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ADVANCES IN PHARMA AND HEALTH SCIENCE RESEARCH VOLUME IV

Editors:
Ms. Shweta Bhandari
Dr. Vishal Garg
Dr. Tarkeshwar Kumar
Dr. Omprakash T. Sangule

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Advances in Pharma and Health Science Research Volume IV

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PREFACE

The field of pharmaceutical and health sciences has witnessed unprecedented advancements over the past few decades, driven by relentless research, technological innovation, and an ever-increasing demand for improved healthcare solutions. The book "Advances in Pharma and Health Science Research" aims to serve as a comprehensive platform to showcase the latest trends, developments, and breakthroughs in these dynamic disciplines.

This volume brings together original research articles, review papers, and case studies from academicians, researchers, healthcare professionals, and industry experts from across the globe. The topics covered span a wide array of subjects—including drug discovery and development, pharmacology, clinical research, biotechnology, health informatics, public health strategies, and emerging therapeutic techniques. Each chapter reflects the contributors' commitment to addressing contemporary challenges while paving the way for innovative solutions in patient care and treatment modalities.

The book is intended for scholars, students, professionals, and practitioners who are engaged in pharmaceutical and health science sectors. We believe that this compilation will not only enhance their knowledge but also inspire collaborative research and interdisciplinary discourse for future advancements.

We are deeply grateful to all the contributors for their scholarly inputs and to the editorial team for their meticulous efforts in bringing this work to fruition. We also extend our sincere thanks to the institutions and organizations that supported the contributors in their research endeavors.

As you delve into the chapters, we hope this book enlightens your understanding, encourages scientific curiosity, and contributes meaningfully to your academic and professional journey.

- Editors

TABLE OF CONTENT

Sr. No.	Book Chapter and Author(s)	Page No.
1.	**DIGITAL THERAPEUTICS (DTx): SOFTWARE AS CLINICALLY VALIDATED MEDICINE** Sunil Kardani, Ghanshyam Parmar, Rajesh Hadia and Dipti Gohil	1 – 19
2.	***MORINGA OLEIFERA*: A MIRACLE HERB AND ITS MEDICINAL BENEFITS** Kinjal Patel, Rajesh A. Maheshwari, Dhanya B. Sen and Ashim Kumar Sen	20 – 29
3.	**EXPLOITATION OF SALT'S POTENTIAL EXTREMOPHILES ON MICROPLASTICS** Swedha Raja and Bhuvaneswari Shanmugam	30 – 41
4.	**COMPUTATIONAL CHEMISTRY IN DRUG DESIGN: TOOLS, TRENDS, AND TRANSFORMATIVE APPLICATIONS** Sowmya P T and Anitha Sudhir	42 – 58
5.	**3D CELL CULTURE: ADVANCEMENTS AND LIMITATIONS** Manoj Patidar	59 – 66
6.	**SYNTHESIS AND SPECTROSCOPIC STUDY OF A THIOSEMICARBAZONE LIGAND AND ITS AU (III) COMPLEX** O. T. Sangule	67 – 77
7.	**WOMEN'S MENOPAUSE - PROBLEMS AND SOLUTIONS** Aruna N. Shende	78 – 85
8.	**ISOLATION OF PSYCHROTROPHIC BACTERIA IN MILK FROM DAIRIES OF AURANGABAD** Syed Nisar Ahmed	86 – 98
9.	**CHRONIC MYELOID LEUKEMIA: MECHANISMS, DIAGNOSIS, AND THERAPEUTIC STRATEGIES** Aarti S. Zanwar, Krupa Joshi, Divya Kanojiya, Sachin B Zanwar and Kirti V. Patel	99 – 109

10.	**RECENT INSIGHTS INTO THE MANAGEMENT AND EMERGING THERAPIES FOR MULTIPLE SCLEROSIS** Sweta B Besh, Maitri Mahant, Rajesh A. Maheshwari, Nirmal Shah	110 – 122
11.	**MATHEMATICAL MODELING IN PHARMACEUTICAL AND HEALTH SCIENCES: APPLICATIONS AND IMPACT** Sheetal R. Gomkar	123 – 126
12.	**FENUGREEK AND HUMAN HEALTH: FROM KITCHEN SPICE TO NATURAL MEDICINE** Rajesh A. Maheshwari, Dhanya B. Sen, Aarti Zanwar and Ashim Kumar Sen	127 – 137
13.	**HEALTH BENEFITS OF SWEET POTATO** Dhanya B. Sen, Ashim Kumar Sen, Rajesh A. Maheshwari and Krupa Joshi	138 – 149
14.	**CUMIN AS CURE: TRADITIONAL USES AND HEALING POWERS OF JEERA** Ashim Kumar Sen, Dhanya B. Sen, Rajesh A. Maheshwari and Dillip Kumar Dash	150 – 160

