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EMERGING INSIGHTS IN PHARMA AND HEALTH SCIENCE VOLUME I

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Dr. Pradip K. Kumbharwad
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PREFACE

The pharmaceutical and health sciences sectors are undergoing a transformative evolution, driven by technological advancements, innovative research methodologies, and a deeper understanding of disease mechanisms. This dynamic landscape demands a continuous exploration of emerging trends, groundbreaking discoveries, and interdisciplinary collaborations to address global health challenges and improve patient outcomes.

"Emerging Insights in Pharma and Health Science" is a scholarly endeavor that brings together a collection of contemporary research findings, critical reviews, and case studies across a broad spectrum of pharmaceutical and health-related disciplines. This volume aims to bridge the gap between fundamental science and clinical application by showcasing the latest developments in drug discovery, pharmacology, biotechnology, nanomedicine, diagnostics, personalized therapies, and regulatory practices.

The chapters in this book are authored by academicians, researchers, and professionals who are at the forefront of innovation in their respective fields. Each contribution reflects a rigorous academic approach while highlighting practical implications and future directions. By presenting multidisciplinary perspectives, the book serves as a valuable resource for students, educators, industry professionals, and policy makers who seek to stay informed about the rapidly evolving paradigms in healthcare and pharmaceutical sciences.

We extend our heartfelt gratitude to all the contributors for their valuable insights and scholarly efforts. We also thank the editorial and review team for their meticulous work in ensuring the quality and coherence of this compilation. It is our sincere hope that this book will inspire further research, foster knowledge exchange, and contribute meaningfully to the advancement of health science and pharmaceutical innovation.

- Editors

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ENVIRONMENTAL IMPACTS OF PHARMACEUTICAL MANUFACTURING

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Abstract:

Pharmaceutical manufacturing is crucial for global healthcare but presents significant environmental challenges. This chapter explores the environmental impacts associated with pharmaceutical production, including waste generation, water contamination, air emissions, and soil pollution. Pharmaceutical waste often contains active pharmaceutical ingredients (APIs) and other toxic chemicals that persist in ecosystems, leading to adverse ecological effects and public health risks. Wastewater from manufacturing plants, carrying unmetabolized drugs, contributes to the accumulation of pharmaceutical residues in water bodies, impacting aquatic organisms and promoting antimicrobial resistance. Air emissions from production facilities contribute to greenhouse gas emissions, while soil contamination from pharmaceutical disposal affects soil health and agricultural productivity. The chapter also discusses regulatory frameworks, including guidelines from the U.S. Environmental Protection Agency (EPA) and European Medicines Agency (EMA), aimed at mitigating environmental risks. Furthermore, it highlights green chemistry approaches, such as biocatalysis and the use of eco-friendly solvents, that offer sustainable alternatives for reducing the environmental footprint of pharmaceutical production. Future research directions include advancing waste treatment technologies, improving environmental risk assessments, and promoting international collaboration for stronger regulations. Emphasis is placed on the importance of sustainable manufacturing practices to ensure environmental protection and public health safety.

Keywords: Pharmaceutical Manufacturing, Environmental Impact, Waste Management

1. Introduction to Pharmaceutical Manufacturing and Environmental Concerns

Pharmaceutical manufacturing encompasses various stages of production, from raw material acquisition to the development of final products like drugs, vaccines, and biologics. The industry is essential in meeting global healthcare needs, yet it presents significant environmental challenges. Environmental concerns associated with pharmaceutical manufacturing are diverse and impact water, air, soil, and biodiversity. They are particularly critical given that pharmaceutical compounds are designed to have

specific biochemical effects, which means their unintended release into the environment can affect non-target organisms.

This industry's environmental footprint can be attributed to high energy consumption, extensive water use, and substantial waste generation. For example, pollutants from pharmaceutical plants can end up in ecosystems, causing long-term harm to wildlife and water bodies (Azzouz *et al.,* 2019). Regulatory frameworks, such as those enforced by the U.S. Environmental Protection Agency (EPA) and the European Medicines Agency (EMA), are evolving to ensure sustainable manufacturing practices that address these concerns.

2. Waste Generation in Pharmaceutical Manufacturing

Pharmaceutical manufacturing produces significant waste, including chemical solvents, active pharmaceutical ingredients (APIs), and by-products. Waste generation is particularly prominent during the synthesis and formulation stages. Organic solvents, used widely in drug synthesis, contribute a large proportion of hazardous waste in pharmaceutical manufacturing (EPA, 2022).

Waste management within the industry often involves methods like incineration, which, while effective, can contribute to air pollution. Alternatively, treatment and recycling methods are employed to reduce waste, though their efficacy varies. Studies suggest that process optimization, green chemistry principles, and alternative solvent usage can minimize waste generation without compromising product quality (Sheldon, 2017). The industry faces challenges in balancing cost-efficiency with environmentally sound practices, particularly in resource-constrained settings.

3. Pharmaceutical Residues in Water Bodies

Pharmaceutical residues in water bodies have emerged as a global environmental concern. These residues enter water systems through manufacturing effluents, improper disposal, and even human excretion, which passes unmetabolized drugs into sewage systems. Conventional wastewater treatment plants are often not equipped to fully remove pharmaceuticals, resulting in the accumulation of compounds like antibiotics, hormones, and analgesics in rivers, lakes, and oceans (Kümmerer *et al.,* 2018).

The ecological impact of pharmaceutical residues is profound, as they can affect aquatic species' reproductive and immune systems. Antibiotic residues, for example, contribute to antimicrobial resistance (AMR), an escalating global health crisis (WHO, 2020). Studies have shown that even trace amounts of pharmaceuticals can disrupt fish

behavior, growth, and hormone regulation, leading to ecosystem imbalances (Boxall *et al.,* 2012). Consequently, advanced treatment techniques, such as ozonation and activated carbon filtration, are being explored to mitigate these effects.

4. Air Emissions and Climate Change Impacts

Air emissions from pharmaceutical manufacturing include volatile organic compounds (VOCs), greenhouse gases (GHGs), and particulate matter. VOCs, emitted during various production stages, contribute to ground-level ozone formation, which can exacerbate respiratory issues in humans and wildlife (EPA, 2021). Moreover, greenhouse gas emissions from pharmaceutical companies contribute to climate change, highlighting the industry's need to adopt more sustainable practices.

The pharmaceutical industry has one of the highest carbon footprints per dollar of revenue among manufacturing sectors (Belkhir & Elmeligi, 2019). The implementation of cleaner technologies, renewable energy sources, and energy-efficient practices is necessary to reduce emissions. Regulatory bodies have begun encouraging companies to disclose and reduce their carbon footprints, aligning with international climate agreements such as the Paris Accord. However, the transition to low-carbon processes remains challenging due to high costs and the technical demands of pharmaceutical production (UNEP, 2020).

5. Land Contamination and Soil Health

Land contamination from pharmaceutical manufacturing is another environmental concern, particularly in areas where waste disposal practices are inadequate. The improper disposal of solid pharmaceutical waste can lead to soil contamination, impacting soil health and agricultural productivity. Pharmaceuticals that persist in the soil, like antibiotics and endocrine disruptors, can interfere with soil microbiota, altering nutrient cycles and reducing soil fertility (Minden *et al.,* 2020).

Pharmaceutical residues in soil can also affect plant growth and enter the food chain, posing risks to animal and human health. Recent studies have documented the bioaccumulation of certain pharmaceutical compounds in crops irrigated with contaminated water, raising concerns about food safety (Wu *et al.,* 2021). Sustainable waste disposal methods, such as bioremediation and phytoremediation, offer potential solutions, but more research is required to enhance their effectiveness and feasibility at scale (Chaturvedi *et al.,* 2019).

6. Ecological and Health Implications of Pharmaceutical Pollution

Pharmaceutical pollution in the environment has significant ecological and health implications, affecting both wildlife and human populations. Pharmaceuticals are designed to elicit specific biological responses, and their presence in the environment can disrupt ecosystems and the physiology of non-target organisms. For instance, exposure to estrogenic compounds from pharmaceuticals can impair reproductive functions in fish and amphibians, leading to population imbalances (Nash *et al.,* 2004). Antidepressants, antibiotics, and analgesics have been detected in surface waters worldwide, raising concerns about their effects on aquatic life.

The presence of antibiotics in water bodies, even at low concentrations, accelerates the development of antimicrobial resistance (AMR), a global health threat (WHO, 2020). AMR reduces the efficacy of antibiotics, complicating the treatment of infections and increasing morbidity and mortality. Pharmaceutical pollutants can also affect soil health and crop quality when contaminated water is used for irrigation, leading to bioaccumulation of drug residues in edible plants (Christou *et al.,* 2017). In humans, these residues may contribute to endocrine disruption, allergies, and antibiotic resistance, although the long-term effects remain to be fully understood (Daughton & Ternes, 1999).

7. Regulations and Policies Addressing Environmental Impacts

To address the environmental impact of pharmaceutical pollution, various national and international regulatory frameworks have been established. Regulatory agencies, including the U.S. Environmental Protection Agency (EPA) and the European Medicines Agency (EMA), enforce policies to limit the release of pharmaceuticals into the environment. The EU Water Framework Directive, for example, lists priority substances, including certain pharmaceuticals, which must be monitored and controlled to protect aquatic ecosystems (European Commission, 2015).

In addition, the Pharmaceutical Management for the Environment (PME) initiative by the International Pharmaceutical Federation promotes sustainable practices in manufacturing and waste management. The U.S. Food and Drug Administration (FDA) and the EMA have established guidelines for environmental risk assessments (ERAs) that require pharmaceutical companies to evaluate potential environmental impacts before product approval (FDA, 2022). Despite these efforts, there is still a need for standardized global policies, as existing regulations are fragmented and vary widely across regions

(Boxall *et al.,* 2012). Strengthening international cooperation and enforcing stricter standards can enhance the effectiveness of these policies.

8. Green Chemistry and Sustainable Solutions

Green chemistry principles offer promising approaches to reduce the environmental impact of pharmaceutical manufacturing. Green chemistry seeks to minimize or eliminate hazardous substances in chemical processes, aiming for safer, more efficient, and sustainable production methods (Anastas & Warner, 1998). In pharmaceutical manufacturing, this involves reducing solvent use, employing safer reagents, and optimizing synthesis routes to minimize waste.

One effective strategy is the use of biocatalysis, which employs enzymes as catalysts, resulting in fewer toxic by-products and reduced energy consumption (Sheldon, 2016). Alternative solvents, such as water and ionic liquids, are increasingly being adopted to replace traditional organic solvents that are hazardous to the environment. Furthermore, companies are developing "benign-by-design" drugs that degrade quickly in the environment, reducing their ecological persistence (Sanderson *et al.,* 2004).

The adoption of circular economy principles can also promote sustainable pharmaceutical manufacturing. Waste from one stage of production can be repurposed or recycled for other uses, reducing overall resource consumption and waste. Collaborative efforts between industry and academia have led to innovations in green chemistry, exemplified by the ACS Green Chemistry Institute's Pharmaceutical Roundtable, which focuses on developing greener synthetic processes (Green Chemistry Institute, 2021). These approaches not only mitigate environmental impacts but also enhance cost-effectiveness and efficiency in pharmaceutical production.

9. Future Directions and Research Needs

Future research is essential to address the gaps in understanding the long-term impacts of pharmaceutical pollution and to develop more sustainable solutions. Emerging contaminants, including new pharmaceutical compounds and their metabolites, require further study to assess their ecological effects accurately. There is also a pressing need for more sensitive analytical methods to detect pharmaceuticals at low concentrations, as current techniques may underestimate the prevalence of these contaminants in the environment (Petrović *et al.,* 2005).

Advances in biotechnology and nanotechnology could offer innovative solutions to tackle pharmaceutical pollution. For instance, nanomaterials can enhance wastewater

treatment by targeting and degrading specific contaminants (Santos *et al.,* 2019). Bioremediation, which uses microorganisms to degrade pollutants, is another promising area, though challenges remain in scaling this approach for industrial use (Sharma *et al.,* 2020).

In addition to technological advancements, interdisciplinary research involving ecotoxicologists, chemists, and public health experts is crucial. This collaboration will help develop comprehensive risk assessment frameworks and create more resilient ecosystems. Educational initiatives that raise awareness of proper drug disposal and responsible consumption can also reduce pharmaceutical pollution at the source (Gaw *et al.,* 2014). Ultimately, achieving sustainable pharmaceutical production and environmental protection will require ongoing research, regulatory support, and a commitment to green innovation.

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AMP C BETALACTAMASES -THE SILENT EMERGING RESISTANCE AMONG ENTEROBACTERALES: A REVIEW

PART 1

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Introduction:

The control of microorganisms is critical for the prevention and treatment of any infectious disease. Modern medicine relies on the antibiotic agents for the treatment of infectious diseases. Ideally these agents act on the pathogenic microorganisms and either inhibit or destroy them to combat the infectious diseases. The antibiotics (Greek, anti- against, bios-life) are chemotherapeutic substances which are active against bacterial infection. Antibiotics without a question have revolutionized the medical practice. The extensive availability of effective antibiotics have translated the practice of medicine from the diagnostic end to highly evolving therapeutic end[1].

Despite the initial optimism, in addition to elimination of the infectious diseases antibiotic use has led inevitably to the emergence of antibiotic resistance. Resistance to antibiotics have been recognized since the beginning of the antimicrobial era[2]. Rapid and extensive development of antimicrobial resistance is mostly attributed to the inadvertent use and over the counter use of antibiotics[3]. Emergence of antimicrobial resistance is the major threat to public health [3].

Enterobacterales inhabit a wide variety of niches, including the human gastrointestinal tract, the gastrointestinal tract of other animals, and various environmental sites[4]. For species capable of colonizing humans, infection may result when a patient's own bacterial strains (i.e., endogenous strains) establish infection in a normally sterile body site[4]. These organisms can also be passed from one patient to another[4]. Such infections often depend on the debilitated state of a hospitalized patient and are acquired during the patient's hospitalization (nosocomial) or other healthcare–associated environment[4]. Genera in the family *Enterobacterales* are important pathogens for three of the four major HAI categories according to the CDC, namely, Central Line-Associated Bloodstream Infections (CLABSI), Catheter-Associated Urinary Tract Infections (CAUTI),

and Surgical Site Infections (SSI)[6]. No collective group of currently defined prokaryotic bacteria has had a greater medical, public health, and veterinary impact on the global community than the family Enterobacterales[6].

β-lactam antibiotics have been a primary choice for physicians to treat bacterial infections caused by *Enterobacterales* due to their high specificity and potent killing effect[9]. The most common β-lactam containing agents belong to 4 major chemical classes, penicillins, cephalosporins, carbapenems and monobactams [8]. Because these agents are so valuable, the medical community is quite alarmed that resistance to these drugs continue to increase in all parts of the world. [8]

Over the last twenty years, many new β-lactam antibotics have been developed that were specifically designed to be resistant to the hydrolytic action of β- lactamases [12]. However, with each new class that has been used to treat patients, new β-lactamases emerged that caused resistance to that class of drug[12]. This may be presumably due to the selective use and overuse of new antibiotics in the treatment of patients which has resulted into emergence of new variants of β-lactamases[12].

To evade the bactericidal effects of β-lactam antibiotics, *Enterobactaerales* have evolved multiple strategies, such as production of β-lactamases, production of novel PBPs with reduced affinity to β-lactam antibiotics, reducing β-lactam antibiotics entry through mutations in porins and expelling these antibiotics out of cells using multi-drug efflux pumps[9]. Of these mechanisms, producing β-lactamases, the enzymes that could hydrolyze β -lactam ring, is still the most efficient strategy[9]. The first "cryptic" β- lactamase, AmpC (originally named AmpA), was identified in β-lactam sensitive *E. coli* K-12 by step wise selection on β-lactam antibiotics containing medium [9].

AmpC class β -lactamases are cephalosporinases that are poorly inhibited by clavulanic acid [11].They can be differentiated from other extended spectrum β – lactamases (ESBLs) by their ability to hydrolyse cephamycins like cefoxitin as well as other extended spectrum cephalosporins [11].They are clinically significant because many confer resistance to a wide variety of β-lactamase inhibitors such as clavulanic acid,sulbactam,tazobactam [5].They may be plasmid-mediated/uninducible which are typically associated with broad multidrug resistance or chromosomal mediated/inducible AmpC wherein they are induced by β-lactam antibiotics such as cefoxitin and imipenem but poorly induced by 3rd and 4th generation cephalosporins [5]. Cephamycin(cefoxitin) resistance in non-AmpC producers

may be due to porin-deficient mutants (as in non-AmpC producing Klebsiella pneumonia)[3,4,5].

Detecting AmpC is clinically important not only because of their broader cephalosporin resistance, but also because carbapenem resistance can arise in such strains by further mutation, resulting in reduced porin expression[13]. Whereas, standardised screening and confirmatory methods for ESBL identification are present (CLSI 2021), no such methods for AmpC detection exist[14]. Although Amp C β- lactamases are less prevalent than ESBLs, *Enterobacterales* producing both ESBL and AmpC have been increasing worldwide. The worldwide prevalence is approxiamtely around 8%- 48% but no clear statistical data is available[24,34,39].

Medically Important Enterobacterales

The genera *Escherichia, Klebsiella, Enterobacter, Serratia* and *Citrobacter* (collectively called the coliform bacilli) and *Proteus* include overt and opportunistic pathogens responsible for a wide range of infections[21].Many species are members of the normal intestinal flora[21].*Escherichia coli* (E.coli) is the most commonly isolated organism in the clinical laboratory[21].A limited number of species, including *E coli, K pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, S marcescens* and *Proteus mirabilis*, are responsible for most infections produced by this group of organisms. [21] The increasing incidence of the coliforms, *Proteus*, and other Gram-negative organisms in diseases reflects in part a better understanding of their pathogenic potential but more importantly the changing ecology of bacterial disease [21].

Enterobacterales are important causes of urinary tract infections (UTIs), bloodstream infections, hospital- and healthcare-associated pneumonias, and various intra-abdominal infections [24].Within this family, *Escherichia coli* is a frequent cause of UTIs, species of *Klebsiella* and *Enterobacter* are important causes of pneumonia, and all of the Enterobacterales have been implicated in bloodstream infections and in peritonitis, cholangitis, and other intra-abdominal infections[24]. Additionally, organisms such as *Salmonella* produce gastroenteritis and, subsequently, in some patients, invasive infection[24].

Antibiotic resistance is not only a major complication but antimicrobial resistance (AMR) is also one of the top ten global public health concerns flourishing all over the world[23].Emerging resistance in *Enterobacterales* is a significant problem[23]. Resistance related to production of extended-spectrum β-lactamases (ESBLs) and carbapenemases is a

major problem in the management of infections with the Enterobacterales[23]. The emergence of carbapenemases in *Enterobacterales* is of particular concern since these organisms often present with extensive drug resistance (XDR) and sometimes even pan-drug resistance (PDR).[23]

β-lactamases

The discovery of β-lactamases predated the clinical use of benzylpenicillin, but the widespread use of these agents in the clinic has, over time, led to the emergence of an astonishing number of β-lactamase variants, which can degrade most or all β-lactam antibiotics[29,30]. β-lactamases are enzymes that hydrolyze the β-lactam ring of all classes of β-lactam antibiotics by one of the two major mechanisms.[25]

The first is mediated by an active-site serine (Ser), via a covalent enzyme intermediate that is rapidly hydrolyzed causing inactivation of the antibiotic. β-lactamases that operate by this mechanism are therefore referred to as serine β-lactamases.[25] This mechanism is reminiscent of that for PBP inactivation by β-lactam antibiotics, as β-lactamases share an active-site Ser-XX-Lys motif with PBPs [25].

The second mechanism is metal-mediated, whereby one or two bivalent metal ions activate a water molecule that attacks the β-lactam ring [31]. These β-lactamases are correspondingly referred to as metallo-β-lactamases. The large number of serine and metallo-β-lactamases is categorized via two different classification systems[25].

The First Ambler classification is based on protein sequence homology that divides β-lactamases into four classes (A, B, C, and D) [25]. Classes A, C, and D are all serine β-lactamases, whereas class B is the metallo-β-lactamases[25]. The second classification scheme in use for β-lactamases, defined by Bush-Jacoby, is based on enzymatic functionality and divides β-lactamases into three major groups: group 1 cephalosporinases (class C), group 2 serine β-lactamases (classes A and D), and group 3metallo-β-lactamases[25]. Each major group is then divided into several subgroups based on specific attributes[29]. Among the β-lactamases, the most common is production of ESBLs & AmpCs [29] (Table 1).

Table 1: Classification of clinically relevant β-Lactamases [3,25, 29]

Molecular Class	Functional Group	Description	Substrates	Representative Families	Representative Enzyme In Clinical Isolates
A	2be	Extended-spectrum β-lactamases (ESBLs)	Penicillins, Cephalosporins monobactam	TEM, SHV, CTX-M, PER, VEB	TEM-3, SHV-2, PER-1, VEB-1, CTX-M-15, CTX-M-9, CTX-M-14, CTX-M-3
A	2br	Inhibitor-resistant β-lactamases	Penicillins, narrow-spectrum cephalosporins	TEM, SHV	TEM-30, SHV-10
A	2f	Serine carbapenemases	Carbapenems, Cephalosporin, cephamycins	KPC, IBC, IMI, NMC, SME, GES, SFC,	KPC-1, KPC-2, KPC-3, SME-1
B	3a	Metallo-carbapenemases	Carbapenems, penicillins, cephalosporins, cephamycins	IMP, VIM, NDM, SPM, GIM, SIM, AIM, DIM, FIM, POM	VIM-1 VIM-2 IMP-1 NDM-1
C	1	AmpC β-lactamases	Cephamycins, Cephalosporins, narrow-spectrum monobactams, and penicillins	CMY, FOX, ACC, LAT, ACT, MOX, DHA, MIR	CMY-1, CMY-2 ACT-1, DHA-1, DHA-2, CMY-13, CMY-4

D	2de	Extended-spectrum β-lactamases (ESBLs)	Cephalosporins, oxacillins	OXA	OXA-10, OXA-13, OXA15, OXA-18, OXA-45
D	2df	Carbapenemases	Carbapenems, oxacillins	OXA	OXA-48, OXA-23 OXA-40, OXA-51, OXA-58

Penicillinase:[25]

Penicillinase is a particular type of β-lactamase, appearing to have specificity for penicillins, hydrolyzing the beta-lactam ring. Weight of penicillinases tend to cluster near 50 kD. Penicillinase was the first betalactamase to be identified. It was first isolated by Abraham and Chain in 1940 from Gram negative *E.coli.* This enzyme degrades the penicillin by hydrolysing the cyclic amide bond in the β-lactam ring of penicillin and thus inactivates the antibiotic.

Extended-Spectrum β-Lactamases (ESBLs)

Extended-spectrum β-lactamases (ESBLs) confer resistance to nearly all β-lactam antibiotics except carbapenems and cephamycins[32]. ESBLs were first identified in the mid-1980s in *K. pneumonia* and *Serratia marcescens* [32]. The occurrence of ESBLs in clinical isolates has been constantly increasing in the past two decades [25].

Early ESBLs evolved from the TEM and SHV enzymes to be able to hydrolyze oxyimino-cephalosporins, and these are molecular class A, functional group 2be[29,30]. Subsequently, the ESBL category expanded to include enzymes such as the CTX-M family, mainly present in *E. coli* and *K. pneumoniae*; the PER family identified in *Pseudomonas*, *Acinetobacter*, and *Salmonella* species; and the VEB family reported in *Acinetobacter baumannii* [29,30]. These β-lactamases are not genetically related to TEM or SHV β-lactamases but have similar hydrolytic profiles and are part of the functional group 2be [29,30]. The most recent ESBLs are the OXA family, originally reported *in P. aeruginosa*, isolated in Turkey and France. The OXA family, in contrast to the other ESBLs, belongs to molecular class D and functional group 2de [25].

ESBLs are prevalent in the clinic and present serious problems in hospital acquired infections, leading to increased mortality worldwide[25]. Another growing family of ESBL's is the OXA-type enzymes that confer resistance to ampicillin and cephalothin, are characterized by their high hydrolytic activity against oxacillin and cloxacillin, and are very poorly inhibited by clavulanic acid[25]. The OXA-type enzyme genes differ genetically from all other ESBLs[25]. To date, over 500 different OXA-type variants have been reported, but not all are ESBLs[25].

The OXA-type enzymes with activity against oxyimino-cephalosporins are OXA- 10 and its variants (OXA-11, OXA-14, OXA-16, and OXA-17), OXA-13 and its variants (OXA-19 and OXA-32), and some other OXA enzymes (OXA-15, OXA- 18, and OXA-45). These enzymes have been identified mainly in *P. aeruginosa* isolates [25,27].

Further, ESBLs are often present on mobile genetic elements with other antibiotic-resistant determinants, including those for aminoglycosides and fluoroquinolones[25,26]. The use of carbapenems to treat infections caused by ESBL producing pathogens is increasing the emergence of carbapenem-resistant strains, starting the debate on how to better treat those pathogens. Using a β-lactamase inhibitor with a β-lactam is in principle a targeted and effective approach [25].

Class C β-Lactamase (AmpC)

The first bacterial enzyme reported to destroy penicillin was the AmpC β-lactamase of *Escherichia coli*, although it had not been so named in 1940[28]. In 1965 systematic Swedish investigators began a systematic study of the genetics of penicillin resistance in *E. coli* [28]. Mutations with stepwise-enhanced resistance were termed *ampA* and *ampB* [38,39]. A mutation in an *ampA* strain that resulted in reduced resistance was then designated AmpC[28]. *ampA* strains overproduced β-lactamase, suggesting a regulatory role for the *ampA* gene.[40] *ampB* turned out not to be a single locus, and such strains were found to have an altered cell envelope[41]. Most of the *amp* nomenclature has changed over the years, but the designation AmpC has persisted[34]. The sequence of the AmpC gene from *E. coli* was reported in 1981[37]. In 1995, the functional classification scheme was published for chromosomally determined AmpC β-lactamases in *Enterobacterales* and also in a few other families[42].

AmpC β-lactamases belong to class C and functional group 1. They confer resistance to cephamycins, such as cefoxitin and cefotetan, and cephalosporins, including oxyimino-

cephalosporins such as ceftazidime, cefotaxime, and ceftriaxone [34]. They are also able to hydrolyze to a lesser extent penicillins and aztreonam[34].

The majority of AmpC β-lactamases are either not inhibited or are only weakly inhibited by inhibitors of class A enzymes such as clavulanic acid, sulbactam, and tazobactam[34]. Some AmpC variants have been reported to be inhibited by tazobactam or sulbactam[34, 35]. Several AmpC β-lactamases are chromosomally encoded enzymes, and this AmpC β-lactamase production is frequently associated with production of multi drug resistance [33]. In the clinical laboratory settings, the commonly detected enzymes causing resistance are AmpC β-lactamases and ESBLs.[36]

Structure, Chemistry, Enzymatic and Physical Properties of Ampc B-Lactamases[11-20,25-29, 30-38]

AmpC β-lactamases have a molecular mass of 34 to 40 kDa and isoelectric point of 8.0, although the isoelectric points of plasmid-mediated FOX enzymes are lower (6.7 to 7.2) [43]. These enzymes are present in the periplasm, some are secreted[34]. They hydrolyse substrates like penicillins, cephalosporins and can also hydrolyze cephamycins, oxyiminocephalosporins and monobactams[34].

AmpC structures are available for four organisms: *E*. *coli*, *E*. *cloacae*, C. *freundii*, and *P*. *aeruginosa*[44]. More than 70 AmpC structures have been solved for *E*. *coli*, including more than 40 with an identical sequence [44].

The known three-dimensional structures of AmpC enzymes of various organisms are very similar [44].There is an α-helical domain on one side of the molecule and an α/β domain on the other [44].The active site lies in the center of the enzyme at the left edge of the five-stranded β-sheet with the reactive serine residue at the amino terminus of the central α-helix [34].The active site can be further subdivided into an R1 site, accommodating the R1 side chain of the β-lactam nucleus, and an R2 site for the R2 side chain [34].The R1 site is bounded by the Ωloop(Omega loop), while the R2 site is enclosed by the R2 loop containing the H-10 and H-11 helices[34,46]. Key catalytic residues in addition to Ser64 for AmpC enzymes include Lys67, Tyr150, Asn152, Lys315, and Ala318, with substitutions at these sites lowering enzymatic activity dramatically[34,45,46]. In the folded protein, most of these essential residues are found at the active site, with Lys67 hydrogen bonded to Ser64 and Tyr150 acting as a transient catalytic base [34,45,46].

Phylogeny:

The ancient enzymes of serine β-lactamases originated more than 2 billion yrs ago[34]. AmpC enzymes are divided from a common ancestor in to class A & class D[34]. AmpC enzymes from organism of same genus cluster together, but, AmpC β-lactamases of *Pseudomonas*, Enterobacterales, *Acinetobacter* are distinctly related [34]. Table 2 enummerates the list of bacteria expressing chromosomally determined AmpC β-lactamases.

