diagnostic and Management Guidelines Group suggested using ICS-based stepwise treatment approach as early as possible during the course of treatment. Initiation of ICS in the early stages of asthma has been reported to have beneficial effects in preventing exacerbations. Delaying initiation of the control drug results in a lower response rate and may require more drugs to achieve a similar response. Frequent exacerbations and progressive loss of lung function may occur over the years in patients not taking ICS. Therefore, it is recommended to start ICS treatment starting at step 1 [64].

25.1.3 Choice of symptom relievers

This treatment can be given as a pharmacotherapy of ICS/SABA combination in fixed-dose or both drugs can be used regularly at the same time. However, if there are symptoms, using ICS/SABA combination may be safer and more effective in asthma treatment. Expert panel emphasizes to use as-needed ICS/FOR as a reliever medication [65].

25.2 COPD

Steroids are one of the most commonly used medications for people with COPD. They help reduce lung inflammation caused by flare-ups. Steroids are available in oral and inhaled forms. Also, steroids and other drugs are included as combination

medications. Each type of steroid works a little differently in controlling or preventing symptom flare-ups. Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines recommend managing chronic obstructive pulmonary disease (COPD) exacerbations with 40-mg prednisone for 5 days, plus oral antibiotics for patients with excessive cough and purulence.

In 2022, the GOLD Reports released data from clinical trials demonstrating that the combination of LAMA, LABA/LAMA, or LABA/ICS with a long-acting beta agonist/long-acting muscarinic antagonist/inhaled corticosteroid (LABA/LAMA/ICS) as triple combinations in comparison with LAMA, LABA/LAMA, or LABA/ICS reduced mortality in a study patients with a history of increased and frequent and/or severe respiratory symptoms [66]. In 2017, the GOLD Reports started to making recommendations for initial treatment in specific GOLD Groups according to their level of dyspnea through the modified Medical Research Council (mMRC) questionnaire and COPD assessment test (CAT): Group A (mMRC 0–1 or CAT < 10; lower number of exacerbations), Group B (mMRC ≥ 2 or CAT ≥ 10 ; lower number of exacerbations), Group C (mMRC 0–1 or CAT < 10; higher number of exacerbations), and Group D (mMRC ≥ 2 or CAT ≥ 10 ; higher number of exacerbations). In clinical practice, few numbers of patients included in Group C; if the risk of the patient's exacerbation is relatively high, the mMRC and/or CAT are typically increased. GOLD's 2023 Report revised the ABCD model by merging Groups C and D into a new Group E [67]. Based on the 2023 GOLD report, the initial treatment for Group A patients is a short- or long-term bronchodilator, while the Group B patients should receive a combination of LABA and LAMA in a single inhaler therapy. The current report also recommends that Group E patients start using the combination of LABA and LAMA and the triple therapy is indicated if EOS $\geq 300/\mu\text{L}$ [64].

These recommendations differ from those previously published by GOLD (2022), which recommended the use of long-acting bronchodilators only in groups B, C and D, and LAMA in particular in groups C and D. In severe symptoms (e.g., CAT-20), consider the LABA/ICS combination in Group D if the eosinophil level is $\leq 300/\mu\text{L}$ in 2022, with no mention of triple therapy as first choice. From 2023, only monotherapy is recommended for patients in Group A [68].

Another update in 2023 Report is that the LABA/ICS combination, previously an option for group D patients with EOS $\leq 300/\mu\text{L}$, will not be recommended. Therefore, the 2023 GOLD report sets a new standard for primary care medicine [64].

25.3 Anaphylaxis

Although intravenous corticosteroids are often prescribed to provide rapid onset in patients with allergic reactions, oral and intravenous corticosteroids are equally effective in the treatment of asthma. In severe cases, corticosteroids are usually given for 2–3 days. In the treatment of asthma, large parenteral doses are usually given acutely, followed by smaller PO doses at different times. Long-acting parenteral preparations may be an alternative and have been shown to be effective in the treatment of asthma. The optimal dose of corticosteroids has not been determined; therefore, multiple doses are given according to published recommendations [69].