Advances in Pharma and Health Science Research Volume IV
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DIGITAL THERAPEUTICS (DTx): SOFTWARE AS CLINICALLY VALIDATED MEDICINE

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Abstract:

This chapter explores Digital Therapeutics (DTx) as clinically validated software interventions within the broader digital health ecosystem. DTx, unlike general wellness apps, are evidence-based and designed to prevent, manage, or treat specific medical conditions. They undergo rigorous clinical testing, often including RCTs, and are subject to regulatory oversight as Software as a Medical Device (SaMD). Key characteristics include a focus on clinical outcomes, patient-centric design, and delivery via software platforms. The chapter distinguishes DTx from wellness apps, highlighting their therapeutic claims and integration into clinical workflows. It further discusses the evolution, categories, clinical validation, regulatory pathways, economic considerations, and challenges associated with DTx, underscoring their growing importance in modern healthcare.

Keywords: Digital Therapeutics (DTx), Clinical Validation, Software as a Medical Device (SaMD) Digital Health

1. An Overview of the Broader Digital Health Ecosystem

The digital health ecosystem represents a dynamic convergence of digital technologies with health, healthcare, and wellness, aiming to enhance healthcare delivery, improve patient outcomes, increase efficiency, and empower individuals in managing their health (1, 2). It encompasses a wide array of tools and platforms that leverage connectivity, data analytics, and software to transform how health services are accessed, delivered, and experienced.[1]

Key components within this ecosystem include:

- **Wearable Technology:** Devices such as smartwatches, fitness trackers, and biosensors continuously monitor physiological data like heart rate, activity levels, sleep patterns, and sometimes even glucose levels or ECG readings (3, 4). This data empowers users with insights into their health trends and provides clinicians with valuable information for remote monitoring and preventative care strategies (5, 6).

- **Telehealth:** This involves the remote delivery of healthcare services using telecommunication technologies (7). It includes synchronous interactions like live video consultations between patients and providers, asynchronous methods like store-and-forward image transmission, and remote patient monitoring (RPM) where patient data is collected remotely and transmitted to healthcare providers (8, 9). Telehealth significantly improves access to care, particularly for those in remote areas or with mobility issues, and enhances convenience (10).
- **Electronic Health Records (EHRs) / Electronic Medical Records (EMRs**): These are digital versions of patients' health information. (11) While EMRs are typically digital records within a single practice, EHRs are designed to be comprehensive and shareable across different healthcare settings (12). They streamline clinical workflows, reduce errors, improve data accessibility for providers, support clinical decision-making through integrated tools, and facilitate better-coordinated care (13, 14).
- **Mobile Health (mHealth) and Wellness Applications:** This category includes a vast number of applications on smartphones and tablets designed for health and wellness purposes (15). They range from fitness tracking, diet and nutrition management, and medication reminders to mental wellness support like meditation and mindfulness exercises (16, 17). While many focus on general wellness rather than specific disease treatment (unlike Digital Therapeutics), they play a significant role in promoting healthy behaviors and patient self-management (18).

These components often interact, creating a connected health environment. Data from wearables can feed into telehealth platforms or EHRs, providing a more holistic view of a patient's health (19). The ultimate goal is to create a more personalized, predictive, preventative, and participatory healthcare system, leveraging technology to improve health for individuals and populations globally (20).

2. Digital Therapeutics (DTx): Evidence-Based Software Interventions

Digital Therapeutics (DTx) constitute a specialized category within digital health, characterized as evidence-based, software-driven interventions meticulously designed to prevent, manage, or treat a specific medical disorder or disease (21, 22). They represent a significant evolution from general wellness apps by delivering direct therapeutic effects underpinned by rigorous scientific validation (23).