Table 2: Taxonomy of bacteria expressing chromosomally determined ampc β-lactamases [34]

Phylum, class, and order	Genus and species
Actinobacteria	*Mycobacterium smegmatis*
Proteobacteria *Alphaproteobacteria*	*Ochrobactrum anthropi* *Rhodobacter sphaeroides* *Chromobacterium violaceum*
Betaproteobacteria *Neisseriales*	*Laribacter hongkongensis*
Gammaproteobacteria *Aeromonadales*	*Aeromonas caviae* *Aeromonas hydrophila* *Aeromonas jandaei* *Aeromonas salmonicida* *Aeromonas veronii* bv. sobria
Enterobacterales	*Buttiauxella agrestis* *Citrobacter braakii* *Citrobacter freundii* *Citrobacter murliniae* *Citrobacter youngae* *Citrobacter werkmanii* *Edwardsiella tarda* *Enterobacter aerogenes* *Enterobacter asburiae* *Enterobacter cancerogenus* *Enterobacter cloacae*

	Enterobacter dissolvens *Enterobacter hormaechei* *Enterobacter intermediusb* *Erwinia rhapontici* *Escherichia albertii* *Escherichia fergusonii* *Escherichia coli* *Hafnia alvei* *Morganella morganii* *Providencia stuartii* *Serratia marcescens* *Shigella boydii* *Shigella dysenteriaec* *Shigella flexneric* *Shigella sonnei* *Yersinia enterocolitica* *Yersinia mollaretii* *Yersinia ruckeri*
Oceanospirillales *Pseudomonadales*	*Chromohalobacter* *Acinetobacter baumannii* *Acinetobacter baylyi* *Pseudomonas aeruginosa* *Pseudomonas fluorescens* *Psychrobacter immobilis*
Xanthomonadales	*Lysobacter lactamgenus*

Epidemiology of AmpC Production [48,86,87]

Chromosomally encoded AmpC genes can be identified in a number of gram-negative organisms including *E. cloacae, Klebsiella* (formerly *Enterobacter*) *aerogenes, C. freundii, S. marcescens, Providencia stuartii, P. aeruginosa, Hafnia alvei,* and *Morganella morganii* [34]. The hallmark phenotypic pattern of these organisms is that they appear to be susceptible to third-generation cephalosporins if AmpC production is not induced (i.e, in the absence of AmpC production), but resistance can develop upon β-lactam exposure as

early as a single day after drug initiation [47]. The extent of β-lactam resistance conferred by these enzymes is associated with the regulatory gene network of the organism and its ability to control expression levels [47].Some frequently encountered *Enterobacterales* are conspicuous by the absence of a chromosomal AmpC gene such as *K. pneumoniae, Klebsiella oxytoca, Salmonella species,* and *Proteus mirabilis* [34].Even species in the same family of the *Enterobacterales* such as *Enterobacter spp., Serratia marcescens, Citrobacter freundii, Providencia spp.* and *Morganella morganii* (ESCPM) organisms may not possess chromosomal AmpC genes, such as *Citrobacter amalonaticus or Citrobacter koseri* [34].The *Enterobacterales* are not homogenous in their level of expression of AmpC production. Derepressed *S. marcescens*, *M. morganii,* and *P. stuartii* strains express AmpC levels that are ~10-fold lower than derepressed *E. cloacae* or *C. freundii* isolates. In recent years, various studies, have been conducted on the occurrence of AmpC producing bacteria in humans. It is being observed more and more AmpC producing bacteria play a major role in health care facilities as the pathogen that cause so called Nosocomial infection or Hospital acquired infection. Risk of infection via food with the different type of pathogens, especially *Salmonella, EHEC, Klebsiella* spp is also prevalent. Wound infection, urinary tract infection, VAP, Meningitis, septicemia, CAUTI have increased mortality and morbidity especially in immunocompromised patients, than in the immune competent [86].To enable bacteria to produce AmpC, they must carry the necessary genetic information i.e. Resistance genes. As they are passed from one bacteria generation to the next during cell division called "VERTICAL TRANSFER', the propagation and distribution of these bacterium also contribute towards the spread of the resistance genes. Poor hygiene, in hospitals, environment, animal shed, & home play a major role in the carryover of the bacteria. Because the resistance gene vary often lie on transmissible gene section, they can also be exchanged between bacteria of same species or different species called "HORIZONTAL GENE TRANSFER". The big problem here is the harmless intestinal bacteria can pass on the genes for AmpC to pathogenic bacteria, such as 'Salmonella' Infections especially by food stock handlers, pet animal handlers, health care workers. So, risk of infections occurs in between animals or animals to human by pet handlers [87]. Here the "One Health" approach by WHO plays a major role in curbing antimicrobial resistance by a multifaceted approach.

AMP C BETALACTAMASES -THE SILENT EMERGING RESISTANCE AMONG ENTEROBACTERALES: A REVIEW PART 2

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Mechanism of Resistance[20-28,40 to 48,51-60]**:**

AmpC resistance can be classified into 3 categories[48]:

1) Inducible chromosomal resistance that emerges in the setting of a presence of β-lactam compound,
2) Stable derepression due to mutations in AmpC regulatory genes, or
3) the presence of plasmid-mediated AmpC genes.

1) Inducible chromosomal resistance that emerges in the setting of a β-lactam compound [48]**:**

Chromosomally encoded AmpC genes can be induced in the appropriate environment. Normally, the regulatory protein AmpR reduces AmpC β-lactamase expression to very low levels. Certain β-lactams induce the production of cell-wall degradation products (eg, N-acetylglucosamine-1,6-anhydro N-acetylmuramic acid oligopeptides). As these peptides accumulate, they compete with uridine diphosphate (UDP)–N-acetylmuramic acid peptides for binding to AmpR, the negative regulator of AmpC. With decreased UDP-N-acetylmuramic acid peptide binding to AmpR, AmpR undergoes conformational changes that disable its function, increasing production of AmpC enzymes. A second recycling protein, AmpD, is responsible for cleavage of residues off cell-wall degradation products, reducing their ability to bind to AmpR but still allowing them to be recycled back into the cell-wall synthesis pathway. AmpG transports oligopeptides involved in peptidoglycan recycling and AmpC regulation into the cytosol. As concentrations of degradation products increase, AmpD is unable to cleave all of the necessary peptides, leading to binding of these products to AmpR, decreasing AmpR repression and subsequently increasing AmpC transcription[48].

The most common cause of AmpC overexpression in clinical isolates is a mutation in AmpD leading to AmpC hyperinducibility or constitutive hyperproduction. AmpR

mutations are less common. Least common are AmpG(inner membrane permease) which result in constitutive low level expression [48].

Additional Features of AmpC Regulation [20-28,40 to 48,51-60]:

1) *E. coli* lacks AmpR gene as in Shigella, consequently AmpC is non inducible but is regulated by promoter and attenuator mechanism.
2) Acinetobacter baumannii also lacks AmpR gene so its AmpC β-lactamase is non-inducible.
3) Serratia marcescens is regulated by AmpR, but AmpC transcript influences the half life.
4) Pseudomonas aeruginosa has 3 AmpD genes which explains stepwise regulation of of AmpC production by successive inactivation of AmpD.
5) AmpC enzyme in Aeromonas.spp controlled by brlAb which has two content regulator.

β-lactam differ in their inducing abilities strong inducers, good substrates for AmpC β-lactamase are ampicillin, amoxicillin, benzyl penicillin & Ist generation of cephalosporins[48]. Cefoxitin, Imipenem are also strong inducers but are much more stable for hydrolysis[34,82,25]. Weak inducers and weak substrates but can be hydrolysed if enzymes are supplemented are the 3rd, 4th generation cephalosporins, piperacillin & aztreonams.

MIC of weakly inducing oxyimino beta lactam are increased with AmpC hyperproduction. But, MIC of strong inducers shows little change with regulatory mutations [34,67,25]. β- lactam inhibitors are also inducers, especially clavulanate, has little inhibitory effect on AmpC, but can paradoxically appear to increase AmpC mediated resistance [34,68,25].

2) Stable derepression due to mutations in AmpC regulatory genes [20-28,40 to 48,51-60]

Mutational inactivation of AmpD also leads to the accumulation of 1,6 anhydromuropeptides and to AmpC hyperexpression, even in the absence of β-lactamase inducers, resulting in a constitutively derepressed resistance phenotype due to AmpC overproduction. In addition to the cytoplasmic AmpD, two periplasmic *N*-acetyl-anhydromuramyl-L-alanine amidases (AmpDh2 and AmpDh3) and that their sequential inactivation leads to a stepwise upregulation of AmpC, reaching full derepression with very high levels (>1,000-fold with respect to the wild-type level) of AmpC expression and clinical β-lactam resistance in the triple mutant.This full AmpC derepression through inactivation of the three amidases has been associated with fitness and virulence

impairment, severely compromising growth rates, motility, and cytotoxicity, the latter being likely due to repression of key virulence factors.

Chromosomal AmpC enzymes [34,25,29]:

With high level AmpC β-lactamase production by mutation, the development of resistance upon therapy is major concern. Combination therapy did not prevent resistance emergence. Low level expression of AmpC β-lactamase in *E. coli*, high level producers identified in clinical specimens as cefoxitin-resistance isolates with stronger AmpC promoters or mutations that destabilize the normal AmpC attenuater [50 to 62].

PCR, and sequencing revealed different promoter or attenuator variants. In a few strains, the integration of an insertion element created a new & stronger AmpC promoter [50 to 62]. These strains are not only resistant to cefoxitin but also typically resistant to ampicillin, ticarcillin, cephalothin, and β-lactam combination with clavulanic acid and have reduced susceptibility or resistant to ESBL[34].

Few *E. coli* strains with promoter mutation have some alteration in bla -AmpC, expanding its resistance spectrum, with loss of outer membrane porins can augment the resistance phenotype further. These strain remain susceptible to cefepime and imipenem, but, may become ertapenem resistance upon therapy is concern[48].

Plasmid-Mediated AmpC β-lactamases[34,25,29]

Plasmid-mediated AmpC (pAmpC) are AmpC β-lactamases encoded on plasmids and hence transferable between species. These enzymes appeared in Enterobacterales that lack chromosomal AmpC enzymes (*Proteus mirabilis, Salmonella spp and Klebsiella spp*) or only express low basal amounts of AmpC like *Escherichia coli and Shigella spp*[53]. The frequency of pAmpC may be of larger concern than initially thought, especially if this resistance threat would mimick the trend that we have seen occurring over the past years for ESBLs[53]. The exact prevalence of pAmpC is still unknown because simple and valid detection methods are not available, hence pAmpC-producing organisms are often missed [53]. More than 20 different AmpC β -lactamases have been found to be mediated by plasmids[54]. They have been found around the world in nosocomial and nonnosocomial isolates, having been most easily detected in those *Enterobacterales* not expected to produce an AmpC β-lactamase [34]. Minor differences in amino acid sequence have given rise to different families [34]. The list of Plasmid mediated AmpC is enlisted in table 3.

Table 3: Plasmid-mediated AmpC β-lactamases [34,25,29]

AmpC β-lactamase	Country of origin	Publication yr	Species of first isolate	Likely source of AmpC gene
CMY-1	South Korea	1989	*K. pneumoniae*	*A. hydrophila*
CMY-2	Greece	1996	*K. pneumoniae*	*C. freundii*
MIR-1	United States	1990	*K. pneumoniae*	*E. cloacae*
MOX-1	Japan	1993	*K. pneumoniae*	*A. hydrophila*
LAT-1	Greece	1993	*K. pneumoniae*	*C. freundii*
FOX-1	Argentina	1994	*K. pneumoniae*	*A. caviae*
DHA-1	Saudi Arabia	1997	*S. enteriditis*	*M. morganii*
ACT-1	United States	1997	*K. pneumoniae*	*E. asburiae*
ACC-1	Germany	1999	*K. pneumoniae*	*H. alvei*
CFE-1	Japan	2004	*E. coli*	*C. freundii*

Like the chromosomally determined AmpC β-lactamases, the plasmid-mediated enzymes confer resistance to a broad spectrum of β -lactams including penicillins, oxyimino-β-cephalosporins, cephamycins, and (variably) aztreonam. Susceptibility to cefepime, cefpirome, and carbapenems is little affected. ACC-1 is exceptional in not conferring resistance to cephamycins and is actually cefoxitin inhibited. The genes for ACT-1, DHA-1, DHA-2, and CMY-13 are linked to *ampR* genes and are inducible while other plasmid-mediated AmpC genes are not, including other CMY alleles and apparently CFE-1. The level of expression of both inducible ACT-1 and noninducible MIR-1 is 33- to 95-fold higher than the level of expression of the chromosomally determined AmpC gene of *E. cloacae.*

Plasmids carrying genes for AmpC β-lactamases often carry multiple other resistances including genes for resistance to aminoglycosides, chloramphenicol, quinolones, sulfonamide, tetracycline, and trimethoprim as well as genes for other β-lactamases such as TEM-1, PSE-1, CTX-M-3, SHV varieties, and VIM-1. The AmpC gene is usually part of an integron but is not incorporated into a gene cassette with an affiliated 59-base element [34]. A variety of genetic elements have been implicated in the mobilization of AmpC genes onto plasmids[34]. The insertion sequence (IS*Ecp)* IS*Ecp1* plays a dual role, it is involved in the transposition of adjacent genes and has been shown able to mobilize a

chromosomal *bla* gene onto a plasmid, and it also can supply an efficient promoter for the high-level expression of neighboring genes [122].

Plasmid-mediated AmpC -lactamases represent a major threat since they confer resistance to 7-β methoxy-cephalosporins such as cefoxitin or cefotetan, are not affected by commercially available -lactamase inhibitors, and can, in strains with loss of outer membrane porins, provide resistance to carbapenems. This resistance mechanism has been found around the world, can cause nosocomial outbreaks, appears to be increasing in prevalence [55].

Plasmid mediated AmpC β lactamase have been discovered world wide, according to resistance produced to the respective antibiotic they have been designated as

CMY ; Cephamycin 60.00.00,000 million

FOX ; Cefoxitin. ; 1.55,00,000 million

MOX ; Moxalatem ; 1.04,00,000 million

LAT ; Latamoxif ; 3,00,000 million

Designated according to the type of β lactamase such as

AmpC type **;**ACT ; 20,00,000

ACC ; (AMBLER CLASS C)

According to the site of discovery they have been designated as

MIR; (Miriam Hospital in Providence) - 69,00,000

DHA ; Dharan hospital in Saudi Arabia-1.07,00,000

In Greece - Plasmid mediated LAT-2, CMY-2, have been found in clinical isolates of *Enterobacter aerogenes* simultaneously with its appearance in clinical strains of *E. coli,* and *Klebsiella.*

In France; plasmid mediated ACC -1 found in *E. coli, Proteus spp*, from urine samples.

In United States Ceftriaxone resistance *Salmonella* was isolated from symptomatic patients.

CMY-2; Spread from Pakistan to UK, India to UK, Algeria to France.

CMY-4; From India to Sweden.

Mox-2; From Greece to France.

ACC; Tunisia to France, FOX - from Guatimala to Germany. Most of the plasmid mediated AmpC enzymes are isolated from ICU, or post operative, post organ transplant cases, malignancy. With increased antimicrobial use AmpC producers causing nosocomial infections are the major cause of outbreaks.

Clinical relevance of diseases produced by pathogens producing AmpC β-Lactamases:[34,25,48]

Infections caused by gram negative organisms expressing plasmid mediated AmpC β-lactamases are usually resistant to all β- lactam antibiotics. Except for cefepime, cefpirome, and the carbapenems constitutive over expression of AmpC β-lactamases in gram negative organisms occur either by deregulation of the AmpC chromosomal gene or by transferable AmpC gene on a plasmid [34]. The transferable AmpC gene products are called plasmid mediated AmpC β-lactamases[55]. Mobilization has occurred from the genome of species carrying inducible/ de- repressed bla/AmpC such as *Citrobacter freundii* and *Morganella morganii* by plasmids in to *E. coli,* and *K. pneumonia* [34,55,56]. The recognition of plasmid mediated AmpC β-lactamases in *E. coli* and *Klebsiella spp* esp.the world wide distribuition AmpC resistance is important. The estimation of the presence of AmpC detection among clinical isolates is also crucial since there are presently no CLSI guidelines for screening and confirmation of the presence of AmpC in routine clinical isolates.

The detection of AmpC production is challenging, since the hyperproduction of enzyme in association with OMP F porin loss in *E. coli* or porin deficiency in *K. pneumoniae* can produce phenotypic resistance [34,55,56]. Detection of plasmid mediated AmpC producing isolates is critical for epidemiological studies, hospital infection control. Because the gene can spread to other organisms. Genes for AmpC β- Lactamases are found on chromosomes of several members of the Enterobacterales family including *E. coli, Enterobacter, Shigella, Providencia, Citrobacter freundii, Morganella morganii, Serratia marcesens*[34,55,56].

In recent years, various studies, have been conducted on the occurrence of AmpC producing bacteria in humans. It is being observed that more and more AmpC producing bacteria play a major role in health care facilities as they cause many Hospitals acquired infection & risk of infection via food with the especially *Salmonella, EHEC, Klebsiella*, and also produce wound infection, urinary tract infection, VAP, Meningitis, septicemia, CAUTI. Mortality and morbidity are increased in immunocompromised patients, then immune competent. So, risk of interlinked transmission of these AmpC β-lactamases is the emerging threat which needs immediate addressing so that it can be curtailed in its very nascent stage itself.

Detection of AmpC[3,4,5]

Detection of the resistance mediated by Class C β- lactamases remains a challenging issue considering that transferable plasmid mediated class C β-lactamases are of worldwide concern. Several methods that use the Kirby- Bauer disk potentiation method with some β- Lactamases inhibitors or the three dimensional methods have been developed and a cefoxitin agar medium based assay that uses preparations of bacterial cell extracts also have been developed. However these methods are technically intricate and interpretation of their results in not sufficiently simple for routine use in clinical microbiology laboratories.

PCR or multiplex PCR analysis are able to provide satisfactory results in the identification and classification of genes for β-lactamases, but equipment availability is limited to medical institutions such as university hospital. They are costly and require time consuming techniques. An enzyme-linked immunosorbent assay has also been developed and has known sensitivity and specificity for the detection of certain class C β -Lactamases. This technique is less costly than genetic methods, but it is not sensitive for the detection of class C β- Lactamases that possess less than 70% homology to CMY – 2 [39]. Thus practical and simple methods for detection of the resistance medicated by plasmid- mediated Class C β - Lactamases are urgently needed for enhanced infection control. There are no CLSI or other approved criteria for AmpC detection. Organisms producing AmpC β- lactamase gives positive ESBL screening test, but fail the confirmatory test and increased sensitivity to clavulanic acid so, confirmatory test are needed.

Phenotypic Method

Screening Tests[34,64]:

Strains resistant to cefoxitin(30µg)(zone diameter ≤18mm) and oxyimino betalactams (ceftriaxone and cefotaxime) by disc diffusion (Kirby-Bauer) method are considered or suspected to be AmpC producers. However, it is not confirmatory as cefoxitin resistance is found in certain carbapenemases and few Class A β-lactamases and also a few AmpC strains have shown susceptibility to ceftriaxone, cefotaxime [50]. Hence confirmatory tests are needed.

Phenotypic Confirmatory Tests:

There are presently no CLSI or other approved criteria for AmpC detection. The methods which are routinely tried are as follows:

1. **AmpC disc test** [57]:
2. **Disc Antagonism Test**[58]:
3. **Ceftazidime- Imipenem disc Antagonism Test** [60]:
4. **Boronic acid inhibitor based test [63]:**
5. **Double disc synergy(Cloxacillin combined disc diffusion test)** [63]:
6. **Modified three dimensional test** [66]:
7. **The Etest**[67]

Molecular Method by Multiplex PCR Method[65-68]

Phenotypic test cannot distinguish various families of plasmid mediated AmpC enzymes but, also can identify chromosomally determined AmpC enzymes as an ESBL[34]. For this, the current Gold standard method for detection of plasmid mediated AmpC is multiplex PCR.Previously it was done by utilizing six primer pairs(MOX, CIT, DHA, ACC, EBC, FOX) now in addition to this a seventh pair for CEF-1β lactamase is added and used for detection[34]. But even these primers could not fully cover the increasing number of alleles deposited in the GenBank database[34]. AmpC screening by MALDI-TOF MS-based direct-on-target microdroplet growth assay (DOTMGA) and Whole genome sequencing are novel methods which are under trials but none of them have emerged as a definite recommendation for Detection of AmpC.

Algorithm For AmpC Detection in *Enterobacterales*[64]:

Combining the most sensitive screening method with the most accurate confirmation assay for AmpC production, consists of (i) cefoxitin as a screening marker for AmpC production and (ii) CC-DDS as phenotypic confirmation, Along with (iii) molecular methods in the case of inconclusive results.

Rationale of Treatment of AmpC Producing Organisms[68] :

β-lactam antibiotics fall within a spectrum of potential for inducing AmpC. Aminopenicillins (i.e., amoxicillin, ampicillin), narrow-spectrum (i.e., first generation) cephalosporins, and cephamycins are potent AmpC inducers. However, organisms at moderate to high risk for clinically significant AmpC induction (e.g., *Enterobacter cloacae*) hydrolyze these antibiotics even at basal AmpC expression levels. Therefore, such AmpC isolates will generally test as non-susceptible to these drugs, averting treatment dilemmas. Imipenem is also a potent AmpC inducer but it generally remains resistant to AmpC hydrolysis because of the formation of stable acyl enzyme complexes.

The induction potential of ertapenem and meropenem has not been formally investigated but, similar to imipenem, they are generally able to resist AmpC hydrolysis. Piperacillin, ceftriaxone, ceftazidime, and aztreonam are relatively weak AmpC inducers. Despite the limited ability of ceftriaxone and ceftazidime to induce AmpC production, the susceptibility of these agents to hydrolysis makes them unlikely to be effective for the treatment of infections by organisms at moderate to high risk for clinically significant inducible AmpC production. Similarly, piperacillin, even with the addition of tazobactam, has the potential to be hydrolyzed in settings of sufficient AmpC production.

Cefepime is an oxyminocephalosporin that is relatively stable against AmpC enzymes and that also has low AmpC induction potential.However, several case reports of therapeutic failure of cefepime against infections caused by AmpC have led to hesitancy in prescribing this agent.Understanding the contribution of AmpC production to cefepime clinical failure is challenging as the drug is generally dosed every 12 hours, co-production of ESBL enzymes is rarely investigated, and the presence of outer membrane porin mutations may have contributed to cefepime treatment failure.

It is preferential to administer carbapenem therapy (i.e., ertapenem, meropenem, imipenem-cilastatin), for infections caused by *E. cloacae*, *K. aerogenes*, and *C. freundii* with cefepime MICs of 4-8 mcg/mL.

Ceftriaxone, ceftazidime, Piperacillin-tazobactam is not suggested for the treatment of serious infections caused by Enterobacterales at moderate to high risk of clinically significant inducible AmpC production. Despite the increased potency of newer β-lactams (i.e., cefiderocol) and β-lactam-β-lactamase inhibitor combination agents (i.e., ceftazidime-avibactam, imipenem-cilastatin-relebactam, and meropenem-vaborbactam) against AmpC infections compared with piperacillin-tazobactam, the panel suggests that these agents be preferentially reserved for treating infections caused by organisms exhibiting carbapenem resistance. Surveillance studies indicate that ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-cilastatin-relebactam exhibit excellent *in vitro* activity against AmpC. Clinical outcomes studies have also reinforced *in vitro* data demonstrating the effectiveness of these newer β-lactam-β-lactamase inhibitor combinations against *Enterobacterales* at moderate to high risk for clinically significant AmpC production.

Ceftolozane was developed to be more resistant to hydrolysis than earlier cephalosporins against *Pseudomonas*-derived AmpC cephalosporinases, but much less is

known about its activity against AmpC. Clinical outcomes data for ceftolozane-tazobactam treatment of AmpC infections are limited.

Preferred treatment options for AmpC uncomplicated cystitis include nitrofurantoin TMP-SMX or a single intravenous dose of an aminoglycoside. Aminoglycosides are nearly exclusively eliminated by the renal route in their active form. A single intravenous dose is generally effective for uncomplicated cystitis, with minimal toxicity. There should be limit in use of oral fosfomycin exclusively for the treatment of *E. coli* cystitis as the *fosA* gene, intrinsic to several other Gram-negative organisms, including organisms at moderate to high risk of AmpC production, can hydrolyze the drug and may lead to clinical failure.

Prevention and control of infection caused by AmpC producing organisms [34,3,4,5]

AmpC producing bacteria are carried in the feces, which may spread via food chain, thereby producing reservoir of multiple resistant bacteria in the gut. It can be spread from person to person by contaminated hand, untrained person handling with urinary catheter in hospitalised patient, so, urinary infection commonly occur in patient after admission, also associated chest infection, wound infection causing septicemia thereby increasing the prevalence of mortality and morbidity. In hospital settings, acquisition is mainly due to indirect transmission between patients with the hands of healthcare workers (HCWs) as vectors. Increased prevalence of colonisation augments the risk of acquiring infection. Such infections represent a serious socioeconomic burden and are associated with a raised mortality, more frequent hospital admissions in comparison with non-carriers and additional costs. Many interventions have been proposed to limit the transmission of multidrug-resistant organisms (MDROs) in hospitals like (1) universal strategies (eg, improvement of hand hygiene among healthcare workers, antibiotic policy & antibiotic stewardship), (2) targeted strategies (eg, screening of patient for ESBL at ICU admission and contact precautions or cohorting of carriers) and (3) mixed strategies (eg, targeted approaches combined with antibiotic stewardship)[68]. One Health recognizes the inextricable link between humans, animals, and the environment to achieve better community health and well-being. One Health is an interdisciplinary and holistic concept considering the interdependent human and animal health in association with the ecosystem, where they live. The leading regulatory authorities such as the International Monetary Fund (IMF), the World Bank, the World Health Organization (WHO), and the G8 declared Antimicrobial resistance as a major global health threat of the 21st century [69]. All these forums affirmed that Antimicrobial resistance needs coordinated and

interdisciplinary efforts because different ecosystems participate in the acquisition, emergence, and distribution of Antimicrobial resistance (Hernando-Amado *et al.,* 2019). The emergence of antimicrobial resistance and transmission dynamics of multi-drug-resistant pathogens comes under One Health suggesting an indispensable collaborative role of human, animal, and environmental professionals in mitigation of global antimicrobial resistance [124].

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THE VIRTUAL REVOLUTION: REDEFINING HEALTHCARE THROUGH TELEMEDICINE AND AI

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Abstract:

Telemedicine, defined as the distant provision of healthcare services through technology, has experienced significant modifications in recent years. This analysis examines the rising trends in telemedicine and their significant influence on healthcare delivery. The article commences with an overview of telemedicine, detailing its development and underscoring its increasing importance in the healthcare sector. The main aim is to elucidate the present condition of telemedicine, discern significant developments, and evaluate their revolutionary impacts on healthcare delivery. The technological environment of telemedicine is analysed, emphasizing advancements in video conferencing, virtual consultations, and the incorporation of electronic health data. Additionally, the function of artificial intelligence (AI) is examined, including diagnostic support via medical imaging algorithms and the incorporation of chatbots and virtual health assistants. Specialized fields of telemedicine, like mental health care and chronic illness management, are examined thoroughly to emphasize particular advancements in these areas. The pivotal element of patient and healthcare provider acceptance is examined, highlighting patient involvement, surmounting technological obstacles, and tackling the difficulties encountered by healthcare professionals in virtual environments. The analysis forecasts the future of telemedicine, highlighting forthcoming advancements like as virtual reality applications and the influence of 5G technology. Concurrently, it tackles ongoing concerns, such as health disparities, the assurance of treatment quality, and ethical issues. This analysis consolidates the present status of telemedicine, highlights transformational developments, and offers insights into the future of healthcare delivery. It necessitates ongoing study, policy formulation, and strategic execution to optimize the potential of telemedicine in establishing a more accessible, efficient, and patient-centered healthcare system.

Keywords: Telemedicine, Healthcare, Delivery, Trends

Introduction:

Technology-based remote healthcare, called "telemedicine," was created in the 1970s. Marginal to revolutionary: healthcare delivery. Telecommunications and medicine have led to new ways to improve patient care, optimize healthcare systems, and bridge geographical distances. Telemedicine's popularity is expanding, but we must first understand its history and rationale. Telemedicine began with phones. Video conferencing expanded telemedicine by letting doctors see patients remotely. Telemedicine is growing quickly because to the internet and digital technologies.[1] Technological advances, regulatory reforms, and public attitudes toward digital health solutions have hampered telemedicine inclusion into traditional healthcare. Early telemedicine faced distance and availability issues, especially in rural or disadvantaged areas. Telemedicine now includes virtual consultations, remote diagnosis and monitoring, and digital health platforms. We must acknowledge telemedicine's past achievements as we look ahead to future advances. Understanding telemedicine's history explains its emergence and provides a foundation for studying how technology is changing healthcare.[2]

Outline of telemedicine

Patients seek treatment remotely utilizing information and communication technologies (ICTs) in the vast field of telemedicine. According to the World Health Organization, telemedicine is "the provision of health care services, where distance is a significant factor, by all health care professionals utilizing information and communication technologies for the exchange of valid information pertaining to the diagnosis, treatment, and prevention of diseases and injuries, research and evaluation, and the ongoing education of health care providers" (WHO, 2010).

Telemedicine now offers several medical treatments. Now including telemonitoring, telediagnostics, and teletherapy, it began with remote consultations. To deliver fast and accurate evaluations, telediagnostics sends diagnostic data and medical images for remote interpretation. Telemonitoring remotely monitors patients' vital signs and health indicators for preventive interventions and tailored therapy.[3] Teletherapy provides mental healthcare via virtual platforms to fulfil the growing need for accessible care. Telemedicine began with health consultations over the phone in the early 20th century. But psychiatry and radiography made significant progress in the 1960s and 1970s. Since astronauts needed remote healthcare, the space race and NASA's efforts helped establish telemedicine. Video conferencing allowed patients and doctors to connect in real time throughout the 1980s and 1990s. More in-depth virtual visits replaced simple phone consultations. Digital health records, wearable technologies, and mobile health applications

were merged in the 21st century, giving telemedicine its promise and setting the foundation for contemporary advances. Current telemedicine is shaped by technological breakthroughs and a growing acknowledgment of its power to break down healthcare obstacles. As this literature research continues, we will analyze telemedicine's essentials, difficulties, and acceptability. [4,5]

Figure 1: Features of Telemedicine for health services

Emerging trends

The impact of emerging technology on healthcare extends beyond telemedicine. This investigation looks at the transformative potential of AI, machine learning, virtual reality, and other cutting-edge developments as medical technology develops. The paradigm shift being architected by these emerging technologies will revolutionize healthcare diagnosis, treatment and delivery.