25.4 Urticaria

Corticosteroids may reduce the pain associated with urticaria resistant to H1 and H2 receptor antihistamine therapies. However, they do not inhibit mast cell

degranulation. They are usually used to treat urticaria attacks or to treat histamine-resistant urticaria or when angioedema is present. Long-term use is not recommended due to long-lasting effects. The corticosteroid agents decrease the inflammation associated with urticaria resistant to H1- and H2-receptor antagonists. However, they do not inhibit mast cell degranulation. They are often used to treat flares of urticaria or as treatment of histamine-resistant acute urticaria or when angioedema is found. Long-term use is discouraged owing to long-term adverse effects [70].

25.5 Allergy rhinitis (AR)

Patients with rhinitis should be treated initially with oral antihistamines and/or intranasal steroids. Immunotherapy should be considered for moderate/inconsistent and mild/severe symptoms to severe/persistent symptoms. When this approach fails, for example in the middle of a major pollen season, short-term corticosteroids are often prescribed, particularly in primary care. Current guidelines recommend the use of oral corticosteroids but there is no scientific support. Intramuscular corticosteroid injections are not recommended due to the risk of side effects. However, there is a long-standing practice of injecting methylprednisolone globally [71].

The severity and occurrence of corticosteroids adverse outcomes are based on the duration of use, dose, drug use, the specific drug used, and individual differences [72].

26. Dermatology

26.1 Atopic dermatitis (eczema)

The topical medications are an important part of eczema treatment. These medications include topical corticosteroids (TCS), topical calcineurin inhibitors, phosphodiesterase 4 inhibitors, Janus kinase (JAK) inhibitors, prescription moisturizers, and emerging investigational drugs. High-potency and intermediate-potency topical corticosteroids were associated with better healing compared with low-potency corticosteroids, but there was no difference in effectiveness between high-potency and intermediate-potency topical corticosteroids. Once-daily corticosteroids may be as good as twice-daily. Weekly treatment (e.g., two consecutive days per week) may prevent recurrence of eczema without the risk of side effects [73].

26.2 Pemphigus vulgaris

Since the introduction of corticosteroids, pemphigus patients have been treated with effective anti-inflammatory agents. The major side effects of corticosteroids have led to the use of immunosuppressive agents (ISAs). However, the combination of systemic corticosteroids and ISAs as conventional immunosuppressive therapy (CIST) has led to severe immunosuppression, resulting in infection and death in some cases. Therefore, biological agents are indicated in the treatment of pemphigus to reduce the adverse events associated with CIST [74].

26.3 Psoriasis

Topical corticosteroids are often used as the first choice for treating patients with mild-to-moderate psoriasis. They reduce inflammation, inhibit cell proliferation, and

constrict blood vessels by down-regulating inflammatory pathways. Corticosteroid strength and dosage should be chosen based on disease location to minimize side effects. The combination of corticosteroids with vitamin D analogs or keratolytic agents (such as halobetasol propionate and tazarotene) is generally more effective and has fewer side effects than when used alone. They can also be used twice weekly for pain relief. However, biologics have changed the treatment landscape of psoriasis, especially for patients with moderate-to-severe disease [75].

27. Endocrinology

27.1 Adrenal insufficiency

Glucocorticoid as replacement therapy is the basis of treatment and is designed to reflect the circadian cortisol profile. Recent guidelines suggested the hydrocortisone use in divided two doses or low-dose (3–5 mg) prednisolone [76].

The hydrocortisone drugs that traditionally used have a short half-life of approximately 1.8 hours and require more frequent doses to maintain therapeutic levels. Although individuals are sensitive to the effects of cortisol, more frequent doses of glucocorticoids can cause nighttime discomfort at supraphysiological levels. Prednisolone has a longer half-life and greater affinity for the glucocorticoid receptor and takes a longer time to dissociate. So, once-daily very-low-dose prednisolone is a safe and effective glucocorticoid replacement therapy and associated with greater patient satisfaction and compliance [77].