The core attribute setting DTx apart is their reliance on robust clinical evidence (24, 25). Similar to traditional pharmaceuticals, DTx products typically undergo rigorous testing, often including randomized controlled trials (RCTs), to demonstrate safety, clinical efficacy, and quantifiable health outcomes for the target condition (26, 27). This evidence is usually published in peer-reviewed journals and is essential for gaining trust from clinicians, patients, and payers (28).

The intervention itself is delivered via software, leveraging platforms such as smartphone apps, web applications, or even video games (29). These sophisticated programs employ algorithms, interactive content, and user feedback mechanisms to deliver therapeutic modalities directly to the patient (30). Examples include software delivering Cognitive Behavioral Therapy (CBT) for insomnia or depression (31), providing personalized coaching for diabetes management (32), or aiding in substance use disorder recovery (33).

DTx are developed with the explicit purpose of impacting a disease state – either preventing its onset, helping patients manage chronic conditions more effectively, or directly treating a diagnosed disorder (34, 35). They target specific, recognized medical conditions as defined by clinical guidelines and diagnostic criteria (36).

Reflecting their therapeutic claims and potential risks, many DTx products are subject to regulatory oversight by bodies such as the U.S. Food and Drug Administration (FDA) or equivalent international authorities, often being classified as Software as a Medical Device (SaMD) (37, 88). This ensures they meet stringent standards for safety, effectiveness, and quality management before reaching patients (39).

In essence, DTx utilize software as a scalable, accessible, and data-driven tool to deliver clinically validated medical interventions, positioning them as a distinct and increasingly important modality in modern healthcare delivery (40).

3. Key Characteristics of Digital Therapeutics (DTx)

Digital Therapeutics (DTx) are distinguished within the digital health field by several core characteristics that underscore their unique nature and purpose (41). These defining features collectively ensure their quality, safety, and efficacy as medical interventions (42).

- **Focus on Clinical Outcomes:** A fundamental characteristic of DTx is the rigorous requirement to demonstrate measurable clinical outcomes (43). Unlike general wellness apps, DTx must undergo robust clinical testing, often including randomized

controlled trials (RCTs), to prove their ability to achieve specific, predefined health results, such as improving disease symptoms, managing physiological markers, or changing patient behaviors related to a medical condition (44, 45). This evidence is essential for validating their therapeutic claims (46).

- **Requirement for Regulatory Oversight:** Reflecting their function as medical interventions, DTx frequently necessitate regulatory oversight by competent authorities like the U.S. Food and Drug Administration (FDA) or equivalent bodies granting CE Marks in Europe (47, 48). They are often classified as Software as a Medical Device (SaMD) and must meet stringent standards for safety, effectiveness, clinical validation, quality management, and often cybersecurity and data privacy before market authorization (49, 50, 51).
- **Patient-Centric Design:** DTx are developed with a strong emphasis on patient-centric design (52). This involves creating intuitive user interfaces (UI) and positive user experiences (UX) to maximize patient engagement and adherence, which are critical for therapeutic success (53, 54). Development incorporates user feedback, usability testing, and principles aimed at making the software accessible and easy to integrate into patients' daily lives (55, 56).
- **Delivery via Software:** The core intervention of a DTx is inherently delivered via software. High-quality, often proprietary software applications, accessible via smartphones, tablets, or web platforms, serve as the mechanism through which the therapy is administered (57). These platforms execute algorithms, deliver content, track patient inputs, provide personalized feedback, and implement the specific therapeutic protocol designed to address the target medical condition (58).

Together, these characteristics validated clinical outcomes, regulatory scrutiny, user-focused design, and software-based delivery define DTx as a credible and distinct class of medical treatments.

4. Distinguishing Digital Therapeutics (DTx) from Wellness Apps

While both Digital Therapeutics (DTx) and general wellness applications utilize digital technology for health-related purposes, they represent distinct categories with fundamental differences in their validation, intended use, and integration into the healthcare ecosystem (59, 60). Understanding these distinctions is crucial for users, clinicians, and regulators (61).

The primary differentiator is the requirement for rigorous clinical validation for DTx (62). DTx must demonstrate safety and efficacy in treating, managing, or preventing a *specific* medical disorder through robust clinical evidence, often including randomized controlled trials (RCTs) published in peer-reviewed journals. This level of evidence aligns DTx with traditional medical interventions. In contrast, wellness apps, while potentially beneficial for promoting healthy habits, generally lack this high standard of specific clinical validation for diagnosed diseases (63, 64). Their evidence, if available, might focus on usability or general engagement rather than validated impact on a specific medical condition (65).