Telemedicine's usage of AI transforms diagnosis accuracy, treatment techniques, and healthcare efficacy. Machine learning algorithms analyze large amounts of data to detect early sickness and recommend treatment. Picture analysis, anomaly diagnosis, and diagnostic process optimization are improved by AI in radiology. Natural language processing (NLP) helps clinicians understand patient data by extracting important insights

from unstructured clinical notes. AI in telemedicine goes beyond diagnosis. Artificial intelligence-powered virtual health assistants provide personalized health information, prescription reminders, and lifestyle advice, enhancing patient engagement and treatment compliance. Integrating AI with telemedicine will alter healthcare delivery by providing quick, data-driven insights that improve patient outcomes. AI in telemedicine might improve diagnosis and therapy.[6]

Research has revealed that AI systems can detect early signs of cancer and cardiovascular disease. AI technologies can quickly evaluate complex medical data to help doctors make better diagnosis and improve patient care. AI-driven telemedicine decision support systems speed diagnosis and personalize therapy. AI algorithms can personalize solutions based on patient-specific genetic, lifestyle, and treatment data. This precision medicine-based approach marks a new era in healthcare where medicines are personalized to each patient. Wearable sensors and gadgets have boosted remote patient monitoring (RPM). Monitoring vital signs, physical activity, and other health data with these technologies lets consumers participate in their healthcare. Real-time data from smartwatches and fitness trackers may be easily shared to doctors for preventative interventions and customized therapy.[7] The addition of biosensors to wearable devices greatly enhances remote patient monitoring. In real time, biosensors can identify biomarkers that reveal diabetic, cardiovascular, and respiratory disorders. This continual observation helps identify health issues early and maintain therapy and prevention. RPM greatly aids chronic disease monitoring. Remote monitoring allows healthcare practitioners to assess the health of people with chronic illnesses including hypertension, diabetes, and COPD without regular visits. The strategy improves patient quality of life and reduces healthcare system pressure. The use of RPM in chronic illness treatment aligns with patient-centered care and preventative healthcare. Wearable technology and continuous monitoring allow healthcare providers to detect tiny health changes, intervene quickly, and empower patients to manage their chronic conditions.[8]

Telemedicine and IoT have created a time when networked devices seamlessly improve healthcare results. Intelligent gadgets, sensors, and data analytics provide real-time monitoring and informed decision-making in healthcare through the Internet of Things. Healthcare practitioners may access patient vitals and environmental data using IoT-enabled telemedicine, enabling a complete and proactive approach. Telemedicine with IoT requires connected medical equipment. Smart blood pressure, glucose, and ECG monitors send accurate, real-time data to healthcare systems. Remote consultations are more effective when healthcare providers can make informed decisions based on current

and complete data. Telemedicine relies more on IoT, making data security and privacy crucial. IoT devices' interconnection increases cyberattack surface, necessitating strict security measures. IoT-driven telemedicine requires encryption, secure authentication, and software updates to protect patient data. Patient privacy is crucial in IoT-enabled telemedicine. Data governance, informed permission, and GDPR and HIPAA compliance are crucial. Telemedicine IoT must balance the benefits of networked healthcare equipment and patient privacy to continue to grow. Telemedicine using VR and AR for medical training and teaching. Virtual reality gives healthcare workers realistic experiences in procedures, surgeries, and emergencies. This immersive training promotes decision-making, skill development, and patient safety.[9]

Augmented Reality (AR) blends the real and virtual worlds. Augmented reality helps telemedicine practitioners by providing real-time guidance, stressing essential anatomical components, and displaying patient information in real time. Augmented reality in telemedicine enhances accuracy and opens new remote collaboration and consulting options. Telemedicine VR and AR boost patient education and interaction beyond professional uses. Virtual reality lets patients see their anatomy, treatment options, and future results in 3D. This participative approach helps people understand medical diagnosis, procedures, and treatment options, enabling informed healthcare decisions. Teleconsultation augmented reality apps improve patient involvement by providing real-time information. Augmented reality may demonstrate medical diagnoses, treatment plans, and pharmaceutical orders, fostering a collaborative and transparent healthcare collaboration. Adding VR and AR to telemedicine is moving healthcare toward a patient-centered approach that stresses education, engagement, and collaborative decision-making. AI, remote patient monitoring, and VR/AR in telemedicine indicate a healthcare revolution. These developments boost telemedicine and help restructure healthcare systems. As telemedicine's future unfolds, we must consider how these innovations will change healthcare delivery.[10]

Healthcare delivery transformations

Telemedicine has altered healthcare by expanding access, especially across borders. Remote consultations and monitoring can bridge urban and rural healthcare access. Virtual consultations might save travel and costs for rural and underprivileged populations who struggle to get treatment. This move improves healthcare access, early intervention, and preventative therapy, lowering health disparities. Telehealth vans with advanced medical equipment illustrate how telemedicine transcends borders. Rural areas receive consultations, diagnostics, and teletherapy via these mobile devices. Telemedicine has

opened up healthcare delivery elsewhere physical institutions, promoting equality and inclusion. Telemedicine helps financially disadvantaged, transportation-challenged, and healthcare desert residents obtain care. At-risk populations like the elderly and chronically ill can be monitored and consulted without frequent hospital visits. Patient-centered care emphasizes individualized healthcare solutions that address individual needs and situations.

Telemedicine reduces demographic gaps in healthcare. Telemedicine reduces healthcare inequities and improves disadvantaged group health. Culturally competent telehealth services ensure that healthcare is accessible and tailored to different demographics. Telemedicine revolutionizes healthcare by prioritizing patients. Virtual consultations allow patients to interact with doctors. A collaborative healthcare approach encourages patients to express complaints, ask questions, and make decisions. This patient-centered approach maximises happiness and health results by considering individual values and preferences. Telemedicine also improves patient education and self-management.[11]

Virtual platforms allow doctors to educate, monitor, and advise on lifestyle modifications. This strategy helps patients understand their health and promotes proactive self-care, improving treatment compliance. Telemedicine improves patient-doctor communication, demonstrating the move to patient-centered care. Virtual consultations make healthcare practitioners more accessible, reducing communication barriers. Patients may voice concerns, ask questions, and get rapid feedback, fostering a supportive healthcare relationship. Patients and physicians can communicate better via encrypted texting, video consultations, and telemedicine. Immediate communications reduce delays in responding to patient requests and concerns. Telemedicine promotes communication, making patients feel valued and included in their treatment decisions. Telemedicine in healthcare has major regulatory and policy implications. The legal system is responding to telemedicine's unique challenges and opportunities. Telehealth regulations are changing to ensure safety, privacy, and efficacy while encouraging innovation. Telemedicine law is shaped by licensing, provider reimbursement, and liability. Telehealth regulations vary per nation, but efforts are underway to standardize them to ensure quality and universality. This legislative change shows how innovation must balance patient safety and ethics. Telemedicine faces license and financing issues.[12]

Telemedicine raises questions about healthcare practitioners' jurisdictional constraints, as state licensing regulations oversee conventional healthcare. National licensing agreements and reciprocity aim to address these challenges and ensure telehealth

service continuity. Telemedicine's widespread adoption depends on reimbursement. Government healthcare programs and private insurers are reevaluating remuneration systems to match telehealth's value and efficiency. Policy interventions are needed to provide equitable reimbursement systems that preserve telemedicine while ensuring patient affordability and access. Telemedicine is changing healthcare access and service, requiring a rethink of legal, regulatory, and policy frameworks. Telemedicine is evolving; therefore, we must consider the regulatory and legislative effects for a sustainable and inclusive healthcare future.[13]

Challenges and Consideration

Increasing telemedicine use raises patient privacy concerns. Patients' sensitive health data is at risk when transmitted and stored online. Cryptography and secure communication are needed in telemedicine systems to prevent data breaches. Policymakers, IT developers, and healthcare professionals must continue to seek a balance between patient privacy and healthcare provider data accessibility. Telemedicine with wearables and remote monitoring raises privacy concerns. These devices collect data constantly, requiring honest communication about data ownership, usage, and the consequences of sharing it with medical professionals. The ethical aspects of telemedicine privacy must start with informed consent and strong privacy frameworks. Without fair access, telemedicine may worsen healthcare inequalities. Telehealth benefits depend on digital literacy, technology, and reliable internet connectivity. Due to a lack of infrastructure and digital resources, vulnerable populations—including low-income ones—may struggle to adopt telemedicine.[14]

A multifaceted strategy that promotes digital literacy, affordable internet access, and technical support is needed to overcome these obstacles. Beyond technology, ethical issues in telemedicine consider social determinants of health, ensuring that everyone can benefit from telehealth. Since telemedicine relies on reliable internet access, speed, capacity, and network reliability may limit its use. Rural and isolated areas may have limited high-speed internet, causing telehealth access discrepancies.[15] Telecoms, legislators, and healthcare organizations work together to improve infrastructure and internet access in underserved areas. Telemedicine's technological issues include the digital divide. Some communities may struggle due to digital literacy and computer, cell phone, or tablet access. Infrastructural improvements and training programs to use virtual healthcare platforms are needed to close this digital gap. There are technological obstacles to interoperability and smooth data transmission when integrating telemedicine into current healthcare systems. Numerous healthcare facilities use a variety of electronic

health record (EHR) systems, and the smooth exchange of patient data depends on interoperability with telehealth platforms. For telemedicine apps and EHR systems to communicate data, standardized protocols like Fast Healthcare Interoperability Resources (FHIR) are essential. And when it comes to solving technical problems, cybersecurity is crucial. To safeguard patient data and preserve the integrity of healthcare systems, strong cybersecurity measures are crucial since the growing use of digital platforms for telehealth services creates new attack vectors. Developing and deploying safe telehealth infrastructures requires cooperation between cybersecurity specialists, healthcare IT professionals, and legislators. Even while telemedicine provides many medical services, there are certain inherent drawbacks to doing some diagnostic tests from a distance.[16] In a virtual environment, physical exams, palpations, and some imaging investigations could be difficult to duplicate. By utilizing the technology at their disposal, working with on-site medical specialists when required, and setting explicit protocols for situations where in-person evaluations are judged appropriate, healthcare practitioners may overcome these constraints. It can be difficult to identify minor clinical signs in telemedicine that are frequently visible during in-person encounters. To make wise judgments, doctors must depend on patient history, efficient communication, and the facts at their disposal. The goal of ongoing research and telehealth technology advances, such as high-fidelity imaging equipment for home usage, is to overcome these diagnostic constraints. The cornerstone of providing healthcare effectively is establishing and preserving trust between patients and healthcare professionals.[17]

Building trust in virtual connections presents special difficulties in telemedicine because of the lack of in-person interaction and the dependence on digital communication. In order to build a trustworthy therapeutic relationship, healthcare practitioners must proactively address patients' concerns over the apparent impersonality of virtual consultations. Additionally, issues with misunderstanding or miscommunication might occur in virtual relationships.

To overcome these obstacles, it becomes imperative to provide compassionate and transparent communication. In order to establish and preserve believe in telemedicine partnerships, healthcare practitioners should get training in virtual communication techniques in addition to using telehealth systems that facilitate safe, high-quality voice and video contact. The ethical, technological, and quality-of-care issues surrounding telemedicine highlight the necessity of integrating technology into healthcare systems in a thorough and nuanced manner. It becomes crucial to create ethical frameworks, resolve

technological issues, and guarantee the provision of high-quality treatment in virtual healthcare encounters as we negotiate these factors.[18]

Future Directions and Innovations

Telemedicine and AI will change healthcare via predictive analytics. Telehealth exchanges, remote patient monitoring, and electronic health data may alert AI systems to health issues. Predictive analytics can target patients for prevention. AI-driven prediction models can enhance community health management by recognizing trends and risk. AI-powered telemedicine can provide personalized health advice, preventative testing, and lifestyle changes. Telemedicine may be proactive and preventative. AI and telemedicine enable precision medicine beyond predictive analytics. AI systems can tailor treatments using genetic data, biomarkers, and health data. Telemedicine allows doctors discuss AI-driven therapy. Telemedicine with precision medicine reduces unwanted effects and unnecessary treatments. Virtual consultations that focus individualized therapy may help AI-driven precision medicine patients worldwide. The future is patient-centered, data-driven telemedicine and precision care. Increasing VR and AR in telemedicine enable remote surgery.[19] Realistic VR environments improve remote surgeons' spatial awareness and accuracy. AR overlays vital information on the surgeon's vision for constant guidance and visibility during remote surgery. VR and AR provide minimally invasive treatments, robotic surgery, and precise interventions. Telemedicine allows expert surgeons and local teams to treat patients overseas. VR and AR enhance telemedicine education beyond patient care. By mimicking medical scenarios, VR simulations help medical students and practitioners enhance their skills and decision-making. Interactive overlays and holograms help medical students. VR/AR telemedicine may democratize high-quality medical education. Virtual dissections, surgical simulations, and clinical case discussions can help remote medical students develop a worldwide healthcare community. Telemedicine affects medical education; thus, VR and AR must offer innovative, inclusive learning. AI-driven mental health telemedicine examinations will enhance diagnosis and therapy. AI systems can assess patients' emotions during virtual mental health consultations by analysing speech, facial expressions, and other behaviors. These tests improve mood disorder, depression, and anxiety diagnosis and treatment. Telemental health AI uses virtual mental health assistance observation. AI can provide between-session assistance, therapy, and mental health tracking. As stigma decreases, AI in telemedicine is crucial for global mental health. Telemedicine expands mental health treatment beyond diagnosis. Secure video conferencing and remote psychotherapy are available with teletherapy. Teletherapy is easy to use, making it appealing to people with social anxiety, transportation issues, or

geographical isolation. Telemedicine for mental health will offer virtual support groups and community projects. Customized online mental health communities may help by connecting people with similar experiences using AI. Comprehensive, patient-centered telemedicine promotes mental health. Telemedicine will prosper with AI, VR, and mental health. These innovations will personalize, access, and cover healthcare. Consider ethical, technical, and regulatory issues while evaluating these techniques.[20]

Conclusion:

Telemedicine is revolutionizing healthcare. Telemedicine's influence on healthcare delivery, trends, obstacles, future directions, and regulatory and policy issues are examined in this comprehensive overview. Telemedicine improves conventional healthcare's accessibility, efficiency, and patient-centeredness. The COVID-19 pandemic increased telemedicine adoption and demonstrated its crisis use. Telemedicine offers convenience, preventative care, chronic illness management, and mental health assistance as it improves. Telemedicine is difficult to implement. Healthcare providers, technology developers, and governments must confront ethical, technological constraints, and access inequities. Patient autonomy and confidentiality are essential in virtual healthcare ethics. Connectivity and telehealth platform integration with healthcare systems require resilient infrastructure and interoperability. Telemedicine quality depends on diagnostic constraints, virtual contact assurance, and healthcare inequities reduction. Future telemedicine advances include AI for predictive analytics and precision medicine. Augmented and virtual reality will transform remote surgery and medical education by making learning immersive and inclusive. Telemedicine's mental health and well-being focus shows holistic treatment. Telemedicine and AI improve mental health evaluations, while teletherapy and virtual support groups provide individualized treatment. Telemedicine regulation and policy are crucial throughout these changes. Telemedicine must be promoted responsibly and fairly by creating standards, resolving reimbursement laws, and upholding ethics. Global telehealth regulations are centered on international collaboration, value-based remuneration, and patient confidentiality. Telemedicine has improved medical access and service. Healthcare stakeholders must collaborate to succeed. The ethical, technological, and regulatory-driven path to an accessible, patient-centered, digitally empowered healthcare future becomes clearer.

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ELASTIC ENERGY AND REHABILITATION: THE ROLE OF SPRINGS IN THERAPEUTIC EXERCISE

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1. Definition and Properties of springs

Springs are mechanical devices capable of storing and releasing energy through deformation. When a spring is stretched or compressed, it stores elastic potential energy, and upon release, it reverts to its original shape by exerting a force in the opposite direction. This behavior is governed by Hooke's Law ($F = -kx$), where F is the force exerted by the spring, k is the spring constant, and x is the displacement from its equilibrium position. In rehabilitation science, these properties allow springs to provide controlled, progressive resistance and assist in functional movement retraining. They also mimic the elastic recoil properties of muscles and tendons, enabling biomechanical efficiency during therapeutic interventions (1).

2. Types of Springs Used in Rehabilitation

Rehabilitative tools incorporate various spring types to suit specific anatomical and functional needs:

- **Compression Springs:** Provide axial resistance and are often found in orthotic devices and joint braces, such as those used for knee realignment or plantar support.
- **Tension Springs:** Stretch under force and are widely used in tools for shoulder abduction and elbow flexion/extension therapy.
- **Torsion Springs:** Provide resistance to rotational motion, ideal for grip strengtheners and forearm pronation/supination training devices. These varied applications allow therapists to customize resistance patterns based on the rehabilitation goals and individual patient profiles (2).

3. Biomechanical Principles of Spring Utilization

The mechanical responsiveness of springs permits the application of variable resistance that adapts to the user's force output. This is beneficial in both concentric and eccentric muscle actions, promoting controlled movement. Additionally, spring-based

devices enable graduated load progression, a principle crucial for neuromuscular re-education and strength recovery. Rehabilitation devices often use this principle to simulate real-world functional tasks while ensuring muscle engagement and joint control (3).

4. Advantages of Spring-based Physiotherapy

Spring-integrated rehabilitation tools are increasingly favored due to their versatile mechanical properties and clinical applicability. They offer unique advantages that make them suitable across a broad spectrum of therapeutic settings, from acute inpatient care to long-term home-based rehabilitation.

1. Lightweight and Portable

Spring-based devices are typically made of compact materials such as stainless steel, plastic, or carbon composites, contributing to their light weight. Unlike bulky motorized machines or cable-based resistance systems, spring-loaded devices do not require electrical power or large structural frameworks. This portability:

- Enables **easy setup at home**, promoting patient adherence and continuation of therapy outside the clinic.
- Allows therapists to carry tools between sessions or clinics, supporting **community-based or domiciliary physiotherapy**.
- Facilitates **on-the-go exercises** for patients, particularly in orthopedic and neurological rehabilitation scenarios.

👉 *Example:* A spring-loaded hand exerciser can be used by a post-stroke patient during daily routines to maintain motor engagement.

2. Cost-effective

Most spring-based rehabilitation tools are **low-cost** in both production and maintenance. They:

- Require **no electrical components** or complex software, which significantly lowers manufacturing expenses.
- Have **minimal maintenance needs**, unlike robotic or motor-assisted therapy devices that need regular servicing.
- Are **more accessible** for low-resource settings or patients who cannot afford high-end rehabilitation devices.

👉 *Implication:* Institutions and clinics in developing regions can **scale up rehabilitation services** without major investments, thereby improving healthcare equity.

3. Customizable Resistance

Springs offer **adjustable and progressive resistance**, which is vital in personalized rehabilitation:

- By changing the **spring constant (k)** or the **range of motion**, the device can cater to various levels of patient strength.
- Resistance can be **progressively increased**, supporting gradual muscle loading as patients recover.
- Springs provide **smooth, continuous force application**, which avoids the abrupt jerks that may occur with weight stacks or pulleys.

👉 *Clinical Relevance:* This property is especially important in **post-operative therapy**, where tissues must not be overloaded too quickly, but still need stimulation to promote healing.

4. Improved Proprioception and Neuromuscular Control

One of the lesser-discussed but powerful benefits of springs is their **ability to enhance proprioception**:

- Springs provide **constant mechanical feedback** throughout movement, helping patients sense joint position and movement more effectively.
- This feedback is crucial for **retraining balance, coordination, and joint stability**, especially in patients with neurological impairments like stroke, multiple sclerosis, or spinal cord injuries.
- It supports **sensorimotor learning**, reinforcing the connection between the central nervous system and musculoskeletal responses.

👉 *Example:* In sitting balance training, using spring-supported unstable platforms improves trunk control and reaction strategies, as shown in studies comparing paraplegic and able-bodied subjects (4).

5. Clinical Applications

Springs are integrated into a wide range of physiotherapy tools due to their ability to store and release energy in a controlled manner. This property is highly advantageous in clinical rehabilitation, where precision, safety, and adaptability are essential.

i. Post-Surgical Rehabilitation

Goal: Restore mobility, minimize stiffness, and initiate early active-assisted movement without overloading healing tissues.

Spring Applications:

- **ACL Reconstruction:** Knee rehabilitation braces with integrated spring elements allow for controlled flexion and extension. These devices:
    - Provide **resistance in one direction** (e.g., flexion) and assist movement in the opposite (e.g., extension), depending on patient needs.
    - Help prevent joint stiffness and promote **gradual tissue loading**.
- **Rotator Cuff Repair:** Shoulder rehabilitation tools use **tension or torsion springs** to support gradual reactivation of the shoulder joint. Springs:
    - Allow **pain-free, low-resistance movement**, especially useful during the early phase when active contraction is limited.
    - Enhance **neuromuscular coordination** as patients regain voluntary control.

Clinical Benefit:

Spring mechanisms promote **active-assisted range of motion (AAROM)** without straining healing structures, thus reducing the risk of re-injury or adhesion formation.

ii. Neurological Rehabilitation

Goal: Re-establish motor patterns, improve strength and coordination, and stimulate neuroplasticity.

Spring Applications:

- **Stroke Rehabilitation:**
    - Spring-assisted **gait trainers** support limb movement while encouraging active participation, mimicking natural walking kinematics.
    - Upper limb devices with spring resistance help patients **practice repetitive movements**, a key element in motor relearning and cortical reorganization.
- **Cerebral Palsy or Spinal Cord Injury:** Orthoses embedded with springs facilitate assisted walking or arm training, aiding in **functional task performance** even with partial muscle activity.

Mechanism:

Springs offer **graded resistance and elastic recoil**, encouraging both concentric and eccentric muscle contractions—crucial for neuromotor recovery.

Clinical Evidence:

Robotic devices utilizing spring elements have been shown to **improve motor recovery and walking patterns** in stroke survivors by facilitating symmetrical and rhythmic movement (5).

iii. Orthopedic Applications

Goal: Correct deformities, provide structural support, and realign musculoskeletal structures.

Spring Applications:

- **Spinal Bracing:** Dynamic spinal orthoses with **tension springs** apply low-level, continuous corrective forces for conditions such as:
    - **Scoliosis**: Encouraging postural symmetry and halting curve progression.
    - **Kyphosis or Hyperlordosis**: Supporting trunk stability while allowing mobility.
- **Limb Alignment:** Braces using **torsional or compression springs** assist in:
    - Correcting valgus/varus knee deformities.
    - Managing conditions like **clubfoot or genu recurvatum** by gradually adjusting joint orientation.

Benefit:

Unlike rigid braces, spring-based systems allow **controlled mobility**, preventing muscular disuse and promoting **functional joint alignment** over time.

6. Role in Passive and Active Therapy

Spring-based systems serve dual purposes in therapy:

- **Passive Therapy:** Continuous Passive Motion (CPM) devices use springs to move joints gently without voluntary muscle contraction, beneficial in acute post-operative care.
- **Active Therapy:** Resistance tools challenge muscle groups during exercises, promoting strength gains and endurance in a safe, controlled manner (6).

7. Evidence-Based Outcomes

Research supports the efficacy of spring-based interventions in improving:

- **Range of Motion (ROM):** Gradual joint mobilization enhances flexibility.
- **Muscle Strength:** Resistance provided by springs helps restore strength.
- **Coordination and Control:** Progressive loading improves neuromuscular integration, particularly in neuro-rehabilitation contexts. Randomized trials show significant improvements in functional outcomes with the inclusion of spring-exoskeletons in therapy (7).

8. Integration with Technology

Modern rehabilitation tools combine spring mechanisms with smart technologies such as:

- **Sensors and Biofeedback:** Allow real-time tracking of movement, resistance, and joint angles.
- **Robotics:** Hybrid devices merge spring mechanics with motors for enhanced precision.

  These integrations facilitate adaptive therapies tailored to patient progress and promote motivation through feedback-driven tasks (8).

9. Design Considerations in Spring-Based Devices

Key factors in device design include:

- **Spring Stiffness (k):** Should be matched to the patient's strength and therapy goals.
- **Joint Alignment:** Springs must align with the natural axis of the joint to avoid abnormal stress.
- **Safety Features:** Limits on range and force prevent overloading.
- **Material Selection:** Stainless steel, carbon fiber, and polymer composites are used for durability and flexibility, depending on the application (9).

10. Future Directions

The evolution of spring-based rehabilitation tools is leaning toward:

- **Smart Adaptive Springs:** Systems that auto-adjust stiffness based on movement or feedback.
- **AI Integration:** Predictive algorithms for therapy customization.
- **Wider Use in Home Settings:** Wearable spring-based exposits and portable devices will allow continuity of care beyond clinical environments (10).

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THE AYURVEDIC TO ALLOPATHIC JOURNEY: A TALE OF TRADITION AND TRANSITION

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Abstract:

The transition from Ayurveda, an ancient system of healing, to Allopathic medicine, grounded in scientific principles, represents a profound shift in the understanding and approach to health. Ayurveda, with its holistic view of health, treats the body, mind, and spirit through personalized regimens including herbal remedies, diet, and lifestyle practices. Allopathy, evolving from scientific exploration, focuses on treating diseases through pharmaceuticals, surgery, and evidence-based methods. The colonial disruption marginalized Ayurveda, but post-independence efforts revived its importance, highlighting the value of integrative medicine. The strengths of herbal treatments, such as fewer side effects, affordability, and preventive care, make them a reliable alternative, especially for chronic conditions. Despite challenges like lack of clinical evidence and regulatory hurdles, herbal preparations continue to gain ground through pharmaceutical advancements. The complementary nature of Ayurveda and Allopathy offers a holistic model of healthcare, combining the preventive and lifestyle-oriented principles of Ayurveda with the precision and fast-acting interventions of Allopathic medicine. This synergy promises a future of patient-centered, sustainable care, incorporating the wisdom of traditional healing and modern innovation.

Keywords: Ayurveda, Allopathy, Herbal Medicine, Integrative Medicine, Pharmaceutical Preparations

Introduction:

Health and healing are intrinsic to human survival, and civilizations across the world have developed diverse systems of medicine over millennia. Among the oldest of these is **Ayurveda**, a holistic Indian system of healing that dates back over 5,000 years. Rooted in the Sanskrit words *"Ayur"* (life) and *"Veda"* (knowledge), Ayurveda emphasizes balance among the body, mind, and spirit through natural means. On the other end of the spectrum lies **Allopathy**, or modern Western medicine, which evolved over the last few centuries with a strong basis in science, technology, and evidence-based practices. The

journey from Ayurvedic to Allopathic medicine is not merely a shift in treatment methods, but a deeper evolution in humanity's approach to health, disease, and care delivery. Herbal medicine, also known as phytotherapy, involves the use of plant-derived substances for therapeutic purposes. Advocates argue that herbal treatments are more reliable than allopathic medicine due to their holistic approach, fewer side effects, and cultural compatibility.

The Foundations Origins and Philosophies of Herbal Medicine:

Herbal medicine has been practiced for thousands of years. Ancient texts such as the Indian *Charaka Samhita* and the Chinese *Shennong Bencao Jing* laid the foundation for plant-based therapies that are still in use today [1]. The reliability of herbal medicine lies in its historical continuity and empirical validation through generations. Medicinal plants like *Withania somnifera* (Ashwagandha), *Curcuma longa* (Turmeric), and *Zingiber officinale* (Ginger) have shown proven therapeutic effects in treating inflammation, metabolic disorders, and microbial infections[2,3]. Ayurveda is more than a system of medicine; it is a way of life. It classifies individuals based on **doshas**—Vata, Pitta, and Kapha—and prescribes personalized regimens involving herbal remedies, diet, yoga, and spiritual practices. Disease in Ayurveda is viewed as an imbalance of energies and is treated by restoring harmony within the body and its environment [4].

In contrast, Allopathic medicine originated from Greek and Roman traditions but truly took form during the 18th and 19th centuries with the advent of the scientific method. It is characterized by a focus on pathogens, anatomy, biochemistry, and pharmacology, relying heavily on pharmaceuticals and surgical interventions [5]. While Ayurveda focuses on the cause and the internal ecosystem, Allopathy focuses on the symptom and the immediate pathology.

Colonial Disruption and Decline of Ayurveda:

The British colonial rule in India was a turning point in the history of Ayurveda. Western medicine was institutionalized and given precedence, while indigenous systems like Ayurveda were marginalized. Medical colleges and hospitals established by the British promoted Allopathic practices, and Ayurvedic texts and practitioners were dismissed as unscientific or obsolete [6]. By the 20th century, Ayurveda had been relegated to rural areas and traditional families, surviving primarily through oral transmission and local use.
Despite this, Ayurveda remained resilient. In various regions of India, Ayurvedic treatments continued to be trusted and practiced, especially for chronic ailments and

lifestyle diseases [1]. However, its reach and formal recognition were severely limited due to lack of integration, research, and state support during colonial times.

Resurgence and Coexistence:

Post-independence, India saw a revival of interest in Ayurveda. The government established bodies like the Ministry of AYUSH (Ayurveda, Yoga, Unani, Siddha, and Homeopathy) to promote indigenous medicine systems [7]. Ayurvedic colleges, research institutions, and pharmacies emerged, and Ayurveda began to regain its credibility. Simultaneously, Allopathy advanced rapidly with breakthroughs in antibiotics, vaccines, surgical techniques, and diagnostic tools. The 20th and 21st centuries marked the dominance of modern medicine globally, especially in emergency and acute care [8]. Yet, despite this progress, limitations such as side effects, antibiotic resistance, and chronic disease management issues led to renewed interest in natural and holistic systems like Ayurveda [9]. In contemporary times, there is increasing appreciation for integrative medicine, which combines the strengths of both Ayurveda and Allopathy. For instance, Ayurvedic therapies like Panchakarma are used to detoxify the body before chemotherapy, and herbs like Ashwagandha are studied for their adaptogenic (stress-relieving) properties [10]. Many patients today prefer using Ayurvedic remedies for lifestyle disorders such as diabetes, arthritis, or stress, alongside Allopathic drugs under medical supervision.

Fewer Side Effects and Better Tolerability:

One of the major criticisms of allopathic medicine is the prevalence of side effects and adverse drug reactions (ADRs). According to Lazarou *et al.,* ADRs are a leading cause of morbidity and mortality in hospitals in the United States [11]. In contrast, herbal medicines tend to have fewer and milder side effects, especially when used correctly under traditional guidelines. For example, green tea (*Camellia sinensis*) has been widely used for its antioxidant properties with minimal reported toxicity [12]. Moreover, herbal remedies typically work by supporting the body's natural healing processes rather than targeting symptoms aggressively, which often results in better tolerability.