27.2 Congenital adrenal hyperplasia

Classical CAH treatment involves striking a balance between three main therapeutic goals: supporting the body's adaptation to adrenal insufficiency, reducing age- and sex-specific adrenal androgens, and avoiding iatrogenic hypercortisolism and its related comorbidities [78].

27.2.1 Glucocorticoid replacement therapeutics recommendations

Hydrocortisone is generally preferred and should be taken two to three times daily, with higher doses in the morning and lower doses in the evening. Children usually require 10–15 mg/m² every 8 hours, while adults require 15–25 mg daily; the last dose should be administered at least 6 hours before bedtime to achieve circadian rhythm. In infants and neonates with newly diagnosed saline (SW), the initial dose of hydrocortisone should be adjusted according to their clinical conditions [79].

27.2.2 Mineralocorticoid replacement therapeutics recommendations

Fludrocortisone is usually the first choice. The usual dosage is 0.05 to 0.2 mg/day (without salt) as a morning single dose. Also, most recipes do not require refrigeration. However, it is important to remember that infants and young children may require more fludrocortisone and may require sodium chloride supplements to maintain adequate sodium levels. The fludrocortisone dose should be titrated to achieve plasma renin activity in the normal/high range that also ensures normal levels of potassium [26].

28. Gastroenterology

28.1 Inflammatory bowel disease

Corticosteroids are anti-inflammatory drugs indicated for the treatment of patients with UC and CD with moderate-to-severe bowel inflammation refractory to high-dose of mesalamine [80].

Corticosteroid preparations in IBD consist of intravenous drugs (hydrocortisone, methylprednisolone, and dexamethasone), oral drugs with systemic effect (prednisone, prednisolone, and deflazacort), local drugs (budesonide, budesonide multimatix [MMX], and beclomethasone dipropionate), and rectally used medications with systemic effect (hydrocortisone, prednisolone, triamcinolone, methylprednisolone, and betamethasone) and with local action (budesonide, beclomethasone, and prednisolone-metasulfobenzoate) [80].

Before prescribing any of these formulations during acute phase of CD or UC, we must take into account not only the severity of the disease but also the extent of the affected area, the patient's medical history, and other medications. These considerations allow us to determine the best treatment strategies to improve the quality of life of IBD patients [81].

28.2 Autoimmune hepatitis

Guidelines for autoimmune hepatitis (AIH) consider corticosteroids, including budesonide, as first-choice therapy for patients without cirrhosis. Azathioprine is indicated to reduce corticosteroid dosage and adverse effects. The drug of choice for AIH includes the corticosteroids prednisone (PD) or prednisolone, either as alone or in combination with azathioprine (AZA). Corticosteroids interfere with the immune system by inhibiting cytokine production and inhibiting T lymphocyte activation. AZA exerts its anti-inflammatory effects by blocking the growth of lymphocyte precursor cells, which takes at least 3 months to complete [82].

AZA is used primarily for its steroid-sparing effects and for therapeutic purposes as maintenance therapy because it is less effective in inducing a response. When steroid response is confirmed and thiopurine methyltransferase deficiency cannot be excluded, AZA may be prescribed according to these guidelines. Genetic variations (up to 0.6% of the population) affect thiopurine methyltransferase, but the consequences of AZA use in these population can be serious [83].

29. Hematology

29.1 Hemolytic anemia

Prednisone (or prednisolone), given at a higher starting dose (100 mg fixed dose or 1-1.5 mg/kg body weight per day) then tapered after 2-3 weeks and approximately 4-6 months after stopping the drug, is still the first-choice treatment for warm-antibody AIHA with approximately 80% of patients responding. However, the other oral corticosteroids have not been shown to be more effective. Many new studies and treatments have emerged over the last 1-2 years, both improving treatment and making it more difficult for patients to choose the best treatment [84].