Consequently, DTx make specific therapeutic claims about their ability to achieve clinical outcomes for particular diseases (e.g., reducing symptoms of depression, improving glycemic control in diabetes). These claims necessitate the supporting clinical evidence and often subject DTx to regulatory oversight as medical devices (66). Wellness apps focus on general health goals like fitness tracking, stress management, or healthy eating, and typically *avoid* making claims about treating, diagnosing, or preventing specific medical disorders (67,68).

Finally, DTx are frequently designed for integration into clinical workflows. They may require a prescription or authorization from a healthcare provider, allow for clinician monitoring of patient progress, potentially integrate data with Electronic Health Records (EHRs), and often pursue reimbursement pathways through health insurance or healthcare systems (69,70). Wellness apps are typically direct-to-consumer products, used independently by individuals without formal clinical oversight or integration into the established healthcare infrastructure.

In essence, the stringent requirements for clinical evidence, the capacity to make specific therapeutic claims, and the potential for clinical integration and regulatory oversight clearly separate DTx from the broader category of general wellness applications.

5. The Importance and Relevance of Digital Health in Modern Healthcare

Digital health technologies, encompassing telehealth, wearables, electronic health records (EHRs), mobile apps, and specialized Digital Therapeutics (DTx), are increasingly vital components of modern healthcare systems worldwide. Their importance stems from their ability to address critical challenges, enhance efficiency, and fundamentally reshape the patient experience. (71)

One key relevance lies in addressing gaps in care (72). Digital tools can extend healthcare reach to geographically isolated or underserved populations who face barriers to traditional in-person services (73). They provide scalable solutions for managing chronic diseases through continuous monitoring and timely interventions, filling gaps between infrequent clinic visits (74). Furthermore, digital platforms, particularly in mental health, can offer access to specialized support where local resources are scarce or stigma presents a barrier (75, 76).

Digital health significantly improves access to healthcare services. Telehealth consultations remove geographical and mobility constraints, allowing patients to connect with providers from their homes. This reduces patient travel time and costs, minimizes time off work, and can decrease waiting times for certain types of appointments (77). Remote patient monitoring allows for proactive care delivery, potentially reducing emergency room visits and hospital readmissions (78).

Crucially, these technologies foster patient empowerment. Access to personal health data through patient portals linked to EHRs, wearables, and health apps enables individuals to take a more active role in managing their health (79). Digital tools provide personalized education, self-management resources (like DTx for specific conditions), and facilitate better communication between patients and providers (80). This shift supports shared decision-making and encourages greater patient engagement and adherence to treatment plans (81).

In summary, by bridging care gaps, enhancing accessibility, and empowering patients, digital health technologies are not just relevant but increasingly essential for creating more equitable, efficient, and patient-centered healthcare in the 21st century.

6. The Evolution of Digital Therapeutics (DTx)

The evolution of Digital Therapeutics (DTx) marks a significant advancement from early digital health applications to a distinct class of evidence-based medical interventions delivered via software. This journey began decades ago with rudimentary computerized health programs and cognitive behavioral therapy tools, often limited by technology and accessibility (82). The true acceleration phase commenced with the widespread adoption of smartphones, ubiquitous internet connectivity, powerful sensors, and cloud computing. These technologies provided the foundation for developing sophisticated, interactive, and personalized software capable of engaging patients directly.

A pivotal conceptual shift occurred when it became clear that software, if rigorously designed and validated, could itself deliver therapeutic outcomes for specific medical conditions. This led to the core definition of DTx: interventions requiring robust clinical evidence, often from randomized controlled trials (RCTs), demonstrating safety and efficacy akin to traditional drug development pathways. Early pioneering companies successfully navigated regulatory landscapes, achieving landmark clearances and approvals from bodies like the FDA for DTx addressing conditions such as type 2 diabetes, substance use disorder, ADHD, and insomnia.

This validation spurred formalization, with organizations like the Digital Therapeutics Alliance establishing core principles and best practices to differentiate clinically validated DTx from general wellness apps. Today, the evolution continues with a focus on generating real-world evidence, integrating DTx seamlessly into clinical workflows and electronic health records, establishing clear reimbursement models with payers, and expanding the therapeutic reach into new disease areas. DTx have thus evolved from a nascent concept into a recognized and rapidly growing category of treatment options within modern healthcare.