Holistic and Preventive Approach:

Herbal treatments emphasize a holistic view of health, integrating mind, body, and spirit. In systems like Ayurveda and Traditional Chinese Medicine (TCM), disease is viewed as an imbalance in bodily energies or functions. Remedies are not just aimed at eradicating disease but restoring balance and preventing future illness. Allopathy, on the other hand, is largely disease-centric and symptom-focused, often overlooking underlying systemic

imbalances [13]. This holistic approach allows herbal medicine to offer long-term benefits rather than short-term relief.

Affordability and Accessibility:

Herbal medicine is also more accessible and affordable than conventional allopathic drugs, especially in low- and middle-income countries. According to the World Health Organization (WHO), up to 80% of the population in some African and Asian countries relies on traditional medicine for primary healthcare due to its low cost and local availability [14]. For instance, neem (*Azadirachta indica*) is used widely across India as a natural antibacterial agent and is far cheaper than pharmaceutical antiseptics.

The primary types of Ayurvedic treatments and their applications in managing a range of acute and chronic conditions.

1. Panchakarma Therapy

Panchakarma is a five-step detoxification procedure meant to eliminate toxins (*ama*) from the body. It includes:

- **Vamana** (therapeutic emesis) is used for Kapha disorders such as asthma, chronic colds, and obesity [15].
- **Virechana** (purgation) helps in Pitta-related conditions like jaundice, skin diseases, and migraines [16].
- **Basti** (medicated enema) is effective for Vata disorders like arthritis, constipation, and neurological issues [17].
- **Nasya** (nasal therapy) is employed in treating sinusitis, headaches, and cognitive dysfunction [18].
- **Raktamokshana** (bloodletting) is used in skin disorders such as eczema and psoriasis [1].

2. Herbal Medicines

Ayurveda relies heavily on plant-based drugs. Formulations may be single herbs or polyherbal mixtures prepared in different forms—*churna* (powder), *kwath* (decoction), *asava/arista* (fermented liquids), and *guggulu* (resins).

- **Ashwagandha (Withania somnifera)**: Adaptogenic herb used in stress, anxiety, and fatigue [19].
- **Guduchi (Tinospora cordifolia)**: Immunomodulatory; used in fever, diabetes, and hepatitis [20].
- **Turmeric (Curcuma longa)**: Anti-inflammatory agent used in arthritis, skin conditions, and cancer prevention [2].

- **Triphala**: A three-fruit formulation beneficial in gastrointestinal disorders, detoxification, and eye health [21].
- **Guggulu (Commiphora mukul)**: Effective in managing cholesterol, obesity, and hypothyroidism [22].

3. Dietary Management (*Ahara Chikitsa*)

Ayurveda emphasizes diet as medicine. Foods are selected based on their qualities (*guna*), post-digestive effect (*vipaka*), and impact on *doshas*.

- **Vata disorders**: Warm, oily, and moist foods are prescribed. Example: Rice gruel with ghee for constipation and anxiety [23].
- **Pitta disorders**: Cooling foods like milk and sweet fruits are recommended. Beneficial in gastritis and liver diseases [24].
- **Kapha disorders**: Light and spicy foods like barley and black pepper are useful in asthma, obesity, and sinusitis [25].

4. Lifestyle and Behavioral Regimens (*Dinacharya* and *Ritucharya*)

Ayurveda prescribes daily (Dinacharya) and seasonal (Ritucharya) routines to maintain equilibrium and prevent disease.

- **Early rising, tongue scraping, oil pulling, and yoga** help maintain oral, gastrointestinal, and musculoskeletal health [26].
- **Ritucharya** prevents seasonal disorders like colds in winter and diarrhea in monsoon [27].
- Behavioral practices (*Sadvritta*) such as ethical conduct and meditation aid in managing stress-related disorders like hypertension and insomnia [28].

5. Rasayana Therapy (Rejuvenation)

Rasayana involves the use of tonics and lifestyle practices aimed at rejuvenation, longevity, and improving immunity.

- **Chyawanprash**: Used to enhance respiratory health and immunity, especially in children and the elderly [29].
- **Brahma Rasayana**: Prescribed in memory loss and neurodegenerative conditions [30].
- **Amalaki (Emblica officinalis)**: Potent antioxidant used in diabetes and premature aging [31].

6. Surgical and Para-surgical Techniques (*Shalya Tantra*)

Ancient Ayurvedic texts describe minor surgical procedures. *Ksharasutra* and *Agnikarma* are two notable methods still in use.

- **Ksharasutra therapy**: Effective for anal fistula, with fewer recurrences compared to conventional surgery [32].
- **Agnikarma**: Thermal cauterization used in chronic pain conditions like sciatica and plantar fasciitis [33].

7. Leech Therapy (*Jalaukavacharana*)

A method of bloodletting using medicinal leeches, classified under *Raktamokshana*.

- Used in **varicose veins**, **non-healing wounds**, and **skin diseases** [34].
- Leech saliva contains anticoagulants and anti-inflammatory agents [35].

8. External Therapies (Abhyanga, Swedana, Shirodhara, etc.)

These therapies use oils, herbal powders, and steam for external application.

- **Abhyanga (oil massage)**: Improves circulation, reduces stress, and is useful in paralysis and muscular dystrophy [36].
- **Swedana (fomentation)**: Used to reduce stiffness in arthritis and back pain [37].
- **Shirodhara**: Pouring medicated oil over the forehead; highly effective in insomnia, anxiety, and migraines [38].

Integration Rather Than Replacement:

Rather than viewing herbal and allopathic medicine as mutually exclusive, an integrative approach could provide the most reliable and comprehensive healthcare. Integrative medicine leverages the strengths of both systems—combining the scientific rigor and precision of allopathy with the holistic and preventive philosophy of herbal treatments. For instance, herbal supplements can be used to manage chronic conditions like diabetes and arthritis, while allopathy handles acute symptoms and emergencies. Pharmaceutical preparations involving herbal extracts represent a growing field within the health and wellness industry. As global interest in natural therapies surges, the integration of herbal compounds into standardized pharmaceutical formulations bridges the gap between traditional healing and modern medicine. Herbal extracts are bioactive substances obtained from plant material, standardized to contain specific therapeutic constituents. These preparations, when developed according to pharmaceutical guidelines, enhance efficacy, safety, and dosage control, thus offering a more reliable alternative to crude herbal use. Herbal extracts provide a concentrated form of these compounds, which can be standardized and formulated into tablets, capsules, ointments, or syrups [39].

Pharmaceutical preparations of herbal extracts offer several advantages:

1. **Consistency and Potency**: Standardized extracts ensure uniformity in concentration and potency, overcoming the limitations of traditional herbal decoctions.

2. **Improved Bioavailability**: Formulations can be optimized to enhance absorption and therapeutic action [40].
3. **Extended Shelf Life**: Proper processing and packaging increase the stability of active ingredients.

Preparation of Herbal Extracts for Pharmaceuticals:

The process of pharmaceutical preparation begins with the selection of suitable plant material based on therapeutic use and phytochemical content. Key steps include:

1. Extraction

The most critical step involves extracting the active constituents using solvents such as ethanol, methanol, water, or a combination thereof. Methods include:

- **Maceration**: Soaking plant material in solvent over time.
- **Percolation**: Continuous solvent flow through a column packed with herb.
- **Supercritical Fluid Extraction**: Uses CO_2 under pressure for high-purity extracts [41].

2. Concentration and Drying

The extract is concentrated under reduced pressure and then dried using techniques such as spray drying or freeze-drying to yield a stable powdered form [42].

3. Standardization

This ensures each batch contains a specific quantity of active constituents. For example, ginkgo biloba extract is standardized to 24% flavone glycosides and 6% terpene lactones [43].

4. Formulation

The dry extract is combined with excipients to produce dosage forms like:

- **Tablets and Capsules**: For oral administration.
- **Topical Creams and Ointments**: For local application.
- **Liquid Syrups**: For pediatric or geriatric use.

Benefits of Herbal Extract-Based Pharmaceuticals

1. **Scientific Validation**: Unlike raw herbal materials, extracts can be analyzed and tested for active compounds and contaminants.
2. **Dose Accuracy**: Precise dosing reduces risks of toxicity or underdosing.
3. **Patient Compliance**: Easy-to-use formats (tablets, capsules) increase patient adherence compared to traditional decoctions or powders.
4. **Global Acceptance**: Herbal pharmaceuticals meet regulatory standards and can be marketed internationally, increasing access to traditional remedies in modern formats [44].

Challenges in the Transition

Despite their benefits, several challenges limit the widespread acceptance and use of herbal extracts in mainstream medicine:

1. Variability in Plant Material

Factors like geography, harvest time, and cultivation conditions can affect phytochemical content. Ensuring batch-to-batch consistency is difficult without rigorous quality control [45].

2. Lack of Clinical Evidence

While in vitro and animal studies abound, clinical trials on humans are limited for many herbal products. This hampers their inclusion in evidence-based medicine [46].

3. Regulatory Hurdles

Herbal preparations often fall into a grey area between supplements and drugs. This lack of regulatory clarity varies by region and complicates approval processes [47].

4. Interactions and Side Effects

Though considered safe, some herbal extracts can interact with pharmaceutical drugs. For instance, St. John's Wort (*Hypericum perforatum*) induces cytochrome P450 enzymes, reducing the efficacy of drugs like warfarin and oral contraceptives [48].

The journey from Ayurveda to Allopathy has not been without controversy and challenges. Without clinical trials, standardization, and regulatory oversight, Ayurvedic medicines struggle to gain mainstream acceptance in modern healthcare systems [49]. On the other hand, overdependence on Allopathy has raised concerns about overmedication, polypharmacy, and impersonalized care. Critics argue that Allopathy often treats the disease, not the patient, and misses the psychosomatic and environmental factors contributing to health [50]. Efforts are now underway to bridge this gap. Institutions like AIIMS and CCRAS (Central Council for Research in Ayurvedic Sciences) are conducting evidence-based studies on Ayurvedic drugs[51]. There are also WHO-supported initiatives to classify traditional medicine under global health frameworks [52].

Globalization of Ayurveda and Allopathy

Interestingly, Ayurveda has found a global audience in recent years. Wellness tourism, yoga, and the organic movement have sparked curiosity about Ayurvedic principles worldwide. Countries like the USA, Germany, and Australia now host Ayurvedic clinics and courses, and international demand for herbal supplements is on the rise. At the same time, India has embraced Allopathic medicine as an essential pillar of its healthcare system. Indian doctors, trained in Allopathy, are among the most sought after globally, and India is a hub for pharmaceutical manufacturing and clinical research. This globalization

underscores a dual acceptance: while Allopathy is essential for acute care, diagnostics, and surgeries, Ayurveda is increasingly recognized for its role in prevention, rejuvenation, and chronic care.

A Complementary Future

Rather than viewing Ayurveda and Allopathy as mutually exclusive, modern healthcare is moving towards complementarity. Ayurveda offers a preventive and lifestyle-oriented framework, while Allopathy provides fast-acting, measurable interventions. Together, they can offer personalized, sustainable, and patient-centered care [53]. Advances in phytochemistry, biotechnology, and nanotechnology are paving the way for next-generation herbal pharmaceuticals.

Techniques such as:

- **Encapsulation using liposomes or nanoparticles:** Encapsulation is a technique used to enhance the delivery and effectiveness of herbal compounds. Liposomes and nanoparticles act as carriers that protect the active constituents, improve their bioavailability, and allow targeted delivery to specific tissues or cells. Nanoparticles are particles in the size range of 1–100 nm made from polymers, metals, or lipids.
- **Genetic engineering of plants for higher yield:** Genetic engineering involves the modification of plant genomes to improve the yield of medicinal compounds, enhance resistance to diseases, and ensure consistent quality of phytochemicals.
- **AI-assisted screening for active phytochemicals write in detail about the methods:** Artificial Intelligence (AI) is revolutionizing drug discovery by enabling the rapid screening and prediction of bioactive compounds from large phytochemical databases.

The integration of traditional knowledge with modern drug discovery offers new hope for safer, effective, and sustainable therapies.

Conclusion:

The evolution from Ayurveda to Allopathy reflects not just a transition in medical systems, but a broader shift in humanity's perception of health, disease, and healing. Ayurveda, with its roots in holistic balance and natural remedies, provides valuable insights into preventive care and personalized treatment through its time-tested principles and plant-based therapies. Allopathy, on the other hand, represents the triumph of modern science with its rapid diagnostics, precision in treatment, and efficacy in acute and emergency care. Both systems offer unique strengths—and notable limitations.

In recent decades, the resurgence of herbal medicine and the global interest in integrative healthcare highlight a growing recognition that these two paradigms need not

be in conflict. Rather, a complementary approach can harness the holistic, low-toxicity benefits of Ayurveda with the evidence-based precision of Allopathy. The incorporation of standardized herbal extracts into pharmaceutical formulations marks a significant step in this direction, improving safety, dosage accuracy, and global accessibility.

Modern innovations—such as nanoencapsulation for targeted delivery, genetic engineering for enhanced phytochemical yields, and AI-assisted compound screening—are revolutionizing herbal medicine, making it more compatible with contemporary drug development processes. While challenges such as regulatory gaps, variable plant quality, and limited clinical trials persist, ongoing research and global collaborations offer hope for overcoming these barriers.

Ultimately, the future of healthcare lies not in choosing between Ayurveda and Allopathy, but in creating a synergistic model that combines ancient wisdom with modern technology. This integrative approach holds the promise of safer, more sustainable, and personalized healthcare solutions for chronic and lifestyle diseases, meeting the diverse needs of a global population while respecting cultural and ecological contexts. Embracing both systems in harmony could lead to a more inclusive, effective, and human-centered approach to medicine.

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ROLE OF AI IN DRUG DISCOVERY

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Introduction:

Artificial Intelligence (AI) has emerged as a transformative force in drug discovery, revolutionizing traditional approaches by enabling rapid and cost-effective identification of novel therapeutics. The conventional drug discovery process is time-consuming, costly, and often yields low success rates. AI offers promising solutions by utilizing machine learning (ML), deep learning (DL), and natural language processing (NLP) to analyze large datasets, predict molecular properties, and identify drug-target interactions (Mak & Pichika, 2019). AI algorithms can process complex biological and chemical data to discover new drug candidates, repurpose existing drugs, and optimize lead compounds. For instance, deep learning models can predict protein-ligand binding affinities with high accuracy, accelerating hit identification and lead optimization (Vamathevan *et al.,* 2019). Additionally, AI tools like AlphaFold have made significant strides in protein structure prediction, a critical aspect of structure-based drug design (Jumper *et al.,* 2021)**.** The integration of AI in drug discovery not only reduces time and cost but also enhances precision and innovation in early-stage development. Despite challenges such as data quality and model interpretability, the continued evolution of AI technologies is set to reshape pharmaceutical research and development profoundly. The evolution of Artificial Intelligence (AI) in pharmaceutical research has transitioned from basic computational methods to advanced machine learning (ML) and deep learning (DL) algorithms that now drive innovation across the drug development pipeline. Early AI applications in the 1980s and 1990s focused on rule-based systems for structure-activity relationship (SAR) analysis and data mining (Chen *et al.,* 2018). However, the recent explosion in biological data, combined with advances in computing power, has catalyzed a new era where AI plays a central role in predictive modeling, target identification, and drug repurposing. Today, AI enables the analysis of omics data, electronic health records, and scientific literature, allowing researchers to generate hypotheses and design experiments more efficiently (Ekins *et al.,* 2019). AI models can predict the pharmacokinetics, toxicity, and efficacy of compounds before synthesis, significantly reducing attrition rates in drug development.

Additionally, generative models are now used to design novel drug-like molecules with specific properties (Zhavoronkov *et al.,* 2019). The integration of AI into cloud platforms and automated laboratories has further accelerated its impact, supporting real-time data analysis and iterative experimentation. As AI continues to mature, it is expected to become indispensable in personalized medicine, biomarker discovery, and beyond.

- **AI Technologies Used in Drug Discovery:**

Artificial Intelligence (AI) encompasses a range of computational techniques that mimic human intelligence and have demonstrated transformative potential in drug discovery. The integration of AI technologies in this field has streamlined processes such as target identification, compound screening, drug design, and clinical trial optimization. Key AI technologies include machine learning (ML), deep learning (DL), natural language processing (NLP), generative models, reinforcement learning, and robotics-driven automation.

1. Machine Learning (ML):

Machine learning forms the foundation of most AI applications in drug discovery. It involves training algorithms on large datasets to identify patterns and make predictions. ML is extensively used for predicting physicochemical properties, biological activity, and toxicity of compounds (Chen *et al.,* 2018). Supervised learning techniques, such as support vector machines (SVM) and random forests (RF), are widely applied in quantitative structure–activity relationship (QSAR) modeling to predict how structural changes affect biological activity. ML models can integrate high-throughput screening data to filter and prioritize compounds for further testing. Moreover, unsupervised learning methods like clustering and principal component analysis (PCA) help identify hidden patterns in complex biological data, aiding in biomarker discovery and patient stratification (Lavecchia, 2015).

2. Deep Learning (DL)

Deep learning, a subset of ML, utilizes neural networks with multiple layers to model intricate relationships in data. DL excels in analyzing high-dimensional data such as genomic sequences, chemical structures, and imaging data. Convolutional neural networks (CNNs) have been used to analyze molecular graphs and protein structures, enabling accurate predictions of binding affinities (Wallach *et al.,* 2015). One significant application of DL is in structure-based drug design. DL models can predict protein-ligand interactions from three-dimensional structures, as seen with tools like AtomNet and DeepDock (Zhou *et*

al., 2020). DL also underpins modern de novo drug design, where it generates new chemical entities with desired pharmacological profiles. Recurrent neural networks (RNNs) and their variants, such as long short-term memory (LSTM) networks, are particularly useful for generating and optimizing molecular sequences, such as SMILES representations of chemical compounds (Segler *et al.,* 2018).

3. Natural Language Processing (NLP)

NLP enables AI to extract information from unstructured text, such as scientific literature, clinical notes, and patents. With the vast and rapidly growing volume of biomedical data, NLP has become essential in mining relevant information to inform drug discovery efforts. NLP models, including Bidirectional Encoder Representations from Transformers (BERT), have been fine-tuned for biomedical texts (e.g., BioBERT and SciBERT). These models help identify drug-gene interactions, disease associations, and adverse event reports (Lee *et al.,* 2020). NLP also facilitates drug repurposing by uncovering hidden relationships in existing literature that might indicate new therapeutic uses for approved drugs.

4. Generative Models

Generative models, particularly variational autoencoders (VAEs) and generative adversarial networks (GANs), have opened new avenues in molecule generation. These models can design novel compounds by learning latent representations of chemical structures and generating new molecules with optimized properties. For example, VAEs trained on chemical libraries can sample new molecules that maintain drug-likeness while exhibiting novel scaffolds (Gómez-Bombarelli *et al.,* 2018)**.** GANs have been used to generate molecular graphs and SMILES strings that satisfy specific pharmacological constraints. The combination of generative models with reinforcement learning (RL) has led to the development of goal-directed molecular design frameworks. In these setups, the generative model proposes new molecules, and the RL component fine-tunes them to optimize a reward function based on desired criteria such as binding affinity, solubility, or novelty (Popova *et al.,* 2018).

5. Reinforcement Learning (RL)

Reinforcement learning is another AI paradigm where agents learn to make decisions by interacting with an environment and receiving feedback in the form of rewards or penalties. In drug discovery, RL has been used to guide molecular optimization, synthesis planning, and even clinical trial design. In molecular optimization, RL agents

modify chemical structures iteratively to improve drug-likeness, efficacy, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties. For example, RL has been applied in scaffold hopping, where the agent searches for bioisosteric replacements that preserve activity while improving pharmacokinetics (Olivecrona *et al.,* 2017). RL has also been used in retrosynthetic analysis, where the goal is to plan a synthesis route for a target compound using available reagents and reactions. Tools like ASKCOS use AI and RL to automate complex retrosynthesis tasks (Coley *et al.,* 2019).

6. AI-Powered Structural Prediction Tools

Protein structure prediction is crucial for understanding molecular mechanisms and for rational drug design. Traditionally, experimental techniques like X-ray crystallography and cryo-EM have been the gold standard but are resource-intensive. AI has brought revolutionary changes with models like AlphaFold and RoseTTAFold. AlphaFold2, developed by DeepMind, predicts protein 3D structures from amino acid sequences with near-experimental accuracy (Jumper *et al.,* 2021). This breakthrough has enabled researchers to model previously uncharacterized proteins, aiding structure-based drug discovery and target identification. These models use attention mechanisms and evolutionary information to infer spatial relationships among residues. The implications of such tools are vast, from understanding disease mechanisms to designing inhibitors for newly discovered protein targets.

7. Robotics and AI-Integrated Automation

AI technologies are increasingly integrated into laboratory automation systems to form "self-driving labs." These setups use robotic platforms to conduct high-throughput experiments guided by AI algorithms. The AI system interprets experimental results in real time and designs subsequent experiments to iteratively improve outcomes. Such closed-loop systems enhance efficiency in tasks like compound screening, formulation optimization, and synthetic route planning. By combining AI with automation, pharmaceutical companies can dramatically accelerate the discovery cycle and reduce reliance on manual processes (Macarron *et al.,* 2020).

8. Cloud Computing and Federated Learning

Cloud-based platforms enable AI models to scale and operate on large datasets from diverse sources. Cloud environments support data sharing, collaborative model development, and remote experimentation, all of which are crucial in the globalized pharmaceutical industry. Federated learning is an emerging approach where models are

trained across decentralized data sources without transferring sensitive data. This is particularly important in drug discovery, where data privacy concerns limit access to clinical or proprietary datasets. Federated learning enables AI systems to learn from diverse datasets while maintaining data confidentiality (Yang *et al.,* 2019).

- **Applications of AI in the Drug Discovery Pipeline:**

The application of artificial intelligence (AI) in drug discovery has significantly accelerated the traditionally long and expensive pipeline, which spans from target identification to clinical trials and regulatory approval. AI technologies are increasingly integrated at various stages of the pipeline, offering improvements in prediction accuracy, time efficiency, and resource allocation. By leveraging large biological, chemical, and clinical datasets, AI enhances decision-making across the spectrum of drug development, including target discovery**,** lead identification**,** lead optimization**,** preclinical testing**,** clinical trials**, and** drug repurposing**.**

1. Target Identification and Validation

Identifying the correct biological target is a foundational step in drug discovery. AI can process high-throughput omics data (genomics, proteomics, transcriptomic) to uncover novel targets associated with disease phenotypes. Machine learning (ML) models have been developed to integrate multi-omics data and infer gene-disease associations, predict protein functions, and identify key signalling pathways involved in disease mechanisms (Ezzat *et al.,* 2019). Natural language processing (NLP) further enhances this process by mining scientific literature and clinical data to extract relationships between genes, proteins, and diseases (Lee *et al.,* 2020). Tools like IBM Watson for Drug Discovery use AI to analyze thousands of research articles and generate hypotheses about new drug targets.

2. Compound Screening and Virtual Screening

Virtual screening is a computational technique used to identify potential drug candidates from large chemical libraries. AI significantly improves this process by predicting the likelihood of interaction between compounds and biological targets. Deep learning models, such as convolutional neural networks (CNNs), have been developed to predict binding affinities and prioritize compounds for experimental testing (Wallach *et al.,* 2015). Structure-based virtual screening (SBVS) has benefited from AI tools like AtomNet, which uses deep learning to evaluate protein-ligand interactions and rank compounds accordingly (Zhou *et al.,* 2020). Similarly, ligand-based virtual screening (LBVS) employs

ML models trained on known active compounds to identify structurally similar candidates with potential activity.

3. De Novo Drug Design

AI plays a transformative role in de novo drug design, where novel chemical entities are generated with desired pharmacological properties. Generative models such as variational autoencoders (VAEs) and generative adversarial networks (GANs) can design new molecules by learning the latent features of existing compounds (Gómez-Bombarelli *et al.,* 2018). Reinforcement learning (RL) is often integrated with these models to iteratively refine generated molecules based on objectives like potency, selectivity, or pharmacokinetic profile (Popova *et al.,* 2018). These AI-driven methods dramatically reduce the time and cost associated with designing drug-like molecules, allowing for the exploration of chemical space beyond traditional medicinal chemistry approaches.

4. Lead Optimization

After identifying potential leads, optimization is required to improve efficacy, safety, and drug-likeness. AI algorithms facilitate multi-objective optimization by balancing various pharmacological and physicochemical parameters simultaneously. Techniques like Bayesian optimization and support vector regression are used to predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles (Schneider *et al.,* 2020). AI also assists in predicting off-target effects, bioavailability, and metabolic stability, helping researchers to prioritize compounds that are more likely to succeed in later stages. By modeling structure-activity relationships (SAR), AI helps in refining molecular structures to enhance activity and reduce toxicity.

5. Preclinical Testing

Preclinical studies aim to assess the safety and efficacy of a compound in biological systems before human trials. AI technologies are employed to predict in vivo responses using in vitro and in silico data. For example, ML models trained on historical toxicology data can predict cardiotoxicity, hepatotoxicity, and genotoxicity with high accuracy (Urban *et al.,* 2021). Animal testing remains a regulatory requirement, but AI is reducing dependence on these models by offering predictive alternatives, thus aligning with the principles of the 3Rs (Replacement, Reduction, and Refinement). Physiologically based pharmacokinetic (PBPK) modeling and AI-enhanced simulations are now used to extrapolate human responses from non-clinical data.

6. Clinical Trial Design and Recruitment

Clinical trials are a major bottleneck in drug development due to high costs, long durations, and high failure rates. AI enhances trial design by analyzing electronic health records (EHRs), real-world data, and genetic information to identify optimal patient cohorts (Bhatt *et al.,* 2021). Predictive modeling can forecast trial outcomes, reduce the risk of failure, and suggest adaptive designs that modify protocols based on interim results. AI-driven tools also assist in identifying trial sites, predicting patient dropout, and optimizing dosage regimens. For instance, machine learning algorithms can cluster patients with similar disease progression patterns, enabling more targeted recruitment and increasing the likelihood of demonstrating efficacy.

7. Drug Repurposing

AI facilitates drug repurposing by discovering new therapeutic uses for existing drugs. This is particularly valuable for rare diseases and emerging health threats where rapid therapeutic development is needed. AI models analyze biomedical literature, molecular structures, gene expression profiles, and patient data to find novel drug-disease associations (Ke *et al.,* 2021).

During the COVID-19 pandemic, AI tools helped identify potential repurposed drugs like remdesivir and baricitinib by analyzing large-scale datasets and predicting antiviral activity. Platforms such as BenevolentAI and Healx use AI to systematically explore repurposing opportunities for unmet medical needs.

8. Post-Market Surveillance and Pharmacovigilance

Once a drug is approved, monitoring its safety and efficacy in the real world is critical. AI enhances pharmacovigilance by analyzing real-world data from healthcare databases, social media, and adverse event reporting systems. NLP algorithms can detect patterns of adverse drug reactions (ADRs) from unstructured data, supporting early detection of safety signals (Sarker *et al.,* 2015). AI also aids in compliance monitoring, identifying prescription misuse, and optimizing medication adherence strategies through personalized digital interventions.

- **Key Tools and Platforms in AI for Drug Discovery**

The rapid adoption of artificial intelligence (AI) in drug discovery has been supported by the emergence of several specialized tools and platforms. These technologies leverage machine learning (ML), deep learning (DL), natural language processing (NLP),

and other AI methodologies to streamline various stages of drug development. Below are some of the most widely used and impactful tools in this field.

1. DeepChem

DeepChem is an open-source Python-based library that provides a suite of tools for applying deep learning to chemical and biological problems. It includes pre-built models for molecular property prediction, molecular docking, and toxicity estimation. The platform facilitates rapid prototyping of new models and integrates with TensorFlow and PyTorch, making it highly flexible for drug discovery applications (Ramsundar *et al.,* 2019).

2. AtomNet

AtomNet, developed by Atomwise, is a pioneering deep learning platform for structure-based drug design. It employs convolutional neural networks (CNNs) to predict the binding affinity between small molecules and protein targets using 3D structural data. AtomNet has been successfully applied to identify lead compounds for diseases such as Ebola and multiple sclerosis (Wallach *et al.,* 2015).

3. IBM Watson for Drug Discovery

IBM Watson utilizes NLP and ML to analyze vast volumes of biomedical literature and generate hypotheses for novel drug targets and repurposing candidates. It extracts relationships between genes, proteins, and diseases from unstructured text, making it a powerful tool for early-stage discovery and hypothesis generation (Ferrucci, 2012).

4. Schrödinger Suite

Schrödinger offers a comprehensive platform for molecular modeling and drug design. Its AI-enabled modules include predictive models for ADMET properties, docking simulations, and lead optimization. Schrödinger integrates quantum mechanics and machine learning to predict molecular behavior with high accuracy, making it a staple in pharmaceutical R&D (Friesner *et al.,* 2004).

5. BenevolentAI

BenevolentAI combines knowledge graphs and machine learning to accelerate target identification and drug repurposing. Its platform integrates data from scientific publications, clinical trials, and proprietary datasets to uncover hidden relationships and novel insights. BenevolentAI played a role in identifying baricitinib as a potential treatment for COVID-19 (Richardson *et al.,* 2020).

6. Insilico Medicine

Insilico Medicine is a leader in generative chemistry and AI-driven drug design. Its platform, PandaOmics, is used for target discovery, while Chemistry42 focuses on de novo molecule generation. The company applies generative adversarial networks (GANs) and reinforcement learning to design novel drug-like compounds with optimized properties (Zhavoronkov *et al.,* 2019).

7. MoleculeNet

MoleculeNet is a benchmarking platform for molecular machine learning developed by Stanford University. It provides standardized datasets and evaluation metrics to train and compare predictive models, supporting transparency and reproducibility in AI research for drug discovery (Wu *et al.,* 2018).