29.2 Leukemia

Corticosteroids have an essential role in cancer treatment by controlling the side effects of chemotherapy, such as nausea and allergic reactions, and by reducing pain. They are particularly effective in some types of leukemia, such as acute lymphoblastic leukemia (ALL). They have an important role in lymphoblastic leukemia treatment and understanding how to cope with short-term and long-term effects and how to improve treatment is critical for continued success. Prednisone and dexamethasone play an important role in the treatment of acute lymphoblastic leukemia (ALL) [85].

30. Rheumatology

30.1 Rheumatoid arthritis

Corticosteroids are more effective anti-inflammatory drugs that are often used to shorten the duration of DMARD use in people with RA. Prednisolone, even at a dose as low as 5 mg per day, can reduce pain and slow joint destruction [86].

Even short-term use of glucocorticoids in the general population is associated with an increase in side effects. Therefore, international guidelines recommend the use of glucocorticoids in RA at the lowest possible dose and for the shortest possible duration, with the aim of stopping glucocorticoids in the first 3 months [87].

30.2 Systemic lupus erythematosus

Glucocorticoids (GCs) have been considered the mainstay of lupus treatment since the 1950s, after Mayo Clinic rheumatologist Philip Hench successfully introduced cortisone for rheumatoid arthritis in 1948 [88].

Glucocorticoids (GCs) are still an important drug in the treatment of lupus erythematosus because no other drug can rapidly reduce the disease. Our main principles are as follows: (a) use methylprednisolone pulses to achieve more than just elimination of severe disease; (b) which drug should it be; and (c) individualize the decision and strategy to stop GCs. Although the disadvantages of long-term use of GCs are well known, they are still the first-line treatment option recommended in SLE guidelines. Therefore, different GC treatment options are urgently needed to achieve rapid and long-term control of lupus activity while minimizing adverse effects [89].

31. Ophthalmology

31.1 Uveitis

Corticosteroid drops are often recommended to relieve anterior segment pain. Anterior uveitis, affecting structures such as the iris and ciliary body, is the most common form of uveitis and is usually treated with topical CS. Integration of systemic CS into uveitis treatment occurs when local interventions are inadequate or when special circumstances require their application. Adverse effects of systemic CS should be carefully evaluated, and the application of a CS-sparing strategy will be helpful in limiting the risks associated with long-term CS use [90].

31.2 Keratoconjunctivitis

Corticosteroids may reduce inflammation and scarring and relieving the acute ocular pain related to corneal ulcer. However, corticosteroids also weaken the response of immune system, which can affect the ability to clear the infection. The safety and effectiveness of corticosteroids based largely on the effectiveness of the antimicrobial drugs indicated to manage the underlying disease. If antibiotics are effective in eradicating or clearing the infection (such as bacterial and herpetic keratitis), topical corticosteroids may be as effective as antibiotics [91].

32. Others

32.1 Organ transplantation

Glucocorticoids (GCs) have long been indicated as induction and treatment immunosuppressive, as well as treatment of acute allograft rejection in patients with solid organ transplant (SOT). However, the problems of GCs make them unsuitable for long-term use. Therefore, steroid-sparing regimens have been used in different types of SOT. New maintenance immunosuppressive agents combined with low-dose CNIs and/or mycophenolate mofetil provide an opportunity for successful steroid-sparing immunosuppression regimens [92].

Most centers performing kidney and pancreatic transplantation are also moving toward steroid avoidance or early or late steroid use [93].

Long-term GC therapy has been the mainstay of immunosuppressive in heart transplant recipients. However, a report from the International Society of Heart and Lung Transplantation Registry on adult heart recipients who were transplanted between 2000 and 2008 reported that early or late GC withdrawal has increased within heart transplant patients. The 10-year survival rate was higher in patients with early (no GC progression within 2 to 5 years after transplantation) or late (no GC progression after 5 years after transplantation) steroid use compared with those with long-term steroid use (>5 years after switch) abstinence (73, 82, and 80%, respectively). Steroid withdrawal within 1 week after transplantation is also indicated in patients with low-risk cardiovascular disease, with a 1-year benefit [94].