7. Categories and Applications of Digital Therapeutics (DTx)

Digital Therapeutics (DTx) represent a diverse class of software-based medical interventions, applicable across a growing spectrum of health conditions. They can be broadly categorized based on their target therapeutic area, their underlying mechanism of action, or their specific intended use within a patient's care plan. This versatility allows DTx to address unmet needs in various medical fields.

One of the most established application areas is mental and behavioral health. Numerous DTx leverage evidence-based approaches like Cognitive Behavioral Therapy (CBT) to treat conditions including depression, anxiety, insomnia, substance use disorder (SUD), and Attention-Deficit/Hyperactivity Disorder (ADHD). These tools offer scalable and accessible alternatives or adjuncts to traditional therapy.

Another major category focuses on chronic disease management. DTx provide significant support for patients managing conditions such as Type 1 and Type 2 Diabetes, hypertension, asthma, and Chronic Obstructive Pulmonary Disease (COPD). They often facilitate improved self-management through personalized coaching, physiological data monitoring integration, medication adherence reminders, and lifestyle behavior modification support.

Beyond these core areas, DTx applications are expanding rapidly. Examples include tools for substance use disorder recovery and relapse prevention (83), management of gastrointestinal conditions like irritable bowel syndrome (IBS), neurological applications for migraine or Multiple Sclerosis symptom management, and supportive care interventions in oncology.

Mechanisms of action vary widely, from delivering specific psychotherapeutic content to employing sophisticated algorithms for personalized feedback and behavior change. Depending on the product and indication, DTx may function as standalone treatments, augment pharmacotherapy, or integrate with other digital health tools. The expanding range of categories and applications underscores the growing role of DTx in modern medicine.

8. Clinical Validation and Evidence Generation for Digital Therapeutics (DTx)

A defining characteristic and absolute requirement for Digital Therapeutics (DTx) is their foundation upon rigorous clinical validation and evidence generation. This commitment sets DTx apart from unregulated wellness apps and underpins their credibility as medical interventions. Generating robust evidence is essential not only to prove safety and efficacy but also to gain trust from clinicians and patients, secure regulatory approval or clearance, and achieve reimbursement from healthcare payers.

The gold standard for establishing the clinical efficacy of DTx typically involves Randomized Controlled Trials (RCTs). These studies are meticulously designed to compare the outcomes of patients using the DTx intervention against a control group (receiving standard care, a sham intervention, or being on a waitlist). Similar to pharmaceutical trials, RCTs for DTx aim to minimize bias and isolate the specific therapeutic effect of the software intervention on predefined, clinically meaningful endpoints, such as symptom reduction, physiological markers (e.g., HbA1c levels), or functional improvements.

In addition to RCTs, Real-World Evidence (RWE) is increasingly crucial in the DTx lifecycle (84). RWE, gathered from data collected during routine clinical practice outside of controlled trial settings, helps demonstrate the effectiveness, usability, and economic value of DTx across broader, more diverse patient populations. It provides insights into long-term adherence, engagement patterns, and impact on healthcare resource utilization in everyday settings.

The evidence generated focuses on clinically meaningful outcomes and patient-reported outcomes (PROs) validated for the specific condition being treated. Transparency

is key, with results expected to be published in peer-reviewed scientific journals and submitted to regulatory bodies. This robust, multi-faceted evidence base is fundamental to substantiating the therapeutic claims and value proposition of any DTx product.

9. Regulatory Pathways and Oversight of Digital Therapeutics (DTx)

As medical interventions making therapeutic claims, Digital Therapeutics (DTx) are subject to rigorous regulatory oversight in major global markets to ensure patient safety, clinical effectiveness, and data security. Unlike general wellness apps, DTx typically fall under the classification of Software as a Medical Device (SaMD), a framework developed and harmonized by international bodies like the International Medical Device Regulators Forum (IMDRF).

Key regulatory agencies governing DTx include the U.S. Food and Drug Administration (FDA) and competent authorities within the European Union overseeing CE marking under the Medical Device Regulation (MDR) and In Vitro Diagnostic Regulation (IVDR). The specific regulatory pathway a DTx must follow depends heavily on its intended use, the significance of the information it provides for healthcare decisions, and its associated level of risk to the patient.