- **Advantages of AI in Drug Discovery:**

Artificial intelligence (AI) offers transformative advantages in drug discovery by improving efficiency, reducing costs, and enhancing decision-making. One of the most significant benefits is the acceleration of drug development timelines. Traditional drug discovery can take over a decade, but AI-driven techniques enable faster identification of drug candidates by rapidly analyzing large-scale biological and chemical data (Vamathevan *et al.,* 2019). Another advantage is cost reduction. AI helps avoid costly failures by predicting pharmacokinetics, toxicity, and off-target effects early in the pipeline (Mak & Pichika, 2019). Furthermore, AI algorithms can process high-throughput screening data and simulate molecular interactions, thereby minimizing the need for extensive wet-lab experiments (Ekins *et al.,* 2019). AI also supports personalized medicine by analyzing genomic and clinical data to identify patient-specific drug responses, which enhances therapeutic effectiveness (Topol, 2019). Additionally, AI enables drug repurposing, identifying new uses for existing drugs, which is especially valuable in addressing urgent health crises such as COVID-19 (Zhou *et al.,* 2020). Overall, AI empowers pharmaceutical researchers to make informed, data-driven decisions, thereby improving success rates and innovation in drug discovery.

- **Challenges and Limitations of AI in Drug Discovery:**

Despite its promise, the integration of artificial intelligence (AI) into drug discovery faces several significant challenges and limitations. One major concern is the quality and availability of data. AI models require large, diverse, and well-annotated datasets to function effectively, but much of the biological and chemical data available is noisy, incomplete, or biased (Vamathevan *et al.,* 2019). Limited access to proprietary

pharmaceutical datasets further hinders model training and validation. Another challenge is the interpretability of AI models. Deep learning algorithms often act as "black boxes," making it difficult for researchers to understand how decisions are made—an issue that complicates regulatory approval and clinical trust (Holzinger *et al.,* 2019). There are also regulatory and ethical concerns. The use of AI in health-related decisions raises questions around data privacy, algorithmic bias, and accountability (Tonekaboni *et al.,* 2019). Furthermore, integration into existing drug discovery workflows can be complex due to the need for interdisciplinary collaboration and cultural adaptation within pharmaceutical organizations. Lastly, generalization of AI models remains a limitation. Many models perform well in silico but fail to translate effectively to real-world biological systems (Mak & Pichika, 2019).

Conclusion:

In conclusion, artificial intelligence (AI) is revolutionizing drug discovery by significantly accelerating and optimizing the traditionally slow and expensive processes of target identification, compound screening, lead optimization, and clinical trial design. With its ability to process and analyze massive datasets, AI offers unparalleled insights into disease mechanisms, molecular interactions, and patient responses. Techniques such as machine learning, deep learning, and natural language processing are enabling the rapid identification of promising drug candidates and repurposing of existing compounds with increased accuracy and efficiency. Furthermore, AI-driven models are enhancing predictive toxicology and pharmacokinetics, thereby reducing failure rates in later stages of drug development. The integration of AI with other advanced technologies, such as high-throughput screening and omics data, is ushering in a new era of precision medicine. Despite certain challenges, including data quality, model interpretability, and regulatory considerations, the continued advancement and validation of AI tools hold immense promise for transforming drug discovery pipelines. As collaborations between pharmaceutical companies, academia, and tech firms expand, AI is poised to play an even more pivotal role in delivering safer, more effective, and personalized therapies to patients worldwide. The future of drug discovery is being reshaped by AI, marking a paradigm shift in modern biomedical research and healthcare innovation.

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ETHNOBOTANICAL INSIGHTS INTO ANTI-ARTHRITIC MEDICINAL PLANTS

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Abstract:

With a prevalence of roughly one in four Americans, arthritis is a chronic, degenerative disease with far-reaching consequences for public health. The two most common and disabling kinds of arthritis, osteoarthritis (OA) and rheumatoid arthritis (RA), are among the many others. Chronic pain, stiffness, inflammation, and loss of joint function are symptoms shared by the two illnesses, which in turn reduce mobility and significantly lower quality of life. To treat symptoms and reduce disease development, many patients eventually need pharmacological intervention, even though non-pharmacological methods like physical therapy, exercise, and lifestyle adjustments are generally recommended initially. A few examples of common pharmaceutical therapies are biologics, disease-modifying anti-rheumatic medications (DMARDs), corticosteroids, and non-steroidal anti-inflammatory medicines (NSAIDs). Nevertheless, these drugs come with a long list of potential adverse effects, such as damage to organs, immunosuppression, cardiovascular risks, and gastrointestinal problems. In addition, these treatments can have a significant impact on healthcare costs over time, therefore patients and providers are looking for safer, more cost-effective options. Consequently, herbal remedies for arthritis have gained a lot of attention. Reduce inflammation, protect tissues from oxidative stress, and ease joint pain with the aid of many plant-based substances that have shown strong anti-inflammatory and antioxidant capabilities. Additionally, new biological mechanisms related to OA and RA have been uncovered by certain herbs, which may lead to the development of alternative treatment options. To confirm the herbal treatments' safety, effectiveness, bioactivity, and ideal bioavailability, large-scale clinical trials that are both well-designed and conducted by experts are necessary, notwithstanding encouraging findings from preliminary and restricted clinical investigations.

Keywords: Arthritis, Osteoarthritis (OA), Rheumatoid Arthritis (RA), Herbal Remedies, Anti-inflammatory Agents

Introduction:

The number of Americans afflicted by arthritis is in the millions [1]. Arthritis patients endure excruciating joint pain, and almost 50% of adult patients report ongoing discomfort [1,2]. There are over a hundred different kinds of arthritis [3]. The two most prevalent forms of arthritis are rheumatoid and osteoarthritis. While rheumatoid arthritis and osteoarthritis both damage joints and their function, the two conditions are distinct in their symptoms, etiology, and approaches to therapy.

Degenerative joint disease, or osteoarthritis (OA), affects more people than any other kind of arthritis [4]. Factors like mechanical and oxidative stress, damage, aging, obesity, metabolic disorders, and mechanical and inflammatory stress all play a role in OA, which is a biomechanical and inflammatory disease [5]. Cartilage deterioration, bone alterations, and synovitis are the hallmarks of osteoarthritis (OA) [6]. Hydrolytic enzymes, like matrix metalloproteinases (MMPs), are linked to cartilage deterioration, and pro-inflammatory and pro-catabolic mediators are localized in synovial fluid. The buildup of innate immune cells, which can cause inflammation and tissue death, might be triggered by the breakdown of the extracellular matrix [7]. Additionally, it has been discovered that signaling pathways and reactions, including those involving NF-κB and mitogen-activated protein kinase (MAPK), have a functioning [5]. Disabilities caused by OA typically manifest later in life because of the disease's gradual onset. Some of the symptoms include stiffness upon waking and after physical exertion, as well as soreness and tenderness in certain joints.

Inflammation and immunological dysregulation characterize rheumatoid arthritis (RA), a systemic disease that impacts numerous joints. As a woman, you are more likely to get RA if you smoke and have a family history of the disease [8]. Seropositive and seronegative RA are distinguished by the presence or absence of antibodies. Upon presentation, seronegative patients show more inflammation than seropositive patients, who develop more inflammation and joint damage as the disease progresses [8]. Seropositive cases or severe illness may present with symptoms outside of the joints. An inflammatory response that includes pain, bone erosions, and anti-citrullinated protein antibodies (ACPA) [9] can persist. This disease's inflammatory nature causes long-term deformities. About 60% of RA patients are unable to work by the time the condition has been around for at least ten years, adding to the significant disability rate experienced by

the disease overall [10]. Rheumatoid arthritis symptoms include sore, heated, and swollen joints, along with morning stiffness and inactivity-induced stiffness [11].

Effective therapy for OA and RA remains a challenge, despite modern disease-state information. The current therapeutic choices have been recommended by the American College of Rheumatology/Arthritis Foundation [12,13]. The following medications are suggested for osteoarthritis (OA): intra-articular corticosteroids, serotonin and norepinephrine reuptake inhibitors, oral analgesics, and topical and oral non-steroidal anti-inflammatory medicines (NSAIDs) [12]. Reducing inflammation and pain is the overarching objective of RA treatment. The severity of the disease and its progression determine the best course of treatment for RA. For patients with modest disease activity levels, it is strongly suggested to undergo standard disease-modifying antirheumatic medication (DMARD) monotherapy, particularly with methotrexate (MTX), for both early and established RA [13]. Tofacitinib, biologics, or standard DMARD combinations are suggested for RA patients with moderate to high disease activity who are currently using DMARD monotherapy. An earlier analysis [14] examined the action mechanism of the medicines now used to treat OA and RA.

Options for pain and RA/OA symptoms alleviation are available in current pharmacology. Nevertheless, these treatments may have limited applicability due to their accompanying negative effects. Nonsteroidal anti-inflammatory drugs (NSAIDs) are not recommended for the long-term management of arthritis due to the risk of gastrointestinal, cardiovascular, and nephrotoxic side effects [15,16]. Acetaminophen has the potential to cause liver damage [17]. The gastrointestinal and central neurological systems can be affected by tramadol [18]. When it comes to treating osteoarthritis (OA), the efficacy of intra-articular corticosteroids is debatable, and they can really do more harm than good [19]. Although they can be costly, hyaluronic acid injections alleviate OA pain in a reasonable and safe manner [19]. While non-biologic DMARDs work wonders in mild to moderate RA, they come with a slew of side effects, including gastrointestinal problems, liver damage, kidney damage, and blood issues [20]. However, biologics are associated with an increased risk of major infections, cancer, and heart failure, and they have poor tolerability [21]. Biologics are effective for mild to severe RA. Last but not least, treating moderate to severe RA with Janus kinase inhibitors comes with the risk of infection and blood problems [22]. The use of pharmaceutical treatment for OA and RA is ultimately a risk-benefit analysis that doctors and patients must do.

Many people are looking for alternative treatments for arthritis because they are worried about the side effects and high expense of conventional medicines. Further, research into herbal remedies has been spurred by the challenge of managing chronic pain in arthritic patients. Herbs have the potential to be a safe and effective alternative or supplement to conventional medicine. New information about the use of herbs to treat arthritis has been covered in this chapter. The purpose of this study was to characterize several herbal remedies for OA and RA, focusing on their mechanisms, safety, and effectiveness (including pain and inflammatory outcomes) [23].

Ginger (*Zinziber officinalis, Zinziberacea*)

As an anti-inflammatory substance, it has a long history of usage in Indian traditional medicine and ayurvedic practices. It is grown all over Asia, both tropical and subtropical, with half of the global supply coming from India. There are five components in ginger that can suppress prostaglandins. The gingerol component of ginger was found to decrease the expression of inducible nitric oxide synthase (iNOS) and NO generation in vitro when tested with lipopolysaccharide (LPS) [24]. Researchers found that giving people ginger oil orally reduced the amount of inflammation that adjuvants may generate [25].

Turmeric (*Curcuma longa*)

Turmeric, whose scientific name is Curcuma longa, is a perennial herb with several medicinal uses in Ayurvedic and traditional Chinese medicine. Originating in the tropics of Southern Asia, this plant can reach a height of three to five feet and flourishes in hot, humid environments. For both culinary and medicinal purposes, the main portion of Curcuma longa is the rhizome, which is the underground stem. The rhizome is a well-known herbal cure and a popular spice when dried and powdered into a bright yellow powder. Turmeric contains the bioactive component curcumin, which is a polyphenol that occurs naturally and is known for its anti-inflammatory, antioxidant, and immunomodulatory effects. Curcumin helps alleviate autoimmune diseases like rheumatoid arthritis (RA) by reducing the chronic inflammatory responses caused by molecules like cytokines, cyclooxygenase-2 (COX-2), and nuclear factor-kappa B (NF-κB).

Curcumin has shown promise as an anti-arthritic in a number of trials, both in humans and in animals. Important symptoms of rheumatoid arthritis, including morning stiffness, joint swelling, and walking time, have been significantly alleviated in individuals who take curcuma-based medicines on a regular basis. These results provide more evidence that curcumin could be a safe and effective supplementary treatment for RA. To

find out how much to take, how quickly it is absorbed into the bloodstream, and whether it is safe to use over the long term, more controlled clinical trials are needed [26].

Matricaria chamomilla L.

For ages, people have turned to Matricaria chamomilla, more often known as chamomile, when they needed a remedy for joint pain. Historically, rheumatic pain and inflammation have been treated with the dried floral component of the plant. Currently, chamomile is listed as a herb that is "generally recognized as safe" by the FDA. German chamomile and Roman chamomile are the two most frequent types of this plant, which belongs to the Asteraceae family and the Compositae family of flowers. Herbal tea is the most common form of chamomile. Glucosides, apigenin, patuletin, quercetin, and luteolin are among the phenolic chemicals found in chamomile. The anti-inflammatory effects of these substances are demonstrated by their ability to reduce cytokines and PGE2, two factors that contribute to the development of arthritis.

A randomized controlled trial was carried out to evaluate the efficacy and safety of topical chamomile oil compared to diclofenac and a placebo in treating knee osteoarthritis. Although chamomile did not influence the results of the WOMAC questionnaire domains, it significantly decreased the requirement for the rescue medication acetaminophen without any negative side effects when compared to diclofenac and a placebo. A different study found that rheumatoid arthritis patients who took 6 grams of chamomile tea daily experienced less joint pain and a slower rate of erythrocyte sedimentation compared to those who took a placebo [27-29].

Saffron (*Crocus sativus, Iridaceae*)

Common folk uses include those of an aphrodisiac, antispasmodic, and expectorant. It is a short-lived perennial flowering plant that can only reach a maximum height of 40 cm. Widely grown from its native Kashmiri region to the Americas, Europe, and Spain. The inclusion of crocetin and carotenoids in saffron stigma gives it an anti-inflammatory activity. Both the water-and alcohol-based saffron flower extracts have anti-inflammatory and radical scavenging properties in xylene and formalin-induced inflammation models [30].

***Withania somnifera* (Ashwagandha)**

Ashwagandha, whose scientific name is Withania somnifera, is an Ayurvedic herb with anti-inflammatory and analgesic properties. Research has shown that W. somnifera extract reduces the activation of the NF-κB and activator protein 1 (AP-1) signaling

pathways, which in turn inhibits the production of TNF-α, IL-1β, and IL-12 [31]. Additionally, by blocking collagenase activity, W. somnifera extract reduced the breakdown of bovine Achilles tendon type I collagen [32]. In a rat model of collagen-induced arthritis, W. somnifera treatment reduced inflammation, redness, deformity, and ankylosis [33]. The anti-arthritic effects of W. somnifera might be because it decreases the activation of ROS, TNF-α, IL-1B, IL-6, MMP-8, and NF-κB, while increasing the secretion of IL-10 [34]. By reducing the gelatinase activity of collagenases, Sumantran et al. [35] revealed that an aqueous extract of W. somnifera significantly protected injured human OA cartilage from further degradation. Its analgesic benefits in OA of the knee have been shown in a recent study. Results showed that compared to baseline and placebo, participants treated with 125 or 250 mg of W. somnifera extract for 12 weeks had significantly lower mean WOMAC and Knee Swelling Index scores. The VAS pain, stiffness, and disability scores also showed a notable decrease. There was a decrease in the requirement for paracetamol rescue medicine, an earlier onset of efficacy (at 4 weeks), and superior physician overall assessments (excellent vs. good vs. fair) with the higher dose compared to the low dose and placebo [36].

Karvi (*Strobilanthus callosus, Acanthaceae*)

Local tribal people in the Indian state of Maharastra have traditionally employed a different medicinal herb to alleviate inflammatory conditions. In carrageenan-induced edema, the Lupeol and 19α-H Lupeol, which were extracted from Strobilanthus callosus roots, showed anti-inflammatory and anti-rheumatic properties [37].

Eremostachys laciniata

The Iranian herb Eremostachys laciniata is commonly used to treat inflammatory disorders, such as arthritis, by making a decoction of its roots and blossoms [38]. The exact mechanism by which this herb works remains unknown. But one investigation found that crude methanol extract or E. laciniate fractions reduced the inflammatory response in rat paws caused by carrageenan [39]. In addition to its significant DPPH radical-scavenging activity and decrease of H2O2- or HOCl-luminal chemiluminescence, the aqueous extract of E. laciniate has demonstrated potential antioxidant activity [40].

The effects of using E. laciniata topically on arthritic pain and symptoms have only been studied once [38]. Patients with arthritis and rheumatoid arthritis reported much less discomfort after two weeks of applying 0.5% E. laciniata ointment to their affected joints compared to those who received a placebo. After one week of using E. laciniata, patients

with arthritis and rheumatoid arthritis reported less pain compared to those using a 0.5% piroxicam ointment, suggesting a more favorable first therapeutic response. But after two weeks, the groups given E. laciniata or piroxicam reported comparable levels of pain. The anti-inflammatory effects of E. laciniata on joints were more pronounced than those of piroxicam throughout the course of the trial. The anti-inflammatory action seen in their arthritis patients might have been caused by iridoid glycosides, which the researchers found to be the most common isolates from their E. laciniata extracts [38].

Chirayita (*Swertia Chirayita*)

Asthma, persistent fever, and anemia are popular uses for this herb, which grows abundantly in the temperate Himalayan highlands. Thrives at altitudes of up to three thousand meters, this plant is native to the Himalayan pastures and slopes. Swerchirin, swertanone, and swertianin are the active ingredients in chirayita that have an anti-inflammatory effect. According to reports, chirayita can lower the high levels of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 in both experimental arthritis and asthma [41].

Paeonia lactiflora

Traditional Chinese medicine has a lengthy history of using Radix Paeonia, the dried root of Paspalum lactiflora Pallas [42]. One study found that decoctions of Radix Paeoniae helped with RA and other inflammatory and autoimmune illnesses [42]. Radix Paeoniae Alba water/ethanol extracts contain total glucosides of paeony (TGP), which include paeoniflorin in particular. Previous studies have demonstrated that peoniflorin and TGP can reduce the production of many pro-inflammatory mediators, including PGE2, leukotriene B4, NO, and ROS. Additionally, paeonia has demonstrated anti-inflammatory effects via reducing microvascular permeability and the infiltration of inflammatory cells. In addition to preventing osteoclast formation and apoptosis caused by TNF-α, the paeoniflorin component of TGP inhibits NF-κB. One randomized clinical experiment evaluated the hepatotoxicity of methotrexate (MTX), leflunomide (LEF), and trigeminal gamma-proliferation (TGP) against those of MTX and LEF alone. At both the midway and the end of the research, the combination that contained TGP was determined to be just as beneficial, with a significant reduction in hepatotoxicity. The hepatoprotective advantages of TGP may be associated with its anti-inflammatory characteristics, which include a decrease in TNF-α, IL-6, and C-Reactive Protein (CRP) [43-45].

Thunder God Vine (*Tripterygium wilfordii Hook F, TwHF*)

This is the gold standard for treating inflammatory and autoimmune disorders in Chinese medicine, including psoriasis, systemic lupus erythematosus, rheumatoid arthritis, and others. It grows in the southern regions of China and Taiwan as a vine-like perennial plant. The anti-inflammatory and free radical scavenging properties are imparted by triptolide, the principal active component. After the first four weeks of treatment, half of all RA patients experience a reduction in symptoms, according to dose-dependent trials of TwHF root ethyl acetate extract. In order to explain its anti-inflammatory effects, the TwHF extract suppresses adhesion molecules such as E-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) [42]. The extract from TwHF inhibits the expression of iNOS, which in turn decreases the activity of nuclear factor-kappa B (NF-κB) DNA binding and leads to the scavenging of ROS [46].

Boswellia spp.

Boswellia, more often known as frankincense, has long been an important component of traditional Ayurvedic therapy. Some of the anti-inflammatory effects of this herb include reducing or activating inflammatory mediators like matrix metalloproteinase (MMP)-9, MMP-13, cyclooxygenase (COX)-2, and nitric oxide (NO) [47-49]. It also inhibits microsomal prostaglandin E2 (PGE2) synthase-1 and 5-lipoxygenase. Boswellia is thought to alleviate osteophytes, enhance the knee joint gap, and decrease inflammatory mediators linked to knee OA, including C-reactive protein and hyaluronic acid, which are the probable causes of its beneficial effects on arthritis.

Boswellia serrata's efficacy and safety have been the subject of numerous studies. If you suffer from osteoarthritis (OA), Majeed et al. [50] found that taking an oral B. serrata extract for 8 weeks was much better than a placebo on several measures, including the Visual Analog Scale (VAS), the Japanese Knee Osteoarthritis Measure (JKOM), and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Another study found that compared to a placebo, oral administration of boswellia extract for more than four months significantly improved physical function in OA patients by reducing pain and stiffness, with no serious side effects. Boswellia Carterii B., or oliban oil, was found to be a potent topical analgesic for OA pain and symptoms when tested against diclofenac and a control group. Conversely, when it came to knee-related ADLs, recreational activities, and quality of life, no improvement could be seen [51, 52].

Conclusion:

Inflammation in various joints is the hallmark symptom of rheumatoid arthritis (RA), an inflammatory disorder that can be both long-lasting and severely disabling. People with RA can have a broad spectrum of symptoms, from occasional minor pain and discomfort to deformity and joint deterioration that severely hinders their ability to go about their everyday lives. The exact cause of RA is still not fully understood; however, it is thought to be a result of a complicated interaction between hereditary, environmental, and immune variables.

The use of DMARDs and non-steroidal anti-inflammatory medicines (NSAIDs) is a part of current RA therapy protocols for the management of inflammation and the slowing down of disease development. Biologic response modifiers are a relatively new type of targeted therapy that have recently entered the market and demonstrated encouraging results in clinical trials. Their possible immunosuppressive effects, hefty costs, and long-term safety profiles are still under intense scrutiny, though. Alternate and complementary therapies, including traditional medical systems, are gaining popularity among RA sufferers, who are part of a larger trend toward integrative medicine as a whole. This development highlights the critical importance of quickly conducting thorough scientific analyses of the many plants utilized in traditional Ayurvedic and Unani medicine from ancient India. New, less risky therapy choices that effectively manage symptoms with little side effects may be able to be developed using these more conventional methods.

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ANCIENT NEEM KNOWLEDGE IN MODERN HEALTH PRACTICES

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Abstract:

This chapter explores the antibacterial, antifungal, antiviral, anti-inflammatory, and antidiabetic potential of neem leaf extracts, emphasizing their relevance in therapeutic applications. A range of bioactive compounds, including alkaloids, flavonoids, saponins, and tannins, were extracted and evaluated for their antimicrobial efficacy against *Staphylococcus aureus*, *Corynebacterium bovis*, and *Escherichia coli*. The complete neem extract demonstrated greater antibacterial activity than the individual phytochemicals, with enhanced effects observed at higher concentrations (75 mg/ml). Additionally, the extracts exhibited notable antifungal activity, especially against *Aspergillus* species and *Candida albicans*, with ethanol-based preparations proving most effective. Anti-inflammatory effects were evidenced by a substantial drop in carrageenan-persuaded paw edema in rats, with dose-dependent improvements. Neem also showed substantial antiviral action, particularly against herpes simplex virus type 1 and several bovine viruses, indicating a broad antiviral spectrum. Furthermore, neem extracts contributed to significant blood glucose reduction in diabetic models, suggesting potential as a natural antidiabetic agent. Collectively, these results highlight neem's diverse pharmacological properties and reinforce its traditional use in herbal medicine. The study underscores neem's promise as a versatile plant with applications in modern therapeutic development.

Keywords: Neem, Antibacterial, Antifungal, Antiviral, Anti-Inflammatory, Antidiabetic Potential

Introduction:

Neem (*Azadirachta indica*), often known as the "village pharmacy," holds a revered and essential role in traditional medicinal systems throughout the Indian subcontinent and beyond. [1] For generations, it has been highly valued in Ayurveda, Unani, and Siddha for its broad range of therapeutic, agricultural, and domestic benefits. Native to South Asia, this hardy tropical evergreen tree is famed for its bitterness, durability, and potent healing abilities. Its uses date back thousands of years and are deeply interwoven with the

everyday health practices, lifestyles, and cultural traditions of indigenous communities. The rich and varied legacy of neem in traditional medicine is well-documented through both ancient texts and oral traditions. Neem has long been viewed as a natural purifier and protective agent. Each portion of the tree—leaves, bark, roots, seeds, flowers, and fruit—is employed in treating a multitude of ailments. Classical Ayurvedic texts like the *Charaka Samhita* and *Sushruta Samhita* detail neem's effectiveness in treating skin conditions, fevers, infections, and metabolic disorders. Within Ayurveda, neem is classified as a "krimighna" (anti-parasitic), "kandughna" (anti-itch), and "twachya" (beneficial for skin), making it a key ingredient in numerous herbal remedies.[2,3]

Traditionally, neem has been used in both external applications and internal treatments. Its leaves are often made into a paste to treat skin issues like acne, wounds, and eczema. Though its bitterness is off-putting to some, neem leaf consumption is traditionally believed to cleanse the blood, stabilize blood sugar levels, and strengthen the immune system. Decoctions made from neem leaves have been used for wound washing, while neem twigs, known as *datun*, have served as natural toothbrushes due to their antibacterial qualities. This oral hygiene practice continues in many rural areas and is increasingly recognized as an eco-friendly alternative to modern dental products.[4] Neem oil, derived from its seeds, is another crucial aspect of traditional neem usage. This oil, rich in fatty acids and therapeutic compounds, is applied to the scalp to combat dandruff and lice, used topically to treat fungal infections, and even incorporated into traditional agriculture as a natural pesticide. Its strong antifungal and insect-repellent properties have made it a staple among farmers for safeguarding crops, protecting stored grains, and deterring pests—without relying on synthetic chemicals.[5,6]

Neem's cultural and ritual uses are equally significant. During springtime festivals like Ugadi and Gudi Padwa, neem leaves are consumed as a symbolic act of accepting both the bitter and sweet aspects of life. In rural homes, neem branches are hung at entrances to fend off evil influences and infectious diseases. Women have traditionally used neem-infused water for bathing during postpartum recovery and seasonal detoxification, underscoring neem's long-standing association with purification and protection.[6,7] Despite technological advances in modern medicine and farming, traditional neem practices continue to thrive. In fact, scientific research is only beginning to confirm what traditional wisdom has long known. Modern studies support neem's antibacterial, antiviral, antifungal, and anti-inflammatory qualities—shedding light on why it has earned such enduring

respect. As interest in natural healing and eco-friendly practices grows worldwide, neem proves to be not just an ancient remedy, but a powerful ally in addressing today's health and environmental needs.[4]

Ultimately, the traditional use of neem exemplifies the strength and sophistication of indigenous knowledge systems. It represents a comprehensive approach to health and harmony, where the lines between medicine, culture, and nature blend seamlessly. The chapters ahead will delve deeper into the specific roles of neem—from treating skin ailments to sustainable farming—illuminating its continued relevance in our modern world.