Corticosteroids are usually a component of immunosuppression treatment in patients with lung transplant. Glucocorticoid receptor (GR) in lung epithelia is important for lung development, and GCs are usually used to manage some lung diseases [95].

Although there are data on the effectiveness of steroid-sparing regimens in patients with SOT, steroids are still used to maintain immunosuppression effect for at least a few weeks, even in hypoimmune transplant types such as liver transplantations. Budesonide has a low systemic bioavailability of approximately 10% due to its initial metabolism in the liver, which may reduce side effects. In contrast, the affinity of budesonide for GR is approximately 15 times greater than that of methylprednisolone and prednisone, and therefore has a strong local anti-inflammatory effect in the liver [92].

Large dose of intravenous GC such as dexamethasone or methylprednisolone or oral such as prednisone or prednisolone has been used historically to treat chronic cell- and antibody-mediated rejection in various types of SOT [96].

A significant complication after SOT is post-transplant malignancy, including post-transplant lymphoproliferative disease (PTLD). Reduction or switching of immunosuppressants is recommended for patients with PTLD. However, GCs are the mainstay of chemoimmunotherapy for some malignancies, including PTLD, and are often preserved in the immune system of SOT recipients with PTLD [97].

32.2 Nephrotic syndrome

Corticosteroids are the first line of treatment for this disease. Corticosteroid therapy (prednisone/prednisolone) is started at the first stage. If relapses are frequent (≥ 2 relapses in the first 6 months following remission of the initial episode or ≥ 3 relapses in any 12 months) or if significant steroid toxicity has occurred, low-dose alternate-day steroids or steroid-sparing drugs (levamisole, mycophenolate mofetil, etc.) can be given to reduce dependence on steroids and minimize the side effects of steroid therapy. In addition, it is also crucial to refer the patient to a pediatric nephrologist who can provide individualized treatment recommendations [98].

32.3 Cerebral edema

Glucocorticoids have been shown to be effective in treating cerebral edema secondary to vasogenic edema, but their use in other types of edema is limited and should be avoided in all cases of trauma [99].

The parent drug, mannitol, used in doses of 0.25 to 1 g/kg body weight, is thought to maximize its effect by reducing blood viscosity and, to a lesser extent, by decreasing blood volume [100].

32.4 Multiple sclerosis

Established treatment strategies include intravenous methylprednisolone (IV-MP) to treat relapses and immunomodulatory or immunosuppressive therapy to prevent relapses and the development of disability. Although not a recommended immunomodulatory or preventive therapy, monthly IV-MP is less routinely used, particularly in low-income countries [101].

33. Conclusion on corticotherapy

Corticotherapy, through the use of corticosteroids, has become a cornerstone in the management of a wide range of inflammatory, autoimmune, and allergic conditions due to its potent anti-inflammatory and immunosuppressive properties. The therapeutic benefits of corticosteroids, such as rapid symptom relief and the ability to control severe disease flares, make them invaluable in clinical practice. They are used extensively across multiple specialties, including rheumatology, pulmonology, dermatology, and organ transplantation.

However, the efficacy of corticotherapy must be balanced against its potential risks. Prolonged use or high doses of corticosteroids can lead to significant side effects, including osteoporosis, diabetes, hypertension, adrenal suppression, and increased susceptibility to infections. These adverse effects underscore the importance of careful patient selection, appropriate dosing, and continuous monitoring throughout the course of treatment.

The future of corticotherapy lies in more targeted approaches, such as optimizing dosing regimens, developing new steroid formulations with fewer side effects, and combining corticosteroids with other therapies to minimize toxicity. As our understanding of corticosteroid pharmacology and the mechanisms underlying their effects continues to evolve, it will be possible to maximize the therapeutic benefits of corticotherapy while minimizing its risks, ensuring safer and more effective treatment for patients.

In conclusion, while corticosteroids remain a powerful and essential tool in modern medicine, their use should always be tailored to the individual patient, with a focus on achieving the greatest benefit with the least harm.

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