In the United States, common pathways include the 510(k) premarket notification process for devices demonstrating substantial equivalence to a legally marketed predicate device, the De Novo classification request for novel low-to-moderate risk devices without a predicate, or the more stringent Premarket Approval (PMA) pathway for high-risk devices (85). In the European Union, DTx must undergo a conformity assessment procedure according to their risk classification (Class I, IIa, IIb, or III) under the MDR before they can bear the CE mark and be placed on the market.

Regardless of the specific pathway, regulators evaluate crucial aspects such as robust clinical evidence validating the DTx's claims, adherence to stringent Quality Management Systems (QMS, often aligned with ISO 13485), software verification and validation, cybersecurity measures to protect patient data, usability engineering, and appropriate labeling for users and clinicians (86). Navigating these complex regulatory requirements is essential for DTx developers to bring safe, effective, and reliable products to patients and providers.

10. Economic Considerations and Reimbursement of Digital Therapeutics (DTx)

The successful integration and widespread adoption of Digital Therapeutics (DTx) into healthcare systems hinge significantly on demonstrating clear economic value and

navigating the complex, evolving landscape of reimbursement. While robust clinical efficacy is the prerequisite, the long-term sustainability of DTx relies heavily on establishing viable business models and securing payment from diverse stakeholders, including public health systems, private insurers, employers, and sometimes patients themselves. This economic viability is crucial for incentivizing continued innovation and ensuring equitable patient access.

The economic value proposition of DTx often extends beyond direct clinical improvements. Key arguments include the potential for significant cost savings across the healthcare system. By improving disease management, enhancing medication adherence, enabling earlier intervention, or providing effective behavioral therapy, DTx can potentially reduce costly downstream events such as emergency room visits, hospitalizations, and complications associated with poorly managed chronic conditions (87). Furthermore, some DTx may substitute for, or reduce reliance on, more expensive traditional therapies or frequent clinician visits. Their inherent scalability as software allows for potentially lower marginal costs per patient compared to resource-intensive interventions. However, substantiating these claims requires rigorous health economic evaluations, including cost-effectiveness analyses (CEA) and budget impact analyses (BIA), often scrutinized by Health Technology Assessment (HTA) bodies where applicable.

Despite this potential, securing reimbursement remains a primary challenge for DTx developers globally. The landscape is fragmented, with no universally accepted pathway. Payers often grapple with assessing the long-term durability of DTx effects, verifying real-world patient engagement and adherence, ensuring data security and privacy, and integrating these novel therapies into existing benefit structures. The lack of standardized billing codes (like CPT codes in the US or specific national codes) and established processes for prescribing, dispensing, and tracking utilization further complicates reimbursement. Payer requirements for evidence often extend beyond clinical trial data to include robust real-world evidence (RWE) and specific economic justifications tailored to their population and budget constraints.

In response, several reimbursement models are emerging, varying by country and payer type. Some DTx are reimbursed via a traditional prescription model, processed through pharmacy benefit managers (PBMs) or medical claims, requiring specific inclusion on payer formularies. Value-Based Agreements (VBAs), where payment is contingent upon achieving predefined clinical or economic outcomes, represent a promising avenue, directly

linking cost to demonstrated value (88). Some payers utilize Per-Member-Per-Month (PMPM) fees for access across their covered population, while employers may offer DTx directly as part of wellness or health benefit packages. Notably, some countries are pioneering specific frameworks; Germany's DiGA (Digitale Gesundheits Anwendungen) pathway, for instance, provides a structured process for the assessment and nationwide reimbursement of approved DTx, serving as a potential model for others (89).

In conclusion, establishing both a compelling clinical and economic value proposition, supported by high-quality evidence, is critical for DTx. Overcoming reimbursement hurdles through the development of clear pathways, appropriate coding, and innovative payment models like VBAs is essential for realizing the full potential of digital therapeutics in transforming healthcare delivery efficiently and equitably.

11. Challenges and Limitations of Digital Therapeutics (DTx)

Despite the significant promise of Digital Therapeutics (DTx) to enhance healthcare delivery and patient outcomes, their path to widespread adoption and seamless integration is fraught with considerable challenges and limitations (90). These hurdles span technological, clinical, regulatory, economic, and user-related domains, requiring careful consideration and strategic solutions for DTx to realize their full potential globally, including in diverse settings like India.