Therapeutic Marvels of Neem

Antibacterial Activity

The study explored the antibacterial potential of various phytochemicals extracted from neem leaves to substantiate its traditional medicinal applications. Researchers isolated specific bioactive compounds, including alkaloids, steroids, tannins, glycosides, flavonoids, and saponins. Both these individual components and the full neem extract were tested at concentrations of 50 mg/ml and 75 mg/ml on three bacterial species—*Staphylococcus aureus*, *Corynebacterium bovi*, and *Escherichia coli*—employing the disc diffusion assay. The diameter of the inhibition zones was used to assess antibacterial potential. Outcomes showed that the complete neem extract exhibited stronger antibacterial effects than any of the isolated compounds. Additionally, the 75 mg/ml concentration consistently showed greater inhibitory action than the 50 mg/ml dose. These outcomes support the traditional use of neem as a natural antimicrobial agent and suggest that the combined effect of its phytochemicals plays a crucial role in its overall antibacterial effectiveness.[8] The study examined the inhibitory effects against microbes of neem leaf and bark extracts on Staphylococcus aureus and Escherichia coli. The results revealed that fresh plant extracts had greater antibacterial activity than dried extracts, and ethanol extracts were more effective than aqueous ones. Among the tested bacteria, only Staphylococcus aureus was sensitive to the neem extracts, while E. coli remained unaffected. Additionally, the study found that the antimicrobial efficiency increased with extract concentration, as reflected by the enlargement of inhibition zones.[9]

The study found that the leaf extract of neem possesses notable antibacterial properties against several human pathogenic bacteria, comprising *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Staphylococcus aureus,*

and *Enterococcus faecalis*. The ethanolic extract was active at all tested concentrations, with the greatest inhibitory effect recorded against *Proteus mirabilis* at 6.25 mg/ml, where it proved more effective than erythromycin. The extract also demonstrated significant antibacterial activity against *Enterococcus faecalis* at 12.5 mg/ml, showing statistically meaningful differences when compared with conventional antibiotics such as ciprofloxacin, erythromycin, ceftriaxone, and gentamicin. These outcomes recommend that neem leaf extract has a strong and broad-spectrum antimicrobial capacity, potentially matching or surpassing the effectiveness of some widely used synthetic antibiotics.[10] The study found that silver nanoparticles synthesized using neem leaf extract effectively sterilized cotton fabric. The nanoparticles were categorised by UV-visible spectroscopy and transmission electron microscopy. Three methods were used to embed them into cotton discs: centrifugation with liquid broth, in-situ coating, and coating with dried nanoparticles. When tested against *E. coli* using the disk diffusion method, the treated cotton disks showed strong antibacterial activity. Additionally, the antibacterial properties remained effective even after repeated washes, demonstrating the potential of these neem-synthesized silver nanoparticles for use in antimicrobial dressings.[11]

Antifungal Activity

The study examined the antifungal properties of water-based, ethyl alcohol, and ethyl acetate neem leaf extracts against several human-infecting microorganisms, including *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Candida albicans*, and *Microsporum gypseum*. All extracts inhibited pathogen growth, with higher concentrations leading to greater inhibition. Among the tested extracts, the 20% ethyl acetate extract showed the highest antifungal efficacy. HPLC analysis identified nimonol as the primary effective constituent in the ethyl acetate extract, which was further confirmed by NMR spectroscopy. However, when nimonol was isolated, the antifungal activity of the 20% ethyl acetate extract decreased, and the purified nimonol itself was not associated with any impact on fungal growth in the microorganisms.[12] The research analyzed the antifungal activity of neem leaf extract against three fungal pathogens: *Aspergillus flavus, Alternaria solani*, and *Cladosporium. Ethanolic* and methanolic extracts were formulated at 25%, 50%, 75%, and 100% concentrations, and their antifungal effects were tested via the disc diffusion method. Ketoconazole was employed as a reference to match the antifungal potency and toxicity of the neem leaf extract.[13]

The investigation determined the antifungal effectiveness of neem leaf ethanol extract (NLE) against *Aspergillus flavus* using disc diffusion and macrodilution methods. Significant differences in inhibition zone sizes were found between the A. flavus samples treated with NLE (1 g/dL) and the control group treated with ketoconazole (0.1 g/dL), with a p-value of 0.034. The minimal inhibitory concentration of NLE against *A. flavus* was 0.5 g/dL. These results suggest that neem leaf ethanol extract exhibits antifungal properties against *A. flavus* in vitro.[14] The study examined the antimicrobial effectiveness of neem leaf extract, 3% sodium hypochlorite (NaOCl), and 2% chlorhexidine (CHX) against *Candida albicans*. Neem extract was prepared with absolute ethanol and its activity was tested using the Agar Diffusion method. The results revealed a substantial alteration in inhibition zones among 3% NaOCl and neem extract when compared to 2% CHX ($p<0.05$). However, no significant difference was found between neem extract and 3% NaOCl. The results indicated that neem extract is on par with 3% NaOCl against *C. albicans* and more effective than 2% CHX.[15]

Blood Sugar Lowering Effect

The study explored the antidiabetic effects of 70% alcoholic neem root bark extract (NRE) in Wistar albino rats. The glucose tolerance test showed that NRE at 800 mg/kg led to a substantial decline in blood sugar levels, though it was inferior in efficacy to the standard drug glibenclamide. In diabetic rats induced by alloxan, glibenclamide significantly lowered blood sugar levels, and NRE at the 800 mg/kg dose showed similar results. The study concluded that neem root bark possesses both antihyperglycemic and hypoglycemic effects, but its effectiveness is lower than that of glibenclamide.[16] The study investigated the hypoglycemic effects of neem in rats with diabetes. A single 250 mg/kg dose of neem extract resulted in reductions in glucose, cholesterol, triglycerides, urea, creatinine, and lipids. In a subsequent study involving multiple doses over 15 days, neem extract continued to lower glucose, creatinine, urea, triglycerides, and lipids. The glucose tolerance test exposed substantial reductions in glucose levels in diabetic rats treated with neem extract related to the normal animal. These outcomes show that neem may serve as a promising alternative for managing diabetes by dropping raised glycemic levels, suggesting the need for further research into its potential use as an oral glucose-lowering drug.[17]

The study investigated the impacts of neem kernel powder (NP) and glibenclamide, both separately and in conjunction, administered to alloxan-induced diabetic rabbits over a 30-day duration. The findings indicated that NP alone and the concurrent use of NP and

glibenclamide effectively lowered serum lipid levels, blood glucose, and the activity of various serum enzymes (alkaline phosphatase, acid phosphatase, lactate dehydrogenase, glucose 6-phosphatase, and HMG CoA reductase) in the liver and intestines. Additionally, all treatments led to an upsurge in liver hexokinase activity. The combination of NP and glibenclamide yielded more substantial outcomes related to NP alone. The study concluded that NP demonstrates significant blood sugar lowering and hypolipidemic properties in alloxan- persuaded diabetic rabbits.[18]

Inflammation Lowering Effect

The study evaluated the inflammation lowering effects of neem seed oil (NSO) in albino rats with experimental paw edema persuaded by carrageenan. It was found that NSO at a 0.25 ml/kg dose had no significant effect on inflammation, but higher doses exhibited enhanced suppression of paw edema, with the highest inhibition of 53.14% observed at a 2 ml/kg dose after 4 hours. Aspirin showed the most significant anti-inflammatory effect. The study concluded that NSO has significant anti-inflammatory properties, with effectiveness increasing at higher doses.[19] The study explored the anti-inflammatory effects of neem extract via the NF-κB signaling pathway, which is involved in cancer, inflammation, and apoptosis. The outcomes showed that NF-κB activity was downregulated by methanolic neem leaf extract in human leukemia cells, both with and without TNF-α stimulation. Neem extract also decreased cell viability, prevented TNF-α-persuaded deprivation of IκB, and inhibited the nuclear translocation of NF-κB p50/p65. Furthermore, it suppressed IKK activity and induced apoptotic cell death, as observed through nuclear fragmentation and flow cytometry. The study concluded that neem extract shows a significant role in modifying pro-inflammatory signaling and apoptosis, offering deeper insights into the mechanisms triggered by neem.[20]

The study explored the anti-inflammatory effects of nimbidin, a compound from the seeds of neem, and compared it with phenylbutazone and prednisolone (a steroid) across various inflammation models. Nimbidin significantly abridged acute paw edema in rats caused by carrageenan and kaolin, eased formalin-persuaded arthritis in the ankle joint, and reduced fluid exudation in croton oil-persuaded granulomas. In the initial phase of the inflammatory response, nimbidin (40 mg/kg) exhibited stronger activity than phenylbutazone (100 mg/kg). The study concluded that nimbidin exerts therapeutic effects in both acute and chronic inflammatory conditions, making it a potential general anti-inflammatory agent.[21] The study examined the mitigating effects of neem leaf extract

(NLE) against pulmonary inflammation triggered by cigarette smoke and lipopolysaccharide. The findings revealed that NLE ominously reduced recruitment of inflammatory cells—namely neutrophils and macrophages—into the bronchoalveolar lavage fluid. A decline was observed in ROS formation, neutrophil elastase activity, and secretion of cytokines like TNF-α and IL-6. NLE also downregulated MCP-1 and iNOS expression in the lungs. The extract also attenuated the activation of ERK and JNK pathways and blocked the phosphorylation of NF-κB and IκB. These outcomes suggest that NLE is likely to have therapeutic capability for treating chronic obstructive pulmonary disease.[22]

Antiviral Activity

The study discovered that a water based extract from neem bark is an effective inhibitor of cellular uptake of herpes simplex virus type 1 (HSV-1. At concentrations of 50 to 100 μg/ml, NBE prevented HSV-1 entry into cells. The effect was seen when the extract was incubated with the virus prior to exposure to the target cells, indicating a direct action against HSV-1. Additionally, NBE-treated virions were unable to bind to cells, suggesting that NBE interferes with the viral attachment process. NBE also repressed HSV-1 glycoprotein-facilitated cell merging and the generation of polykaryocytes. These findings suggest that NBE has the potential to be advanced as a new antiherpetic treatment.[23] The investigation looked into the in-vitro and in-ovo antiviral effects of neem bark extract on Newcastle disease virus.. Various concentrations of the extract were tested using spot assays, micro-hemagglutination tests, and in-ovo evaluation through injections into 11-day-old embryonated eggs. The results showed that both the stock solution and 1:2 dilution had antiviral activity, but they also caused cytotoxicity. Higher dilutions (1:8) did not display significant antiviral effects. A similar trend was observed in the in-ovo tests, where higher dilutions failed to show significant antiviral activity. In summary, no considerable differences in antiviral effects were found between the different concentrations of neem bark extract, with exposure duration being a vital factor influencing cytotoxicity.[24]

The report explored the antiviral effects of four fractions derived from alcohol extracts of neem seed kernel on the duck plague virus in vitro. The findings revealed that fractions 1 to 3 showed no activity. In contrast, fraction 4 demonstrated significant antiviral effects, with a median inhibitory concentration (IC50) of 10.9 μg/mL. It suppressed virus protein expression in infected cells and notably decreased plaque formation compared to the negative control ($P < 0.01$). Fraction 4 also enhanced the

survival of infected cells, minimized cytopathic effects, and abridged the level of virus protein in the cells. Its antiviral action was effective throughout the viral infection process.[25] The study included 35 hepatitis C patients who had not responded to conventional treatments. These patients were administered neem leaf extract orally, and HCV seropositivity was assessed before and after the treatment using quantitative HCV RNA analysis via Polymerase Chain Reaction. Additionally, ALT, AST, and total protein levels were measured using standard methods. The findings revealed high HCV seropositivity in patients prior to treatment. After taking the neem extract, there was a notable reduction in both HCV seropositivity and enzyme levels (ALT and AST). However, serum protein levels remained unchanged. The study concluded that neem leaf extract may be a promising treatment for hepatitis C due to its ability to inhibit the protease involved in viral replication.[26]

The study investigated the virus-inhibiting properties of NBE against various bovine viruses, including Bovine Corona Virus (BCoV), Bovine Herpes Virus-1 (BHV-1), Bovine Parainfluenza Virus-3 (BPIV-3), and Bovine Entero Virus (BEV) in vitro. The aim was to assess whether NBE affected the viral entry or replication phases. The WST-1 test indicated that NBE concentrations under 0.87 mg/mL were not toxic to MDBK cells. Although NBE did not notably influence the attachment of BPIV-3 and BEV to host cells, it led to a 100-fold reduction in the TCID50 of BCoV and completely blocked BHV-1 replication. The study concluded that NBE exhibited strong antiviral activity against BCoV and BHV-1, suggesting the need for further in vivo testing and additional virus screenings to support future antiviral drug development.[27] The study explored the antiviral properties of crude water based neem leaf extract and the pure neem compound Azadirachtin against Dengue virus type-2 in both in vitro and in vivo settings. In vitro, the aqueous neem leaf extract inhibited the virus in a dose-dependent manner in C6/36 cells, completely blocking 100–10,000 TCID50 of the virus at its maximum non-toxic concentration of 1.897 mg/mL, with no cytopathic effects observed. In vivo, neem leaf extract at non-toxic concentrations of 120–30 mg/mL effectively prevented virus replication in suckling mice, as shown by the absence of clinical symptoms and the lack of a virus-specific 511 bp amplicon in RT-PCR. In contrast, Azadirachtin, the pure neem compound, did not exhibit a marked any inhibitory effect on Dengue virus type-2 replication in either the in vitro or in vivo models.[28]

Conclusion:

This chapter confirms the multifaceted therapeutic potential of neem through its significant antibacterial, antifungal, antiviral, inflammation lowering, and antidiabetic properties. Neem leaf extracts demonstrated strong antimicrobial activity, particularly in ethanolic forms and at higher concentrations, with full extracts often outperforming isolated compounds. Notably, neem exhibited wide-range antifungal efficacy against various pathogenic fungi and was equally potent as standard antifungal agents. Its anti-inflammatory effects were evident through reductions in induced edema and cytokine levels in animal models. Neem also exhibited promising broad-spectrum antiviral activity, encompassing viruses like HSV-1, NDV, BCoV, and dengue virus. Furthermore, its hypoglycemic action suggests neem's potential in diabetes management. These findings collectively reinforce the traditional medicinal use of neem and highlight its promise as a natural, multifunctional therapeutic agent.

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THERAPEUTIC POTENTIAL OF CATECHIN: A COMPREHENSIVE REVIEW OF ITS HEALTH BENEFITS

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Abstract:

Growing health concerns, including lifestyle-related disorders like cancer, dementia, diabetes, and inflammatory diseases, have led to a rising interest in the health benefits of foods. Flavonoids are phytochemical compounds found in ariel parts of medicinal plants, with promising applications in medicinal chemistry. They offer numerous health benefits. These affordable medicinal compounds exhibit significant biological activities, and their efficacy has been demonstrated in treating various diseases. Catechin is a highly valued phytochemical derived from the abundant riches of nature. It is one of the most promising naturally derived compounds because of its intrinsic pharmacological properties. It is proved to be potential therapeutic agent against oxidative and inflammatory associated disorders. Additionally, it has a good potential to suppress the growth progressive disorders such as dementia, diabetes as well as cancer. The management of wounds through utilization of catechin is also known traditionally and scientifically as well. This review highlights recent research on the pharmacological effects of catechins, underscoring their potential as safe, affordable, and versatile agents in medical and healthcare applications.

Keywords: Anti-Cancer, Anti-Alzheimer's, Antioxidant, Anti-Inflammatory, Catechin, Phytochemicals.

Introduction:

For millennia, indigenous cultures around the globe have turned to traditional herbal medicine to treat various ailments. Over time, plants have proven to be an invaluable source of affordable natural compounds, especially secondary metabolites. These compounds, renowned for their intricate structural complexity, are often difficult or impossible to synthesize artificially. They exhibit diverse biological activities, including antitumor effects. Secondary metabolites are primarily small organic molecules produced by an organism that are not essential for its growth or reproduction. [1, 2]

Herbal Medicines:

Herbal medicines are plant-based substances naturally sourced and utilized in local or regional healing traditions to treat various illnesses. These remedies are intricate blends of organic compounds that can be derived from any raw or processed part of a plant. The main understanding of the medicinal uses of plant-based natural products comes from centuries of human experimentation, often involving trial and error through taste tests or accidental poisonings, as people sought food and remedies for various illnesses. Herbal medicine is deeply rooted in cultures across the globe. While various traditional medicine systems differ in their philosophies and practices based on geographic location, they all share a common emphasis on a holistic approach to life. The use of healing herbs enables individuals to flourish by addressing their overall well-being rather than focusing solely on a specific ailment, which often stems from an imbalance between the mind, body, and environment. Rooted in ancient cultures, herbal medicine utilizes plants for treating illnesses and promoting overall health and well-being. Also referred to as herbalism or botanical medicine, this system relies on the use of plants or their extracts, which can be consumed or applied topically. Throughout history, herbal medicine has been utilized by diverse cultures worldwide to treat various conditions, such as malaria, warts, bowel disorders, heart issues, and chronic pain. Much of this knowledge stems from pharmacists and doctors building upon traditional folk practices. [1,3,4]

While plants are widely recognized for providing nutrition and shelter, their role as a source of medicine is often undervalued. Human societies have relied on plants for food, shelter, and medicinal purposes for nearly the same duration throughout history. Nature offers an extensive array of therapeutic compounds, many of which have been incorporated into modern medicine. In developing nations, traditional medicine remains one of the most accessible forms of treatment. It is estimated that around 80% of the global population in some regions relies on traditional medicine to address their primary healthcare needs. Many pharmacologically active compounds and innovative drugs have been developed from plants, as evidenced by the widespread use of medications that can be traced back to plant-derived origins. In recent years, millions of dollars have been invested in the search for promising medicinal herbs. Although these significant research efforts in traditional herbal medicine remain relatively small compared to the overall pharmaceutical industry, they indicate a growing shift among researchers away from conventional drug development toward alternative and natural treatment options. [5,6]

Phytochemicals:

Phytochemicals include a diverse group of plant-derived compounds that are believed to be important in preventing various diseases, commonly associated with diets rich in fruits and vegetables. Phytochemicals are non-essential compounds, meaning they are not necessary for the survival of plants. However, thousands of these phytochemicals play important roles in the treatment of various diseases. These compounds seem to act both independently and synergistically, potentially alongside vitamins and other nutrients, to prevent, halt, or slow the development of diseases. The vast variety of plants worldwide and their by products such as fruits and vegetables remains largely unexplored, with over 85% of their chemical compositions and contents still undiscovered. Therefore, it is important to focus on consuming whole diets rather than relying solely on supplements. Phytochemicals are frequently found in high concentrations within the pigments of fruits and vegetables, underscoring the advantages of incorporating a variety of brightly coloured produce into the diet. The use and significance of natural substances for treating various illnesses have been recognized for centuries. While natural products have many medical applications, ongoing research in this field remains essential. [7, 8]

Epidemiological research indicates that eating fruits and vegetables high in polyphenols may help prevent and potentially reverse the negative effects of aging on brain communication and behaviour. For example, phytochemicals especially phenolics present in fruits and vegetable are the main bioactive compounds known for providing various health benefits. The health benefits of polyphenolic compounds are primarily attributed to their antioxidant properties and their ability to combat various chronic ailments. Flavonoids are a class of compounds defined by two phenyl rings linked by phenolic hydroxyl groups, connected via a central three-carbon framework. Flavonoids, commonly found and generally low in toxicity, can be safely incorporated into the diet and show significant effectiveness in combating inflammation and oxidative processes. The medicinal value and biological activities of different plant parts are attributed to the presence of bioactive phytochemical compounds within them. To date, thousands of flavonoid compounds have been isolated and identified. These compounds are naturally produced via the phenylpropanoid pathway, with their bioactivity influenced by how they are absorbed and their bioavailability. Flavonoids have been utilized in natural dyes, cosmetics and skincare products, as well as in anti-wrinkle treatments. The most significant use of these polyphenols is in medicine. Flavonoids have been widely applied as treatments for cancer,

antimicrobial and antiviral purposes, among many other serious diseases. The majority of these flavonoids are widely recognized for their therapeutic properties. Flavonoids help reduce symptoms of various diseases by acting through several mechanisms, such as regulating the immune system and reducing inflammation. Examples of flavonoids include anthocyanin, kaempferol, fisetin, and catechin. [8,9]

Catechin:

Catechins offer numerous benefits, such as preventing or reducing skin damage. They are key components of tea leaves, possessing strong antioxidant properties and notable physiological activities. As members of the polyphenol compound group, catechins are found in a variety of sources**.** Catechins belong to the polyphenol compound group commonly found in many medicinal plants. The primary sources of catechins are *Camellia sinensis* and *C. assumica*. Green tea, a notable source, comprises more than 79% water and polyphenol compounds. Catechins make up over 70% of the polyphenol compounds found in tea leaves. Catechins, a type of polyphenol within the flavonoid family, exist in various isoforms and are found in numerous fruits and plant leaves. While catechins are not essential for human nutrition, they contribute to disease prevention and overall health improvement. Catechins consist of two stereoisomers, (+)-catechin and its enantiomer, and include compounds such as epigallocatechin gallate (EGCG) & epigallocatechin (EGC) as well as epicatechin gallate (ECG). The structure of catechins consists of two or more aromatic rings: an A ring resembling resorcinol and a B ring similar to catechol, each with at least one hydroxyl group. These rings are connected by a carbon bridge and a dihydropyran heterocycle (C ring) that includes a hydroxyl group. In the figure 1, structure of catechin is presented. [10,11]

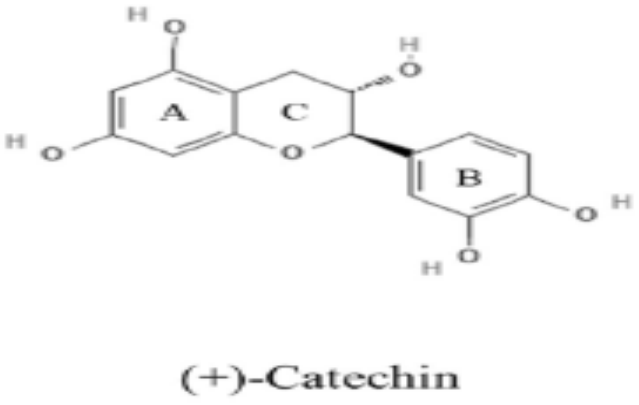

Figure 1: Catechin structure. [10]

Pharmacological activities of Catechin:

1. **Antioxidant Activity:** Catechins are extensively researched compounds known for their proven antioxidant properties. Efforts have been made to enhance their stability and improve their absorption rate in the human body. Recent research has concentrated on optimizing the effectiveness of antioxidants. Catechins are potent antioxidants, primarily due to their ability to neutralize free radicals and reduce oxidative stress in the body. Their antioxidant activity stems from the presence of hydroxyl groups on their aromatic rings, which allow them to donate hydrogen atoms or electrons to stabilize reactive oxygen species (ROS) and free radicals. Their antioxidant effect arises from hydroxyl groups that enable them to donate electrons or hydrogen atoms, helping to stabilize reactive oxygen species. They also bind metal ions that facilitate radical generation, suppress enzymes linked to oxidative reactions, safeguard cell membranes from harm, and enhance the body's natural antioxidant systems. [11,12]
2. **Anti-Alzheimer's Activity:** Dementia is a broad term used to describe a variety of progressive conditions that impair memory, thinking, and behaviour, significantly affecting a person's ability to carry out everyday household activities. Alzheimer's disease (AD) is the most common type of dementia, accounting for more than sixty two percent of all cases. Presently, it affects over 4.5 million individuals, with estimates indicating that this figure could surpass 12.5 million by the year 2050. The most common type of dementia and a rapidly growing global health crisis, often referred to as senile dementia, is an age-related neurodegenerative disorder and the most widespread form of dementia. As the global population grows older, the number of individuals affected by Alzheimer's disease continues to rise. Growing evidence suggests that excessive production of Reactive Oxygen Species plays a major role in the development of this condition. It is characterized by the accumulation of beta-amyloid and tau proteins in the brain, which interferes with normal cognitive functions. This typically presents as loss of memory, swinging of mood, impaired thinking impairment, difficulty with judgment, and behavioural change. In neurological disorders, an imbalance between the generation and removal of Aβ results in the development of senile plaques. Previous studies have shown that catechins are highly effective in inhibiting the formation of beta-amyloid plaques and tau proteins. Moreover, catechins help shield neurons from oxidative

stress and inflammation which are the key factors in cognitive decline and therefore potentially slowing the progression of the disease and enhancing brain function.[13, 14]

3. **Anti-Diabetic Activity:** Diabetes mellitus (DM) is a long-term metabolic disorder characterized by persistently elevated blood glucose levels, known as hyperglycemia, which over time leads to serious and irreversible damage to multiple organs. The number of people worldwide with DM is expected to exceed 635million by 2030 and reach more than 778 million by the year 2045. Diabetes mellitus causes the body to either produce insufficient insulin or respond poorly to it, leading to an inability to regulate proper blood sugar levels. This imbalance results in various negative effects and complications associated with the disease. Diabetes mellitus causes hyperglycemia, which is a key factor in the development of oxidative stress. It plays an active role in hindering insulin action, impairing insulin function, and reducing its secretion. There is substantial evidence indicating that oxidative stress plays a significant role in the connection between stress and complications related to diabetes mellitus. Additionally, antioxidants play an important role in managing complications in diabetic patients. Oxidative stress occurs in the body due to the production of free radicals, which causes cellular damage and the destruction of pancreatic beta cells. Medicinal plants contain various chemical compounds, primarily flavonoids & phenols as well as catechins. Catechins demonstrate significant antidiabetic effects through various mechanisms. They enhance insulin sensitivity and promote glucose uptake by cells, supporting improved blood sugar control. Catechins also decrease oxidative stress and inflammation, factors associated with diabetes complications. Furthermore, they may block enzymes responsible for carbohydrate digestion, resulting in a slower release of glucose into the blood. [15, 16]
4. **Anti-Cancer Activity:** Cancer has impacted multicellular organisms for more than two hundred million years, and the ancestors of modern species have experienced cancer for hundreds of thousands of years. Cancer ranks as the second most common cause of death among both children and adults. Each type of cancer has a distinct age range and pattern of occurrence, with its prevalence varying across different genders. Cancers are the result of malignancies that develop through gradual processes characterized by the accumulation of successive mutations over

time. In the year 2018, there were approximately more than eighteen million new cancer cases and more than 9.5 million cancer-associated deaths worldwide. By 2025, the number of new cases is projected to rise to above twenty million globally. Despite significant progress and sustained efforts in cancer prevention and treatment over the years, it continues to be a leading cause of morbidity worldwide. Although significant progress has been achieved in identifying drug targets and therapeutic molecules for treating cancer cells, the resistance of cancer cells to existing anticancer drugs remains a major challenge in cancer therapy. Given that surgical and use of radiation methods can sometimes cause adverse side effects and lead to drug resistance, there is an increasing interest in utilizing natural products as therapeutic agents. Furthermore, cancer stem cells, limited bioavailability, and the side effects of anticancer drugs are significant obstacles in advancing cancer treatments. These challenges drive the need to explore innovative alternative treatment strategies to address the limitations of current cancer therapies. Natural sources with chemo preventive properties against the carcinogenesis process have gained greater recognition and broader use in cancer chemotherapy. Epidemiological studies have indicated that consuming green tea is linked to a modest reduction in cancer risk. Molecules derived from various natural bioresources, including plants and animals, exhibit potential for cancer therapy. Among natural products, Camptothecin as well as Taxol originally isolated from the bark of *Camptotheca acuminata* and numerous fungal species. Both have been well-established as effective against multiple types of cancer and are currently undergoing human clinical trials. Additionally, several other plant-derived compounds demonstrate anticancer properties, such as hematoxylin, which is extracted from the heartwood of *Haematoxylon campechianum.* Likewise, many natural compounds with anticancer potential have been identified in marine sources. Notably, the Caribbean sponge was the first marine organism to provide anticancer compounds, such as arabino nucleosides and cytosine arabinose. Catechin is believed to impact the carcinogenesis process, including tumor initiation, proliferation, and growth. This understanding comes from studies conducted on animal models and cancer cell lines. The available literature showed that catechin is having anticancer potential as it is effective in arresting cell cycle,

fragmentation of DNA, condensation of chromatins, increment in apoptosis process, inhibiting process of formation of new blood vessels. [17, 18]

5. **Anti-Inflammatory Activity**: Inflammation is a protective response that defends organs against external injury and infection. Inflammation can be categorized as either acute or chronic, with both types involving a similar fundamental mechanism. When a trigger is detected, the inflammatory cascade begins at the cell surface receptors, leading to the production of inflammatory markers and the activation of inflammatory cells. Initially, the process ends once the trigger is removed or resolved; however, in the other case, the body is unable to repair the damage or eliminate the cause. In response to changes causing tissue damage, the immune system elevates the activity of immune cells and various inflammatory mediators. When the inflammatory response is triggered by excessive stimulation, it can become chronic, which contributes to the development and advancement of diseases in different tissues. Acute inflammation is triggered by an immediate reaction to a microbial or viral infection, whereas chronic inflammation arises from a slow and prolonged response. These inflammatory responses spread throughout the body via the blood and lymphatic vessels, worsening the onset and symptoms of various diseases. An elevated chronic and systemic inflammatory response is recognized as a key characteristic of diseases including diabetes, cancer cardiotoxicity as well as respiratory & metabolic disorders. Catechins function by blocking important inflammatory pathways and decreasing the production of pro-inflammatory substances like cytokines and enzymes. By regulating the immune response, catechins help reduce inflammation at the cellular level, protecting tissues from damage and aiding in the prevention and treatment of several long-lasting inflammatory ailments. Research conducted on animal models and cell cultures has shown that catechins can effectively inhibit both acute as well as chronic inflammation, emphasizing their potential as anti-inflammatory agent. [10, 19]
6. **Wound Healing Activity:** Skin wounds pose a major global health issue, frequently involving high costs and limited treatment success. They are characterized by a disruption in the cellular and structural integrity of the skin's tissue layers. Wounds impair the skin's protective function by disrupting the continuity of the epithelial tissue, potentially affecting other tissues as well. Chronic wounds represent a major healthcare challenge and have a significant impact on disease burden. They affect

patients' quality of life, increase treatment costs, and continue to pose a significant clinical challenge worldwide. The epidemiology of chronic wounds in India estimates their prevalence to be 4.5 cases per 1,000 people. Historically, natural products and substances derived from nature have been used for wound healing because they possess antibacterial, antioxidant, activity against inflammatory reactions, bacteria & fungi as well as angiogenic, and promotion of cellular regeneration properties. Catechins assist in lowering oxidative stress and inflammation at the wound site, thereby facilitating quicker tissue repair. It also promotes the synthesis of collagen and other vital elements necessary for regeneration of tissues, strengthening the structural integrity of skin. Research has shown that catechins expedite the healing process by promoting cell proliferation and angiogenic reactions, establishing their value as effective agents in wound care and management. [20, 21]

Conclusion:

Catechins a flavonoid class of phytochemical, found in plants like tea leaves, beans & berry fruits and they exhibit a range of pharmacological activities. Since ancient times, catechin has been extensively utilized as a therapeutic agent in clinical practices. Catechin also demonstrates significant therapeutic potential and may contribute to the treatment and prevention of various conditions, including management of wounds, inflammatory & autoimmune disorders as well as cancer. It is also proved beneficial in memory dysfunction ailments such as Alzheimer's. Catechins are reported to have health-enhancing properties and beneficial effects in combating numerous sicknesses. This review presents compelling evidence that catechins are effective in mitigating inflammation-related diseases.

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HUNTINGTON'S DISEASE: CLINICAL INSIGHTS AND EMERGING THERAPIES

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Abstract:

Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by the progressive deterioration of motor function, cognition, and behaviour, ultimately leading to significant disability and early mortality. A mutant huntingtin protein (mHTT) is produced when the CAG trinucleotide repeat in the HTT gene on chromosome 4 expands, causing the disease. Symptoms typically manifest in midlife, around the age of 40, and include chorea, psychiatric disturbances, and cognitive decline, with a disease progression spanning 15 to 20 years. The clinical presentation of HD is marked by a combination of motor, cognitive, and psychiatric symptoms, with early signs often including clumsiness and mood changes. As the disease advances, patients may experience severe motor impairments, including dystonia and parkinsonism, alongside significant cognitive decline leading to dementia. Genetic testing is essential for diagnosis, particularly in individuals with a family history of the disease, while MRI can reveal characteristic brain atrophy. Management of HD involves symptomatic treatment to alleviate motor and psychiatric symptoms, with pharmacological options including dopamine-depleting agents and antidepressants. Non-pharmacological strategies, such as physical and occupational therapy, play a crucial role in maintaining quality of life. Recent advances in research, including gene therapy and neuroprotective agents, offer hope for future disease-modifying treatments. Understanding the complex pathophysiology of HD is vital for developing effective therapeutic strategies that could improve the lives of those affected by this devastating condition.

Keywords: Huntington's Disease, Chorea, Neurodegeneration, Genetic Inheritance, Recent Advances in Huntington's

Introduction:

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder that leads to a progressively worsening decline in motor function, behaviour, and cognition,

ultimately resulting in significant disability and premature death [1]. The first known description of a patient with what we now call Huntington's chorea was made by Waters in 1842. However, it was George Huntington who provided a detailed account of the disease in 1872, highlighting its hereditary nature, associated psychiatric and cognitive symptoms, and its typical manifestation between the ages of 30 and 40 [2]. Huntington famously noted the relentless progression of the disease, stating, "Once it begins, it clings to the bitter end" [3].