A primary challenge lies in achieving broad clinician and patient adoption. Many healthcare providers lack awareness or training regarding DTx, face difficulties integrating them into existing clinical workflows, express skepticism about the evidence base, or have concerns about time constraints and potential liability (91). For patients, the "digital divide" remains a significant barrier; unequal access to smartphones, reliable internet, and the necessary digital literacy skills prevents equitable uptake, particularly among older adults, rural populations, and lower socioeconomic groups (92). Furthermore, building patient trust, especially concerning data privacy and security, and sustaining long-term engagement and adherence outside the controlled environment of clinical trials are persistent difficulties. "App fatigue" and the preference for human interaction can also limit sustained use.

Generating sufficient and convincing clinical and economic evidence poses another hurdle. While initial randomized controlled trials (RCTs) are crucial, demonstrating long-term effectiveness, durability of outcomes, and cost-effectiveness through real-world evidence (RWE) that satisfies skeptical payers and clinicians is complex and resource-

intensive. The rapid iteration cycles common in software development also present challenges for maintaining validation across product updates and ensuring evidence generalizability to diverse patient populations encountered in routine practice.

Technical and infrastructure limitations are also significant. Achieving seamless interoperability between DTx platforms and existing Electronic Health Record (EHR) systems is often problematic, hindering integrated care and data sharing. Ensuring robust data security and privacy in compliance with varying global regulations (e.g., HIPAA in the US, GDPR in Europe, India's Digital Personal Data Protection Act) is a complex but critical requirement (93). Basic infrastructure, including consistent internet connectivity and device availability, cannot be taken for granted in all regions. While progress is being made, navigating complex, evolving, and often fragmented regulatory pathways and securing consistent, adequate reimbursement remain persistent obstacles in many markets worldwide, limiting scalability and investment. Finally, ethical considerations, such as the potential for algorithmic bias to exacerbate health disparities, ensuring transparency in data usage, defining data ownership, and proactively working to ensure equitable access, must be addressed to prevent DTx from inadvertently widening health inequities .Overcoming these multifaceted challenges requires collaborative efforts among DTx developers, healthcare providers, patients, regulators, and payers to build trust, streamline processes, ensure equity, and ultimately integrate these innovative tools effectively and responsibly into the fabric of modern healthcare.

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MORINGA OLEIFERA: A MIRACLE HERB AND ITS MEDICINAL BENEFITS

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Abstract:

Moringa oleifera (MOL), a plant known for its wide-ranging medicinal and nutritional properties, has shown considerable therapeutic promise, especially in the area of cancer treatment. Recent research underscores MOL's anticancer effects, particularly its ability to promote apoptosis, inhibit tumor progression, and reduce reactive oxygen species (ROS) in cancer cells. A cold water extract of MOL has demonstrated the ability to suppress over 90% of targeted genes, suggesting it disrupts RNA production and inhibits cancer cell survival. Additionally, the extract selectively targets cancer cells while sparing healthy ones, positioning MOL as a potentially safer alternative to conventional chemotherapy. Moreover, aqueous MOL extracts have been shown to effectively curb tumor growth in vivo, with minimal toxicity and preservation of normal tissues, further affirming its potential as an anticancer agent. In addition to its anticancer properties, MOL has been studied for its anti-inflammatory, antimicrobial, and antioxidant actions. Its anti-inflammatory effects have been validated in several models, where MOL extracts alleviated edema and pain, working through mechanisms similar to traditional anti-inflammatory medications. Antimicrobial research has found that MOL extracts, particularly from seeds and roots, exhibit significant antibacterial and antifungal properties, strengthening its therapeutic profile. Furthermore, MOL's antioxidant effects help reduce oxidative stress, offering potential benefits in managing chronic conditions like diabetes. Collectively, these findings highlight the increasing importance of *Moringa oleifera* in contemporary medicine, not only as a cancer treatment but also as a powerful agent for managing inflammation, infections, and oxidative stress. Ongoing research into MOL's biological mechanisms could pave the way for sustainable, plant-based therapies for various health issues.