The onset of HD typically occurs around 40 years of age, with a disease course that spans 15 to 20 years, eventually leading to death [1]. Early symptoms often include clumsiness and chorea, which gradually worsen into more severe motor issues like parkinsonism and dystonia, ultimately rendering the patient bed-bound [1]. Behavioural symptoms, such as depression, irritability, and repetitive behaviours, usually precede motor changes and progressively worsen, leading to generalized dementia [1].

Huntington's disease is clinically marked by progressive, unintentional chorea, subcortical cognitive impairment, behavioural changes, and depression, with symptoms generally starting in midlife [4]. The disease is caused by the repetition of the cytosine, adenine, and guanine (CAG) trinucleotide on chromosome 4p16.3 in the huntingtin gene, leading to the production of a mutant huntingtin protein (mHTT) [5]. Although a family history is a significant factor in HD cases, 1-3% of patients have no known family history of the disease [5]. The mutation is passed on in an autosomal dominant manner, with a 50% chance of inheritance from an affected parent [5].

The renaming of the condition from Huntington's chorea to Huntington's disease (HD) in the 1980s reflected a broader understanding of its extensive non-motor symptoms [2]. In 1983, a significant milestone was reached with the identification of a genetic linkage on chromosome 4, and by 1993, the gene responsible for HD was found [2]. This discovery led to a surge in interest in HD and other neurogenetic disorders, opening new research avenues and providing the first real rationale for treating this devastating disease [2]. While many symptomatic treatments are now available, there remains a critical need for more effective disease-modifying therapies [2].

The global prevalence of Huntington's disease is approximately 2.7 per 100,000 people, with those carrying a higher number of CAG repeats showing more severe disease symptoms [5]. Juvenile-onset HD, which constitutes about 5% of cases, is often mistaken for Parkinson's disease due to its similar symptoms. The first signs of juvenile-onset HD are

often learning difficulties at school, and many patients experience epileptic fits [5]. Notably, in 75% of juvenile-onset cases, the disease is transmitted by the father [5].

Epidemiology

The prevalence of Huntington's disease in Western populations varies between 10.6 and 13.7 cases per 100,000 individuals. Significant regional variations in HD prevalence were observed. Asians had an overall HD prevalence of 0.40/100,000, which is significantly lower than the 5.70/100,000 prevalence in Europe, North America, and Australia. In South Africa, where black populations had lower rates than white and mixed populations, Japan, Taiwan, and Hong Kong have significantly lower incidences of HD, with 1-2 cases per million. The variance in the occurrence of disease among different ethnic groups can be attributed to genetic polymorphisms in the HTT gene. Longer average CAG repeat lengths are found in high prevalence groups. For example, those of European ancestry typically fall between 18.4 and 18.5, while those with Asian ancestry typically fall between 16.9 and 17.45 [6][3].

Etiology:

Genetic Cause:

- Huntington's disease (HD) is an autosomal dominant condition caused on by the huntingtin (HTT) gene on chromosome 4p16.3 expanding its CAG trinucleotide repeat [2].
- A mutant huntingtin protein (mHTT) with an unusually extended polyglutamine tract is generated as a result of this mutation [2].

CAG Repeat Lengths and Disease Onset:

- Individuals with more than 39 CAG repeats are certain to develop HD, while those with 36-39 repeats may have reduced penetrance or a later onset of symptoms [3].
- The length of the CAG repeat and the age of onset are inversely correlated; the longer the repetition, the earlier the onset of symptoms [2].
- Juvenile Huntington's Disease (JHD) is associated with CAG repeat lengths exceeding 55, often manifesting before the age of 20 [2][7].

Anticipation Phenomenon:

- The anticipation phenomenon, where HD symptoms appear at an earlier age in successive generations, is commonly observed in the paternal line due to the greater variability in CAG repeat length during spermatogenesis [7].

Role of Huntingtin Protein:

- The normal huntingtin protein is involved in synaptic function and may have protective anti-apoptotic effects [3].
- The mutant huntingtin protein (mHTT) disrupts these functions, leading to neuronal degeneration [3][7].
- mHTT may cause both a gain of toxic function and a loss of normal huntingtin function, contributing to the pathogenesis of HD [3][7].

Pathophysiology

Huntington's Disease (HD) is a complex neurodegenerative disorder characterized by the degeneration of specific neuronal populations within the brain. The initial pathology involves the degeneration of medium spiny neurons in the putamen, caudate, and cerebral cortex, particularly affecting enkephalin-containing neurons in the basal ganglia, which results in chorea. Additionally, the loss of substance-P containing neurons leads to the development of dystonia and akinesia [7].

The progression of HD is marked by the accumulation of mutant huntingtin (mhtt) protein aggregates within neurons, which is a hallmark of the disease. These aggregates disrupt normal cellular functions, contributing to oxidative stress, excitotoxicity, and apoptosis, all of which drive the neurodegenerative process [8]. As the disease advances, the neuronal loss extends to the putamen and eventually to the cerebral cortex, causing widespread cognitive decline and behavioural changes such as depression and anxiety [8].

At the molecular level, the mutation in the HTT gene leads to the production of the mhtt protein, which interacts with various cellular pathways, causing excessive calcium influx and neuronal stress, ultimately leading to apoptosis [9]. Furthermore, the presence of mhtt protein disrupts axonal transport and synaptic function, exacerbating the neuronal degeneration observed in HD [7]. MRI studies have shown that striatal and cerebral white matter atrophy can be detected even before motor symptoms appear, indicating that neurodegenerative changes occur early in the disease process [9].

It is crucial to understand these interrelated pathophysiological mechanisms, such as neuronal loss, protein aggregation, and hereditary variables, in order to create targeted treatment plans that can hinder the progression of HD and enhance the quality of life for people who are impacted [8][9].

Clinical Manifestations

Cognitive decline and mental disorders are among the non-motor and motor signs of Huntington's disease (HD). The clinical onset of HD is defined by extrapyramidal motor signs and symptoms.

In Juvenile HD compared to motor characteristics, the neuropsychiatric load is significantly larger than in adult forms, making it the most debilitating symptoms. While childhood-onset starts before age 10, JHD starts before age 20. Both variations have the biggest CAG expansions. Its 5% mean incidence rate is likely understated because of the unusual appearance. Compared to adults, the clinical aspects are often distinct and significantly more variable. In addition to behavioral and neuropsychiatric abnormalities, learning impairments, and early and severe cognitive impairment, the main characteristics include dystonia and parkinsonian symptoms. Furthermore, childhood patients may exhibit developmental delay, spasticity, myoclonus, autism, cerebellar characteristics, and developmental delay, making an appropriate diagnosis extremely difficult [7].

In Prodromal HD, it is possible for subtle clinical characteristics to appear 10–15 years prior to motor symptoms. Since the creation of the Predict HD project, two variables—the number of CAG and the present age—have been used to determine the prodromal HD stage and the predicted years to motor diagnosis. Patients begin to show mild cognitive and motor abnormalities. There may be behavioral changes, including despair and indifference. Imaging alterations are seen [7][10].

To manifest HD there are noticeable impairments in motor function, cognition on quality of life is seen. Early in the illness, the majority of the motor symptoms are hyperkinetic and include chorea, or involuntary movements. These symptoms are progressive. These motions usually start off small-degree and distally before becoming more axial and amplitude-driven. Since motions are frequently a part of spontaneous, natural movements, they may initially seem like plain restlessness. While motor symptoms are hyperkinetic in the early stages, they tend to be hypokinetic with bradykinesia and dystonia in the later stages of the disease. Atypical eye movements and motor impersistence are additional characteristic indications. Aspiration is widespread and pneumonia is a common cause of mortality; dysphagia becomes a symptom with significant morbid effect late in the disease.

In most cases, cognitive impairment is present. Early on, there may be some restrictions on the executive function, leading to issues with planning, organizing, making

decisions, and multitasking. As these symptoms worsen over time, a more comprehensive picture of cognitive impairments becomes apparent, and dementia is ultimately diagnosed. Generally speaking, HD dementia is regarded as "subcortical," emphasizing the role played by the corticostriatal systems. One of the main distinctions between typical cortical dementia and HD dementia is that, in memory tests, people with HD dementia may recall objects more readily when cued, indicating that the disease may be caused by inefficient memory search rather than a memory deficit. Memory loss often happens later in HD [10][11].

Diagnosis

The diagnosis of Huntington's disease (HD) is primarily based on the combination of clinical symptoms and genetic testing. When a patient presents with motor symptoms, such as chorea, along with psychiatric or cognitive disturbances, and has a positive family history, HD is often suspected. The onset and progression of these symptoms can vary, leading to delays in diagnosis or even misdiagnosis in some cases [2].

Clinical Diagnosis

A thorough clinical evaluation is crucial for diagnosing HD, especially when the patient's parent has a confirmed case of the disease. This process includes a detailed personal and family history, with an emphasis on identifying motor, psychiatric, and cognitive changes. The combination of these three types of symptoms, in conjunction with a family history, is often sufficient for a clinical diagnosis [2].

Genetic Testing

Genetic testing is the gold standard for confirming a diagnosis of HD. The test typically involves analyzing the number of CAG repeats in the huntingtin gene on chromosome 4. Individuals with 26 or fewer repeats are not considered at risk for HD. However, those with 36 or more repeats are likely to develop the disease, with the risk increasing with the number of repeats [7]. Genetic counseling is recommended before testing, especially since a positive diagnosis can have significant implications for the patient's family [7].

Imaging and Other Diagnostic Tools

Magnetic resonance imaging (MRI) can also play a role in diagnosing HD, particularly in differentiating it from other neurodegenerative disorders. MRI can reveal striatal atrophy in the caudate nucleus, which is a characteristic finding in HD. However, these changes may be subtle or absent in the early stages of the disease [12]. Other

laboratory tests might be performed to rule out HD-like syndromes, such as McLeod syndrome or Wilson's disease, which can present with similar clinical features [12].

Prenatal Diagnosis

There is prenatal diagnostic available for couples who run the risk of passing on HD to their children. This can be accomplished via amniocentesis between the 15th and 17th week of pregnancy, or by chorionic villus sampling between the 10th and 12th week. These techniques make it possible to identify the fetus's CAG repeat expansion, giving vital information for family planning[7].

Management

Huntington's Disease (HD) leads to a wide array of physical, cognitive, and psychiatric symptoms, making management a complex and multifaceted challenge. Effective management of HD involves a combination of pharmacological interventions, non-pharmacological strategies, and emerging treatments aimed at modifying the disease course.

Pharmacological Management

Pharmacological treatment forms the cornerstone of managing Huntington's Disease, particularly in addressing the motor symptoms, psychiatric disturbances, and cognitive decline associated with the disorder.

1. **Symptomatic Treatment**: The primary aim of pharmacological treatment in HD is symptom management. The most prominent symptom, chorea, is often managed using dopamine-depleting agents such as tetrabenazine and deutetrabenazine. These medications are the only drugs currently approved specifically for treating chorea in HD patients. They function by inhibiting the vesicular monoamine transporter 2 (VMAT2), which decreases dopamine release in the brain. This reduction in dopamine is crucial for alleviating chorea, which is characterized by involuntary, erratic movements. The effectiveness of these drugs, however, is often balanced against their side effects, which may include depression and other psychiatric symptoms [1].

   In addition to dopamine-depleting agents, antipsychotic medications like haloperidol and olanzapine are commonly used to manage both motor and psychiatric symptoms in HD. Haloperidol, a typical antipsychotic, is effective in controlling chorea and aggression but comes with the risk of extrapyramidal side effects, which can exacerbate movement disorders. On the other hand, olanzapine,

an atypical antipsychotic, is preferred for its dual efficacy in managing psychiatric symptoms and chorea, with a more favourable side effect profile [2].

2. **Antidepressants**: Psychiatric symptoms such as depression, anxiety, and obsessive-compulsive behaviours are common in HD and significantly impact the quality of life. Selective serotonin reuptake inhibitors (SSRIs), like fluoxetine, are frequently prescribed to manage these symptoms. Serotonin is a neurotransmitter that helps elevate mood and lessen anxiety. SSRIs function by raising serotonin levels in the brain. These drugs do have some negative effects, though. For example, fluoxetine may cause drowsiness and weight gain, which could make managing HD more difficult, particularly for people who already have fatigue and decreased mobility [2].

   Furthermore, based on the patient's reaction to SSRIs and their tolerance for side effects, additional classes of antidepressants, such as tricyclic antidepressants (TCAs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), may be utilized. Each class of antidepressants offers different mechanisms of action, allowing for tailored treatment approaches based on the patient's specific symptom profile and needs.

3. **Neuroprotective Agents**: Although the current pharmacological strategies are primarily aimed at symptom management, there is ongoing research into neuroprotective agents that might slow the progression of HD. Compounds such as coenzyme Q10, creatine, and various antioxidants have shown potential in preclinical studies for their neuroprotective properties. These compounds are believed to reduce oxidative stress and mitochondrial dysfunction, which are key factors in the neuronal damage seen in HD. However, despite promising results in animal models, translating these findings into effective clinical therapies has been challenging. Large-scale clinical trials are needed to establish the efficacy and safety of these agents in HD patients [3].

   The exploration of neuroprotective agents is part of a broader effort to develop disease-modifying treatments that could alter the course of HD, rather than just alleviate symptoms. As research progresses, the hope is that these agents might be used in conjunction with symptomatic treatments to offer a more comprehensive approach to HD management.

Non-Pharmacological Management

Non-pharmacological approaches are essential components of a comprehensive HD management plan. These strategies focus on maintaining functionality, improving the quality of life, and addressing the social and psychological needs of both patients and their families.

1. **Physical Therapy**: In particular, physical treatment is essential for preserving mobility and lowering the risk of falls when managing Huntington's disease. Tailored exercise programs are designed to strengthen muscles, enhance coordination, and improve balance. To preserve motor function for as long as possible, these programs may incorporate strength training, flexibility training, and aerobic exercises. Physical therapists also work with patients on gait training and posture correction, which are critical as HD progresses and movement becomes more difficult [3].

   Additionally, physical therapists may use assistive devices such as walkers, canes, or orthotics to aid in mobility and reduce the risk of injury from falls. Regular physical therapy sessions can help slow the decline in motor function, allowing patients to maintain a higher level of independence for a longer period.
2. **Occupational Therapy**: As HD progresses, patients often struggle with daily living activities, such as dressing, bathing, and eating. Occupational therapy focuses on helping patients maintain their independence in these activities for as long as possible. Occupational therapists evaluate the patient's living space and suggest changes to increase accessibility and safety. This could be making a living area more accessible by moving furniture, employing adaptable cookware, or adding grab bars to bathrooms [2].

   Moreover, occupational therapists work with patients on cognitive tasks to help preserve mental functioning. This might involve memory exercises, problem-solving tasks, and strategies to manage time and organize daily activities. The goal is to help patients continue to engage in meaningful activities and maintain their quality of life, despite the cognitive and physical challenges posed by HD.
3. **Psychosocial Support**: The psychological and emotional impact of HD on patients and their families is profound. Coping with a diagnosis of HD and the inevitable progression of the disease can lead to significant stress, anxiety, and depression. Psychosocial support, including counselling, support groups, and educational

resources, is crucial in helping patients and families navigate these challenges. Mental health professionals, such as psychologists and social workers, can provide therapy to address the emotional aspects of living with HD and offer guidance on coping strategies [2].

Support groups offer a valuable space for patients and families to connect with others who are experiencing similar challenges. Sharing experiences and advice in these groups can reduce feelings of isolation and provide emotional comfort. Additionally, education about HD is important for both patients and caregivers, helping them understand the disease process and what to expect as it progresses.

Recent Advances in Treatment

In recent years, there have been significant advances in the understanding and treatment of Huntington's Disease. These developments hold promise for the future, offering potential new avenues for treatment that could alter the disease's progression.

1. **Gene Therapy**: One of the most exciting areas of research in HD is gene therapy. This approach focuses on silencing or modifying the expression of the mutant huntingtin gene (mHTT), which is responsible for HD. The potential of strategies like small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) to lower the production of the harmful huntingtin protein is being studied. These treatments function by focusing on the mutant gene's messenger RNA (mRNA) and blocking its translation into the huntingtin protein [1].

   Early clinical trials of ASOs have shown promising results, with reductions in the levels of the mutant huntingtin protein in the cerebrospinal fluid of patients. This suggests that gene therapy could potentially slow or even halt the progression of HD. To ascertain these treatments' long-term safety and effectiveness, extensive research is necessary as they are still in the experimental stages.
2. **CRISPR Technology**: Another ground-breaking development in HD research is the use of CRISPR/Cas9 gene-editing technology. This technique aims to directly correct the genetic mutation that causes HD by excising the expanded CAG repeat in the huntingtin gene. If successful, CRISPR technology could provide a permanent solution to the genetic defect that underlies HD [3].

   While CRISPR-based therapies are still in the early stages of development, the potential for a one-time treatment that could effectively "cure" HD is an exciting prospect. However, significant challenges remain, including the need to deliver the

CRISPR machinery to the appropriate cells in the brain and ensuring that the gene-editing process is both precise and safe.

3. **Neuroinflammation Research**: Recent studies have highlighted the role of neuroinflammation in the pathology of HD. It is now believed that inflammation in the brain may contribute to the progression of neurodegeneration in HD. As a result, there is growing interest in developing therapies that target inflammatory pathways [3].

   Drugs that modulate the immune response in the brain are being explored for their potential to slow or prevent the neurodegenerative process in HD. By reducing neuroinflammation, these therapies could protect neurons from damage and potentially slow the progression of the disease. This area of research is still in its early stages, but it represents a promising new direction in the search for disease-modifying treatments for HD.

Conclusion:

Huntington's disease (HD) represents a complex interplay of genetic, clinical, and therapeutic challenges, underscoring the need for a multifaceted approach to management. As a hereditary neurodegenerative disorder, HD profoundly impacts not only motor function but also cognitive abilities and emotional well-being, leading to significant disability and a reduced quality of life. The identification of the CAG repeat expansion in the HTT gene has revolutionized our understanding of the disease, paving the way for genetic testing and counselling, which are crucial for at-risk individuals and families. Despite the availability of symptomatic treatments aimed at alleviating the diverse manifestations of HD, there remains a pressing need for effective disease-modifying therapies. Recent advances in research, including gene therapy and neuroprotective strategies, offer hope for future interventions that could slow disease progression and improve patient outcomes. Furthermore, a comprehensive management plan that incorporates pharmacological and non-pharmacological approaches is essential for addressing the multifaceted needs of patients and their families. In conclusion, ongoing research and clinical advancements are critical in the fight against Huntington's disease. By enhancing our understanding of the underlying mechanisms and developing innovative therapies, we can aspire to improve the lives of those affected by this debilitating condition, ultimately striving for a future where the burden of HD is significantly reduced.

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NOVEL ADVANCEMENTS IN COLON TARGETING DELIVERY SYSTEMS

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Abstract:

Colon-targeted drug delivery systems have emerged as a critical focus in pharmaceutical research due to their potential for localized treatment of colonic diseases such as inflammatory bowel disease, colorectal cancer, and infections, as well as for systemic delivery of drugs that benefit from colonic absorption. While traditional strategies—such as pH-dependent, time-controlled, and microflora-activated systems—have laid the groundwork, they often face limitations in precision, efficiency, and reproducibility due to variability in gastrointestinal conditions. Recent advancements have introduced novel delivery technologies that offer greater specificity, stability, and therapeutic efficacy. These include nanoparticle and microparticle carriers, bioresponsive hydrogels, smart polymers, 3D-printed dosage forms, and microbiota-responsive systems. Additionally, the integration of biopolymers and enzyme-sensitive materials has enhanced site-specific drug release while improving biocompatibility and safety profiles. Cutting-edge approaches such as artificial intelligence in formulation design, microbiome-engineered delivery vehicles, and CRISPR-based targeting are pushing the frontiers of personalized and precision medicine. This chapter provides a comprehensive overview of the latest innovations in colon-targeted delivery systems, discussing their mechanisms, materials, design strategies, evaluation methods, and therapeutic applications. It also explores regulatory considerations, commercial prospects, and the future trajectory of this dynamic field.

Keywords: Colon targeting, Nanotechnology, Novel approach, Improved bioavailability

Introduction:

Colon-targeted drug delivery refers to the strategic design and development of drug delivery systems that release therapeutic agents specifically in the colon, with minimal or no release in the upper gastrointestinal (GI) tract. This targeted approach is especially valuable for treating local diseases of the colon such as ulcerative colitis, Crohn's disease, colorectal cancer, and irritable bowel syndrome (IBS), as well as for the systemic absorption of drugs that are unstable in the stomach or small intestine[1,2].

1. Rationale for colon targeting

The colon offers several unique advantages as a site for drug delivery:

- **Localized therapy**: Direct delivery to the colon enhances therapeutic efficacy and reduces systemic side effects.
- **Improved bioavailability**: Some drugs are better absorbed in the colon due to longer retention time and reduced enzymatic activity.
- **Protection from degradation**: Drugs sensitive to acidic pH or enzymatic degradation in the upper GI tract can remain more stable until reaching the colon.
- **Chronotherapeutic applications**: Synchronizing drug release with circadian rhythms is useful in diseases that follow diurnal variation, such as asthma or arthritis.

2. Anatomical and physiological considerations

The colon is the final section of the digestive tract, with a near-neutral pH (~6.8–7.4), lower enzymatic activity compared to the stomach and small intestine, and a diverse and dense microbial population. Transit time through the colon is relatively long (up to 24–48 hours), which provides a prolonged window for drug absorption. However, inter-individual variability in GI transit, pH, and microbiota composition presents challenges for consistent drug release[3,4].

3. Challenges in colon-targeted drug delivery

- **Physiological variability**: pH, transit time, and microbiota can differ significantly between individuals and even within the same individual under different conditions.
- **Premature drug release**: Ensuring that the drug is not released or degraded before reaching the colon is critical.
- **Formulation complexity**: Designing systems that can navigate the upper GI tract unaltered and activate only in the colon often involves sophisticated materials and technologies.

Limitations of conventional delivery systems

Despite the promise of colon-targeted drug delivery, conventional systems such as pH-dependent, time-dependent, and microbiota-activated formulations have faced several limitations that hinder their reliability and clinical success. These systems often suffer from a lack of site-specific precision, variability in patient physiology, and insufficient control over drug release, prompting the need for more advanced approaches[5-7]

1. pH-dependent systems

These systems rely on polymers that dissolve at the higher pH levels typically found in the distal small intestine or colon (e.g., Eudragit® coatings). While simple and cost-effective, they face several issues:

- **Inter-individual variability in pH**: GI pH can vary due to diet, age, disease state, and co-administered drugs, leading to premature or delayed drug release.
- **Incomplete targeting**: Drug release often begins in the ileum or late jejunum, resulting in partial absorption before reaching the colon.
- **Failure under altered GI conditions**: Conditions like diarrhea or inflammation may change local pH, reducing the effectiveness of the delivery system.

2. Time-dependent systems

These systems release drugs after a predefined lag time, assuming a relatively constant GI transit:

- **Unpredictable transit times**: Gastric emptying and intestinal transit times vary greatly between individuals and can be influenced by food, stress, or disease.
- **Risk of premature release**: Rapid transit may cause drug release in the small intestine, compromising therapeutic efficacy in the colon.
- **Limited adaptability**: Time-controlled systems do not respond to environmental cues or real-time physiological conditions.

3. Microbiota-activated systems

These systems utilize polysaccharides or prodrugs that are metabolized by colonic bacteria:

- **Variability in microbiota**: The composition and activity of colonic microflora vary significantly among individuals and populations.
- **Delayed activation**: Some systems require a threshold level of microbial enzymes, leading to slow or incomplete drug release.
- **Potential instability**: These formulations may degrade prematurely if exposed to bacterial enzymes present earlier in the GI tract due to dysbiosis or infection.

4. Pressure-controlled systems

These rely on the increased luminal pressure in the colon to trigger drug release:

- **Sensitivity to motility**: Colonic pressure varies with motility patterns and may not be sufficient to activate all formulations.
- **Limited specificity**: Pressure changes may occur in other parts of the GI tract under certain conditions, leading to off-target drug release.

5. General limitations across conventional systems

- **Lack of real-time responsiveness**: These systems are not equipped to respond dynamically to changes in GI conditions.
- **Poor control over drug release kinetics**: Ensuring sustained or pulsatile release is often challenging.
- **Stability and scalability**: Some materials and coatings used in conventional systems are difficult to scale up or lack long-term stability under storage conditions.

Conventional colon targeting strategies

Conventional colon-targeted drug delivery systems have laid the foundation for treating colonic diseases and improving systemic absorption of certain drugs. These systems aim to protect the drug from the harsh environment of the upper gastrointestinal (GI) tract and ensure its release upon reaching the colon. While innovative in concept, traditional approaches are often limited by physiological variability and inconsistent performance. This section outlines the major conventional strategies used for colon-specific drug delivery[8-10].

1. pH-dependent systems

These systems employ enteric coatings that dissolve at higher pH levels typically found in the terminal ileum and colon (pH ~6.8–7.4). Common pH-sensitive polymers include:

- **Eudragit® S100 and L100**
- **Cellulose acetate phthalate (CAP)**
- **Hydroxypropyl methylcellulose phthalate (HPMCP)**

Advantages:

- Simple formulation design
- Commercial feasibility

Limitations:

- Variable GI pH among individuals
- Risk of premature drug release in the small intestine
- Unpredictable release in pathological conditions (e.g., IBD)

2. Time-dependent (delayed-release) systems

These systems are designed to release the drug after a fixed lag time, assuming the drug reaches the colon within that window.

Technologies used:

- Coated tablets or capsules with hydrophobic layers

- Pulsatile release systems

Advantages:

- Independent of pH and microbiota
- Useful for drugs with circadian rhythm relevance

Limitations:

- Highly variable gastric emptying and intestinal transit times
- Not responsive to real-time physiological changes

3. Microflora-activated systems

These systems exploit the presence of colonic bacterial enzymes (e.g., azoreductase, glycosidase) to degrade specific carrier materials or prodrugs and trigger drug release.

Materials commonly used:

- Polysaccharides (e.g., pectin, guar gum, chitosan, inulin)
- Azo-linked prodrugs

Advantages:

- High specificity to colonic environment
- Biocompatible and biodegradable materials

Limitations:

- Variability in microbial populations
- Risk of incomplete degradation in dysbiotic or diseased colons

4. Pressure-controlled release systems

These systems utilize increased luminal pressure in the colon due to peristalsis and water absorption to rupture the dosage form.

Dosage forms:

- Osmotic systems
- Pressure-sensitive capsule coatings

Advantages:

- Triggered by physiological conditions unique to the colon

Limitations:

- Inconsistent pressure levels
- Lack of precision and predictability

5. Prodrug approaches

This strategy involves chemical modification of the active drug into a prodrug that remains inactive until enzymatically cleaved in the colon.

Examples:

- Sulfasalazine (cleaved by azoreductase into 5-aminosalicylic acid and sulfapyridine)

Advantages:

- High specificity and minimized systemic absorption in upper GI tract

Limitations:

- Complex chemical synthesis
- May require individualized enzymatic activity for activation

Novel and advanced delivery approaches

In response to the limitations of conventional colon-targeted drug delivery methods, a wide range of novel and advanced technologies have been developed to enhance site-specificity, improve drug stability, and offer programmable release profiles. These systems often integrate responsive materials, nanoscale carriers, and innovative engineering to overcome physiological variability and deliver drugs more efficiently to the colon.

1. Nanoparticle-based delivery systems

Nanoparticles (NPs) offer high surface area, enhanced drug loading, and controlled release characteristics, making them ideal for colon targeting.

Types of nanoparticles:

- **Polymeric nanoparticles** (e.g., PLGA, chitosan)
- **Lipid-based nanoparticles** (e.g., solid lipid nanoparticles, nanostructured lipid carriers)
- **Mesoporous silica nanoparticles**

Advantages:

- Enhanced mucoadhesion and retention in colonic mucosa
- Protection of drug from enzymatic degradation
- Targeted delivery via surface modifications (e.g., ligand conjugation)

2. Liposomes and vesicular systems

Liposomes and other vesicular systems such as niosomes and transfersomes can encapsulate both hydrophilic and lipophilic drugs[11,12].

Key features:

- Biocompatibility and biodegradability
- Potential for surface modification with colon-specific ligands

Challenges:

- Stability during GI transit
- Manufacturing complexity

3. Prodrug strategies (advanced modifications)

Modern prodrug approaches go beyond simple azo-linkages by incorporating advanced chemical linkers that respond to colonic enzymes or pH[13].

Examples:

- Enzyme-sensitive linkers cleaved by glucuronidase, azoreductase
- Redox-sensitive prodrugs for inflamed colonic environments

Benefits:

- Higher specificity and minimized systemic side effects
- Controlled activation in colonic environment

4. Mucoadhesive and bioadhesive systems

These systems use polymers that adhere to colonic mucosa, enhancing the residence time and localized action of the drug[14,15].

Common materials:

- Chitosan, polycarbophil, carbopol

Advantages:

- Prolonged contact time with colonic tissue
- Potential to bypass transit-time-related limitations

5. Smart polymeric carriers

Smart or stimuli-responsive polymers change their properties in response to specific triggers in the colon (e.g., pH, redox environment, enzymes).

Triggers and responses:

- pH-sensitive swelling or degradation
- Enzyme-responsive breakdown
- Thermo-responsive or redox-sensitive polymer matrices

Benefits:

- On-demand drug release
- Potential for personalized treatment profiles

6. Hybrid and multifunctional systems

Combining multiple mechanisms—such as pH sensitivity, enzyme activation, and mucoadhesion—into one system for enhanced targeting and reliability.

Examples:

- Core–shell structures
- Multi-layer coatings with sequential activation
- Dual-responsive nanoparticle systems

Advantages:

- Improved robustness against GI variability
- Greater control over drug release timing and location

These novel and advanced delivery approaches represent a paradigm shift in colon-targeted drug delivery. By overcoming the limitations of traditional methods, they offer promising avenues for treating colonic diseases with higher precision, improved efficacy, and reduced systemic toxicity. The integration of biotechnology, materials science, and patient-specific data continues to propel the field toward more intelligent, responsive, and effective delivery systems.

Biopolymer and natural polymer-based systems

Biopolymers and natural polymers have gained significant interest in colon-targeted drug delivery due to their biocompatibility, biodegradability, non-toxicity, and ability to respond to specific physiological triggers in the colon—particularly microbial enzymatic activity. These polymers serve as carriers or coating materials in advanced drug delivery systems, facilitating site-specific release of therapeutic agents within the colonic environment.

1. Rationale for using natural polymers

The colon houses a dense and diverse microbial population capable of secreting enzymes (e.g., glycosidases, esterases, reductases) that can degrade polysaccharide-based polymers. Natural polymers remain mostly intact in the stomach and small intestine, but are selectively degraded by colonic microflora, making them ideal for targeted delivery.

2. Commonly used biopolymers in colon targeting[16,17]

a. Pectin

- A polysaccharide derived from citrus fruits or apples.
- Degraded by pectinase enzymes produced by colonic bacteria.
- Often used in combination with other polymers (e.g., ethyl cellulose) to control swelling and premature dissolution.

b. Guar Gum

- A galactomannan obtained from *Cyamopsis tetragonoloba*.
- Resists digestion in the upper GI tract and is fermented by colonic bacteria.
- Used in matrix tablets and film coatings for colon-specific delivery.

c. Chitosan

- A deacetylated derivative of chitin obtained from crustacean shells.
- Exhibits mucoadhesive and antimicrobial properties.

- Can be chemically modified to enhance solubility or target-specific responsiveness (e.g., thiolated or carboxymethyl chitosan).

d. Inulin

- A non-starch polysaccharide composed of fructose units.
- Resistant to gastric and small intestinal enzymes but fermented by colonic bacteria into short-chain fatty acids (SCFAs).
- Used in prodrug conjugation and tablet formulations.

e. Xanthan Gum

- A microbial polysaccharide produced by *Xanthomonas campestris*.
- Used for controlled release and mucoadhesive applications in colon-specific formulations.

f. Amylose

- A linear polysaccharide component of starch.
- Fermented in the colon by amylolytic bacteria.
- Often combined with ethylcellulose to form resistant film coatings (e.g., *COLAL-PRED*® technology).

3. Applications in drug delivery systems

- **Matrix tablets**: Natural polymers are used as matrix formers to allow microbial-triggered drug release in the colon.
- **Coating materials**: Polymers like guar gum and pectin are used to coat tablets or capsules, enabling enzymatic degradation in the colon.
- **Microparticles and nanoparticles**: Biopolymer-based micro/nanocarriers provide improved drug protection and targeted release.
- **Hydrogels**: Crosslinked biopolymer hydrogels swell and degrade in response to colonic enzymes, providing a controlled release platform.

4. Advantages of natural polymer-based systems

- Biodegradable and renewable
- Generally recognized as safe (GRAS)
- High compatibility with hydrophilic and hydrophobic drugs
- Selective degradation by colonic microbiota
- Minimal systemic toxicity

5. Challenges and considerations

- Batch-to-batch variability in natural polymer composition
- Limited mechanical strength compared to synthetic polymers

- Need for combination with synthetic polymers for fine-tuned release
- Sensitivity to storage conditions (moisture, temperature)

Innovative technologies in colon targeting

The evolution of drug delivery science has led to the development of cutting-edge technologies that surpass the limitations of conventional colon-targeted systems. These innovative technologies offer enhanced precision, responsiveness, and personalization, leveraging advances in materials science, nanotechnology, engineering, and digital health. This section highlights some of the most promising and transformative technologies in colon targeting.

1. Smart stimuli-responsive systems

These systems are engineered to respond to one or more physiological triggers present specifically in the colon, such as[18]:

- **pH** (e.g., pH-sensitive polymers like Eudragit® variants)
- **Enzymes** (e.g., glycosidase-, azoreductase-sensitive linkers)
- **Redox environment** (e.g., glutathione-sensitive carriers)
- **Inflammatory markers** (e.g., reactive oxygen species-responsive systems)

Benefits:

- Site-specific and on-demand drug release
- Enhanced targeting in diseased states (e.g., IBD, colorectal cancer)
- Reduced off-target toxicity

2. 3D Printing for personalized dosage forms

3D printing technologies, such as fused deposition modeling (FDM) and stereolithography, are revolutionizing oral drug delivery by allowing the design of customizable, compartmentalized, or multilayered dosage forms[19].

Applications:

- Print-on-demand colon-targeted formulations
- Complex release profiles tailored to individual patient needs
- Integration of sensors for real-time monitoring

Example: Multi-layer tablets with enteric and enzymatically degradable layers printed using polymer filaments.

3. Microbiota-guided delivery systems

With increasing understanding of the gut microbiome, systems are now being designed to exploit microbial composition and activity for colon targeting=.

- Microbiota-responsive hydrogels and coatings

- Prebiotic-loaded carriers that synergistically modulate microbiota and deliver drugs
- Bacteria-directed targeting using phage or bacterial membrane-coated nanoparticles

Potential: Personalized delivery platforms based on a patient's microbiome profile.

4. Microneedle capsules and ingestible devices

Advanced oral capsules with microneedles or electronic components are under development to release drugs directly into the colonic mucosa.

Examples:

- **IntelliCap®**: A programmable capsule that releases drugs at a precise GI location using pH and temperature sensors.
- **Microneedle capsules**: Painlessly inject drugs into the colonic wall after localization.

Advantages:

- Precise, site-specific delivery
- Real-time monitoring and feedback
- Improved bioavailability of biologics and peptides

5. CRISPR and nucleic acid delivery systems

With the rise of gene therapy, delivery of nucleic acids (e.g., siRNA, CRISPR-Cas9 components) to the colon is gaining traction, especially for diseases like colorectal cancer and inflammatory bowel disease.

Innovative carriers:

- Lipid–polymer hybrid nanoparticles
- Stimuli-sensitive hydrogels
- Colon-activated viral vectors

Innovative technologies are significantly advancing the landscape of colon-targeted drug delivery. By integrating responsive materials, nanoscale engineering, personalized formulation, and digital monitoring tools, these systems are moving drug delivery closer to the ideals of precision medicine. Continued interdisciplinary collaboration will be essential to translate these technologies from lab to clinic, ensuring safer and more effective treatment options for colonic and systemic diseases.

Future trends and research directions

Colon-targeted drug delivery continues to evolve rapidly, driven by emerging biomedical technologies, a deeper understanding of gastrointestinal physiology, and increasing demand for precision therapeutics. As conventional and current novel strategies

still face challenges such as inter-patient variability, scalability, and regulatory hurdles, ongoing research is focused on overcoming these limitations and paving the way for next-generation systems.

1. Personalized and precision medicine approaches

Future colon-targeted therapies will increasingly be tailored to individual patients' physiological and genetic profiles, including:

- **Microbiome-based personalization**: Leveraging individual gut microbiota composition to predict and optimize drug release.
- **Genetic and biomarker-based targeting**: Customizing delivery systems based on genetic predisposition or disease-specific biomarkers.
- **Digital twin models**: Simulating patient-specific GI conditions for in silico optimization of delivery systems.

2. Integration with digital health technologies

Smart, connected delivery systems that interact with digital devices are expected to play a significant role:

- **Ingestible sensors**: For real-time monitoring of pH, temperature, or drug release location.
- **Digital feedback loops**: Linking drug release with real-time disease monitoring or wearable biosensors.
- **Theranostic platforms**: Systems capable of both diagnosing and treating colonic diseases simultaneously.

3. Advanced materials and responsive polymers

Material science is poised to significantly impact colon delivery:

- Multi-stimuli-responsive polymers (e.g., dual enzyme-pH sensitive systems) for more precise targeting.
- Bioinspired and biomimetic materials that mimic the GI environment.
- Self-healing and shape-memory polymers to adapt to GI motility and stress.

4. Enhanced biologic delivery

Colon targeting of biologics—such as peptides, proteins, nucleic acids, and live biotherapeutics—remains a major frontier:

- Encapsulation technologies to protect labile molecules from enzymatic degradation.
- Mucus-penetrating particles and nanoemulsions to enhance bioavailability.
- Gene-editing tools (e.g., CRISPR-Cas systems) for localized genomic therapy in colonic disorders like colorectal cancer and IBD.

5. Microbiome engineering and modulation

The gut microbiome is being explored not only as a trigger for drug release but also as a therapeutic target and delivery vehicle:

- Engineered probiotics to produce and deliver drugs in situ.
- Microbiota-responsive hydrogels and coatings that adapt to microbial signals.
- Co-delivery of prebiotics/probiotics alongside drugs to modulate gut health and enhance treatment outcomes.

6. Green and sustainable formulation practices

With growing environmental concerns, future systems will emphasize:

- Biodegradable and renewable polymers with minimal ecological impact.
- Eco-friendly manufacturing processes and solvent-free formulation techniques.
- Waste reduction in large-scale production through additive manufacturing and continuous processing.

7. Regulatory and translational considerations

Future research must also address translational and regulatory challenges:

- Establishing standardized in vitro–in vivo correlation (IVIVC) models for colon-specific formulations.
- Developing predictive animal models and non-invasive imaging tools to evaluate colon targeting.
- Ensuring scalability and GMP compliance of complex nanocarriers and smart systems.

Conclusion:

The future of colon-targeted drug delivery lies in convergence—of biology with digital health, materials with nanotechnology, and personalization with real-time adaptability. With sustained interdisciplinary collaboration and innovation, these emerging trends promise to reshape how we treat local and systemic diseases via the colonic route, making drug delivery more targeted, efficient, and patient-centric than ever before.

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EFFECTS OF ELECTROMAGNETIC FIELDS EXPOSURE ON THE ORGANS AND TISSUES

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Abstract:

An increasingly major new source of human-caused pollution, electromagnetic radiation (EMR) has emerged in recent decades. Many people around the world are curious about the biological effects of EML. The brain in particular is the focus of most studies because of the potential dangers that EMR poses to human organs. A large number of studies have shown that the neurological system is an important target organ system that responds to electromagnetic resonance (E MR). Disruption of cellular ionic balance and breakdown of large molecules are two of the many chemical effects of electromagnetic fields (EMF). Oxygen is essential for life, but it may also be a source of reactive oxygen species (ROS), which are harmful consequences of biological reactions. Numerous biological components are susceptible to destruction by reactive oxygen species, including DNA, lipids, and proteins. To protect living things from the harmful effects of free radicals, antioxidant defense mechanisms work by reducing their production. There are numerous potential sources of free radicals, including ultraviolet light, drugs, lipid oxidation, immunological reactions, radiation, stress, tobacco use, alcohol, and cellular redox reactions. We experience oxidative stress when our antioxidant defense system is unable to halt the harmful effects of free radicals. Electromagnetic fields are known to affect the recombination of radical couples, as well as to increase concentrations of free radicals and their traceability.

Keywords: EMR, Brain, Neurotransmitter, Free radical, Antioxidant

1. Introduction:

Electromagnetic radiation (EMR) has close ties to human existence; it originates from a variety of electrical systems, such as electronic devices, communication base stations, microwave ovens, mobile phones, and high-voltage lines. Elevated magnetic resonance (EMR) radiation is becoming more prevalent in human habitations due to the wide range of electromagnetic waves it generates[1]. The ionization process can be triggered

by cosmic rays, gamma rays, and other high-frequency electromagnetic waves. Many common electromagnetic fields are utilized in everyday life, including radio waves, microwaves, infrared, ultraviolet, visible, and extremely low-frequency electromagnetic fields (RF-EMFs, 30 kHz-300 GHz) from electrical sources, and extremely low-frequency electromagnetic fields (ELF-EMFs, 3 Hz-3 kHz) from communications. Another frequent name for RF is microwave (MW) radiation. Furthermore, gradually drawing attention is the effect of EMR on human health; human body was shown to exhibit modulation of brain functional connectivity[2,3].

Many natural and man-made sources that are vital for daily existence produce electromagnetic fields (EMF). Every day, around 3 billion individuals all around are exposed to EMF. Since lifetime EMF exposure has the ability to produce important modifications and negative impacts in biological systems, research on this topic is growingly important in science[4]. One can classify the biological effects of EMF as thermal and nonthermal. Thermal impacts relate to the heat produced in a given area by EMFs. This process uses changes in temperature derived from radiofrequency (RF) fields[5]. Every contact between RF fields and live tissues could induce an energy transfer that raises temperature. Usually absorbing the non-thermal radiations emitted by mobile phones, the skin and other superficial tissues generate a negligible rise in the temperature of the brain or other organs in the body[6].

Generation of reactive oxygen species (ROS) has been found to be mediator of this impact. ROS participate in several cell processes. For cellular homeostasis, they might be either quite harmful or absolutely needed. Their cytotoxic properties come from peroxidation of membrane phospholipids[7]. This causes loss of membrane integrity and changes the conductivity of the membrane. EMF has been shown to produce more free radicals in the cellular environment. Glutathione (GSH), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) are among the anti-oxidative mechanisms living entities use to minimize damage ROS and its products cause[8]. This defense system controls or reduces the chain reaction set off by ROS. In this situation, an agent causing overproduction of ROS, including EMF, compromises antioxidant defense mechanisms, hence generating oxidative stress. Recent studies have shown that many diseases, including diabetes and cancer, have their mechanism mostly related with free radicals. Due to the fact that EMR's effects on biological systems may depend on radiation strength, intensity, and frequency, EMR parameters provide a challenge for a literature review. In the frequency range of 100 kHz to 10 GHz, the specific absorption rate (SAR) measures how

much energy the human body absorbs when exposed to electromagnetic fields[9]. Quantification of absorbed power per mass of tissue is done using the watt-per-kilogram (W/kg) unit. Tissue structure, incidence direction, frequency, and E-polarization direction determine the SAR value. The SAR values thus far range from 10−4 to 35 W/kg in those published studies on the bioeffects of microwave radiation[10].

The neurological system is an important target organ system that responds to electromagnetic resonance (EMR), according to numerous investigations. When exposed to electromagnetic fields, the nervous system could experience changes in structure and function. During synaptic transmission, some chemicals in the nervous system called neurotransmitters act as messengers[11]. Several investigations have shown that EMR affects neurotransmitter metabolism and trafficking. The interplay between various brain regions and neurotransmitters allows the brain to carry out its duties, as is well-known to be based on the anatomical principle of neural circuitry. Hence, EMR's regulating effect on neurotransmitter levels in various brain regions may be crucial to brain functioning. The effects of radiofrequency electromagnetic radiation on the brain's neurotransmitters have been the subject of much investigation[12].

Presynaptic cells need neurotransmitters from nerve cells. Calcium ion channels triggered by action potentials transmit information between pre- and post-synapses. From synaptic terminals, transmitters spread across the cleft. They then connect with postsynaptic neuron or effector cell receptors. Residual neurotransmitters are recycled by presynaptic vectors from the synaptic cleft into vesicles[13]. Neurotransmitters like dopamine (DA) can be inactivated by enzymatic hydrolysis by mitochondrial monoamine oxidase and cytoplasmic catechol-O-methyltransferase (COMT). Neural circuit creation, maturation, and differentiation during brain development depend on neurotransmitters. Depression, schizophrenia, Alzheimer's, and Parkinson's diseases are linked to dysregulation of neurotransmitters, which allow neurons to communicate. In the brain, neurotransmitters are categorized by their chemical makeup into four types. DA, E, NE, 5-HT, and others are biogenic amines[14]. Amino acids include glycine, glutamate, acetylcholine (Ach), γ-aminobutyric acid (GABA), and others. Some endogenous opioid peptides are neurotransmitters. The other groupings contain additional transmitters like NO and substance P. The relevant research on the four types of brain neurotransmitters after EMR exposure is summarized in this review to describe their metabolism and receptor modifications[15].

2. Biochemical amine neurotransmitters and EMR

2.1 Dopamine (DA) Impact of EMR

Key neurotransmitter in the pituitary and hypothalamus, DA is a precursor of norepinephrine. It is mostly in charge of activities in the brain linked with reward, learning, emotion, motor control, and executive processes. DA also connects to neurological and psychiatric diseases including Huntington disease, multiple sclerosis, and Parkinson's disease. DA has been proposed to reduce the release of gonadotropin-releasing hormone; axonal link and interaction between DA and gonadotropin-releasing hormone in nerve terminals is also indicated. Patients with Parkinsonism show DA deficits in the basal ganglia. DA also plays some part in schizophrenia; striatal DA is raised and cortical DA transmission is changed[16].

DA was influenced by EMR in several studies. After two months of exposure and one month after stopping exposure, hippocampus DA dropped significantly with a specific absorption rate (SAR) of 0.843 W/kg, power density of 0.02 mW/cm2, and daily E MR exposure for one hour at 1,800 MHz. EMR exposure may diminish hippocampus DA production, affect rat arousal, and impair learning and memory, according to this study. Maaroufi et al. exposed rats to 900 MHz EMF, 1 h/day, for 21 days with SARs ranging from 0.05 W/kg to 0.18 W/kg dependent on field position. EMR-exposed hippocampi had lower DA. Additionally, hippocampus and striatum DA and dihydroxyphenyl acetic acid differ considerably in the EMR-exposed group. For 12 weeks, C57BL/6 mice exposed to an 835 MHz RF-EMR with a SAR value of 4.0 W/kg for 5 h/day dropped their striatum DA levels[17]. Microwave radiation may cause hippocampus and striatal monoamine neurotransmitter metabolic abnormalities, according to the research above. Exposed adult rats to 2,450 MHz microwave radiation at 5 and 10 mW/cm2 for an hour. DOPAC content in the pons and medulla oblongata increased only at 10 mW/cm2, while DA turnover rates and DOPAC:DA ratio increased only in the striatum and cerebral cortex. In any brain region, this power density did not modify DA content. These studies reveal that EMR may cause brain monoamine neurotransmitter metabolic issues and abnormal emotional behavior, depending on radiation exposure[18].

2.2 The Impact of EMR on Norepinephrine and Epinephrine

Mostly synthesised and secreted by sympathetic postganglionic neurons and adrenergic nerve terminals in the brain, norepinephrine is a neurotransmitter. Adrenal medulla generates an inadequate amount of norepinephrine as a hormone. Though it mostly binds to α receptors (including α1 and α2), it can bind to two varieties of adrenergic

receptors: α and β. N-methylation allows norepinephrine adrenaline to be transformed into epinephrine[19]. Many activities, including stress, attention, sleep, inflammation, and autonomic nervous system reactions, depend on norepinephrine released in the brain. Megha et al. discovered that the levels of norepinephrine and epinephrine in rat hippocampal tissue were much lowered following 30 days (2 h/day, 5 days/week) of continuous 1,800 MHz, 1 mW/cm2 microwave radiation, suggesting that some conditions of microwave radiation could lead to a reduction in norepinephrine and epinephrine contents in the brain. Cao and associates subjected male LACA mice to 900 MHz microwave radiation. The radiation intensity applied was 0, 1, 2, and 5 mW/cm2; the SAR values were 0, 0.22, 0.44, and 1.1 W/kg, respectively; mice were exposed for one hour every day for thirty-five straight days. The results revealed that while exposure intensity was 2 or 5 mW/cm2, there were no clear changes in norepinephrine content; rather, the brain norepinephrine content increased dramatically when E MR intensity was 1 mW/cm2. This implies even further that low-intensity EMR exposure can raise norepinephrine content in the brain, which would theoretically influence epinephrine content and cause problems with the synthesis of neurotransmitters[20].

2.3 Exploring the Impact of EMR on 5-Hydroxytryptamine

While only a tiny amount is generated in the neurological system, 5-hydroxytryptamine (5-HT) is extensively created in the gastrointestinal tract—mostly in enterochromaffin cells. Mostly concentrated in the raphe nuclei, 5-HT cell bodies in the brain send axons to practically every brain region. Mostly found in the pineal gland and hypothalamus, 5-HT is an inhibitory neurotransmitter that particularly affects the cerebral cortex and neuronal synapses. 5-HT helps control physiological processes including mood, eating, cognition, memory, pain, sleep, and body temperature maintenance; these processes have been observed to be markers of brain damage caused by electromagnetic radiation[21]. As such, 5-HT may be crucial in the neurobiological impacts of EMR. The effect of microwave radiation on 5-HT is not well known from any studies. Rats were reportedly subjected to 1 hour of 2,450 MHz microwave radiation at 5 and 10 mW/cm2, at power densities. Following microwave treatment at power densities of 5 and 10 mW/cm2, the 5-hydroxyindoleacetic acid (5-HIAA) concentration in the cerebral cortex was much raised. At a power density of 5 mW/cm2 the 5-HT turnover rates and the 5-HIAA:5-HT ratio in the cerebral cortex rose noticeably. On the other hand, 5-HT content in the brain of microwave-exposed rats did not clearly change. In the pons, medulla oblongata, and hypothalamus at a power density of 10 mW/cm2, the 5-HT turnover rate was consistently much raised. With

the maximal power level of 5 kW at 2,450 MHz and radiation durations of 0.5 and 1.5 s, the effect of microwave radiation on monoamine metabolism was also examined in the cortex, striatum, and hippocampal of the rat brain. The quantities of intracerebral monoamines and their metabolites were ascertained by high-performance liquid chromatography (HPLC) with electrochemical detection. The 0.5 s radiation lowered the amounts of norepinephrine, DA, and 5-HIAA. Although 1.5 s radiation raised the amounts of these monoamines. In contrast, another study on pregnant rats exposed to 900 MHz cellular phones revealed no appreciable variation in the content of 5-HT of unborn rats in different intensity of microwave radiation groups. More research is required overall to clarify the function of 5-HT in EMR-induced learning and memory malfunction as well as morphological changes in the brain[22].

2.4 EMR impact on amino acid neurotransmitters

The main excitatory neurotransmitters in the neurological system is glutamate. Glutamate receptors abound in glia of the brain and spinal cord as well as in neurons. Glucose sources the C-terminus and carbon backbone of glutamate. Glucose is broken down to pyruvic acid in the cytosol by glycolysis following astrocytic end foot across the blood-brain barrier. α-ketoglutarate is then produced when pyruvic acid then joins the tricarboxylic acid (TCA) cycle. At last, pyruvic acid is passed to get an amino group provided by leucine, isoleucine and valine, aspartate, γ-aminobutyric acid (GABA), and alanine etc. Furthermore, present in several amino acid-based derivatives, including the antioxidant glutathione, glutamate also serves as a metabolic precursor of GABA. All of the glucose is finally turned to glutamate in the central nervous system, according to metabolic studies, suggesting the fundamental importance of glutamate in many facets of brain physiology[23].

Besides glutamate, aspartate is a major central nervous system excitatory neurotransmitter. Glutamate and aspartate synthesis and metabolic enzymes are found in neurons and glial cells, especially in the mitochondria of neurons involved in the TCA cycle of glucose metabolism. Aspartate is generated and stored in axon terminals by aminotransferase from oxaloacetic acid. Glutamate and aspartate are released by the presynaptic membrane and quickly diffuse into the postsynaptic membrane, where they bind to their receptors and activate sodium and potassium channel gates to cause excitatory effects. The presynaptic membrane releases a small amount of glutamate and aspartate to glial cells[24].

Following 10 minutes of microwave radiation, 30 mW/cm2 Wistar rats underwent HPLC to measure hippocampus aspartate and glutamate levels at 1, 7, 14, and 28 days. Acute EMR exposure reduced hippocampus excitatory amino acid concentrations of aspartate and glutamate one day after radiation. Ahmed et al. repeatedly probed how EMR altered hippocampus, striatal, and hypothalamic amino acid neurotransmitters in young adult and juvenile rats. For 1, 2, and 4 months, the exposure group underwent 1,800MHz EMR with SAR value of 0.843 W/kg, power density of 0.02 mW/cm2, 1 h daily. After one month, EMR decreased hippocampal glutamate and glutamine levels. EMR may diminish hippocampal excitatory amino acid neurotransmitters, impacting neuron excitatory-inhibitory balance and learning and memory[25].

3. Potential processes for alterations in neurotransmitter changes caused by EMR

3.1 Electrophysiological Changes

The effects of EMR on neurotransmitters can be better understood if we delve into the neurophysiological mechanisms at work, particularly electrophysiological alterations. Various neuroimaging techniques shed light on the interference between EMR and brain electrical activity. Consider the following examples: functional magnetic resonance imaging (FMRI) can detect regional changes of blood oxygen utilization during neuropsychological performance; positron emission tomography (PET) reflects cerebral metabolism; electroencephalography (EEG) measures changes of extracellular electrical potential in the cortex. Neuronal membrane potential fluctuations are the initial sources of electrical activity in the brain. Possible reflections of neurotransmission modulation include the postsynaptic potential and subsequent synaptic transmission that follow nerve impulse transduction[26].

Research suggests that brain electrical activity alterations, such as increased cortical excitability and efficiency, may last for a few minutes after EMR exposure ends. Further effects of EMR exposure include an upregulation of cerebral metabolism (PET), a downregulation of alpha activity, an upregulation of high beta and gamma frequency activity, a quickening of reaction time, and an interruption of sleep EEG. It appears that the frontal and temporal areas are more vulnerable, according to multiple techniques such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), EMF-elicited event-related potentials (ERPs), event-related desynchronization (ERD), and interhemispheric synchronization. Evidence suggests that electromagnetic fields (EMFs) influence cortical excitability and efficiency through a number of mechanisms, such as modulation of the stress response, changes to cellular calcium homeostasis, altered

dependent Na-K trans-membrane ionic channels, and enhanced cellular excitability. Methodological variations, statistical power, and interpretation criteria may account for the observed heterogeneity in results, which is accompanied by several contradictory findings. In sum, EMR-induced regulation of neurotransmission may cause alterations in neurotransmitters, which may be reflected in aberrant brain electrical activity[27].

3.2 Cell Membrane Damage

It is well-known that EMFs first and foremost aim at cell membranes. Alterations to neurotransmitters in the brain may be the outcome of cell membrane injury. Finding out where EMR goes in cells requires an understanding of how it affects neurotransmitters. For example, alterations in calcium concentration, ionic distribution, and ion permeability can occur as a result of EMR. An imbalance of calcium homeostasis can disrupt numerous cellular activities; calcium is a crucial signalling molecule[28]. Research has shown that electromagnetic radiation (EMR) can change cell membrane calcium channels and receptors, impacting the movement of calcium ions across the membrane. These changes have profound implications for cell signaling pathways and, ultimately, for neurotransmitter responses. There was some evidence that EMF exposure increased the number of open calcium channels, which could explain why intracellular calcium concentrations rise when exposed to EMRs. Additionally, abnormal synaptic activity or neuronal death can be triggered by changes in intracellular calcium levels. As a result, this has the potential to impact the neurotransmission involved in learning and memory[29].

Furthermore, in numerous cell types, the increased activity of voltage-gated calcium channels (VGCCs) following EMR exposure has been characterized. In order to determine whether ion channels were altered by microwave radiation, earlier research looked at VGCC activity. The function of the synaptic vesicular membrane can be inferred from the levels of neurotransmitters, which in turn can be used to describe membrane features, such as the expression level of proteins linked with synapses. According to reports, VGCCs are activated by EMR, leading to a surge in intracellular calcium, nitric oxide, and peroxynitrite levels. Nevertheless, a recent investigation into the impacts of 2.856 GHz pulsed microwave radiation on primary hippocampus neurons found that exposure to microwaves reduced total cellular calcium, endoplasmic reticulum calcium levels, and mitochondrial calcium levels, indicating calcium efflux during microwave radiation. The effects of EMR on neuronal calcium efflux and influx have been the subject of several animal research, however the conclusions concerning EMR's effects on membrane permeability and integrity remain unclear. Damage to membrane integrity, brought on by alterations in

membrane permeability, can alter the neurotransmitter imbalance in the brain. Regarding this matter, additional research utilizing different EMR durations and doses is necessary to examine how EMR impacts the connection between neurotransmitters and cell membrane permeability[31].

4. Discussion:

A number of cellular calcium functions are affected by EMR. Numerous studies have shown that exposure to radiofrequency electromagnetic fields alters calcium metabolism, but the exact processes by which this occurs remain unclear. Molecular apoptosis pathways may be activated after calcium activation, which according to some research may be the first event that causes a change in protein structure, followed by the production of reactive oxygen species (ROS). According to Lushchak et al., EMR exposure may cause the brain to create free radicals, which are then transformed to ROS. Several biomolecules within the cell are vulnerable to assault when ROS levels are elevated. An increase in reactive oxygen species (ROS) can cause calcium release, which in turn can activate the genetic pathways that cause DNA damage[32]. Changes in gene and enzyme expression levels have the potential to activate signal transduction pathways; in particular, the mitochondria-dependent caspase-3 pathway, which in turn can trigger neuronal death and induce a variety of behavioral and physiological abnormalities. Electromagnetic radiation (EMR) exposure raises intracellular calcium and reactive oxygen species (ROS) production, which in turn change's cellular function and causes a cascade of biological effects, including an imbalance in neurotransmitters[33]. Possible combined effects in different brain areas, such as neurophysiological alterations, increased calcium and ROS, cell membrane damage, and downstream signaling modifications, could account for the wide range of neurotransmission effects seen in animals exposed to EMR[34]. Behavioural changes might occur even in the absence of overt structural alterations if neurotransmitter changes led to an unbalanced excitation-inhibition ratio in neurons. The exact neurochemical processes via which EMR radiation works remain unknown at this time. To get a better understanding of the brain mechanisms affected by EMR, additional research is required in this area.

Conclusion:

In conclusion, EMR is slowly but surely improving neurotransmitter metabolism and transport in the brain. However, experimental results are not very consistent or comparable because of the various EMR parameters, experimental objects, and situations. Consequently, EMR's impact on neurotransmitter metabolism and transport remains

unclear. Also, the neurobehavioral dysfunction caused by EMR and the part played by neurotransmitters have not been uncovered. Additional in-depth research is required. It is challenging to differentiate between the main and secondary alterations of each neurotransmitter due to their interaction, transmission, and coregulation, as well as the brain's rich diversity of neurotransmitters. The complex neuronal circuits that underpin the brain's ability to carry out its many activities are themselves formed by the interplay of many neural nuclei. The EMR-induced neurotransmitter imbalance may, therefore, involve the control of neuronal circuits.

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