Keywords: *Moringa oleifera,* anti-Inflammatory, Oxidative Stress, Antimicrobial

Introduction:

Moringa Oleifera, commonly known as the "Miracle Herb," has gained international acclaim for its rich nutritional profile and numerous medicinal properties. Originally native to the lower slopes of the Himalayas in northwestern India, this rapidly growing, drought-tolerant tree is now extensively cultivated in tropical and subtropical regions across Africa, Asia, and South America. [1] Every part of the plant—leaves, pods, seeds, and even flowers—is infused with beneficial compounds, making Moringa highly adaptable and valued in traditional medicinal systems such as Ayurveda and various African healing traditions. Its reputation as a “miracle” plant stems from its exceptional ability to deliver significant health benefits naturally and sustainably.[2] The leaves, in particular, are known for their dense nutritional value. They are abundant in essential vitamins such as A, C, and E, which aid as dominant antioxidants that help battle oxidative damage, boost immunity, and promote skin health while reducing the risk of long-term illnesses like cancer. Moreover, Moringa leaves are a potent source of minerals like calcium, iron, magnesium, and potassium—key elements in maintaining bone health, muscle function, and overall vitality. The seeds are also prized for the oil they produce, which is widely used in cooking and skincare due to its anti-aging and antioxidant effects.[2] In addition to its dietary contributions, Moringa is highly respected for its therapeutic properties. Historically, it has been utilized to address a wide spectrum of health issues, ranging from joint inflammation and arthritis to gastrointestinal problems like ulcers and constipation. Its inherent anti-inflammatory and pain-relieving qualities make it particularly effective in easing swelling and discomfort linked to injuries or chronic conditions. Furthermore, Moringa possesses antimicrobial activity, making it a natural remedy for combating infections and supporting general health. It also plays an important role in managing blood sugar levels, making it beneficial for those with diabetes. Its strong antioxidant profile aids in neutralizing free radicals, helping to prevent cellular damage, slow down aging, and reduce the likelihood of chronic disease development. [2] One of Moringa’s most impressive traits is its comprehensive support for overall health. It aids digestion, elevates energy, improves metabolic function, and even supports cognitive health. Thanks to its high levels of vitamins, minerals, and amino acids, it promotes optimal functioning of multiple bodily systems, including the immune and nervous systems. Additionally, it has been linked to weight management benefits by helping regulate fat storage and metabolism.[3] Moringa’s detoxifying properties further contribute to its ability to cleanse the body, especially

benefiting liver and kidney function. For lactating women, studies have suggested that Moringa can help increase breast milk production, making it an essential dietary addition for new mothers. Despite its vast advantages, Moringa should be consumed with caution. Overuse, particularly of certain plant parts like the roots, can lead to adverse effects due to the presence of potentially harmful compounds. While the health benefits of Moringa are widely recognized, ongoing scientific studies continue to explore its full range of medical applications. Still, *Moringa Oleifera* remains a popular choice among those seeking natural remedies and nutritional enhancement, thanks to its potent blend of health-promoting elements. As more people turn to holistic and plant-based solutions, Moringa stands out as a true miracle herb, playing an increasingly important role in both traditional and modern wellness practices.[1-5]

Anticancer Activity

The study provides an expanded view of the remarkable therapeutic potential of *Moringa oleifera* leaves (MOL), a plant widely valued in traditional medicine and nutrition for its wide-ranging health benefits. Although *Moringa* has long been used to address numerous health issues, contemporary scientific research is increasingly confirming and extending its medicinal applications, especially its antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and, most significantly, anticancer effects. Within this framework, the researchers examined a cold water extract of MOL (prepared at 4°C with a concentration of 300 μg/mL) to evaluate its viability as a natural anticancer treatment. The findings revealed that this extract effectively triggered apoptosis, or programmed cell death, and significantly inhibited the growth and multiplication of several types of cancer cells, including those from human lung tissue. Furthermore, the extract was shown to lower the levels of reactive oxygen species (ROS) within the cells—molecules that are often associated with cancer development and cellular harm. One of the study's most noteworthy outcomes was the gene expression analysis, which showed that more than 90% of the genes tested were suppressed by at least twofold following treatment with the MOL extract. This extensive downregulation is thought to result from the disruption of normal RNA production, likely due to ribosomal RNA (rRNA) degradation. This interference with vital cellular functions may play a key role in the extract's ability to hinder cancer cell survival. Notably, the MOL extract also exhibited selective cytotoxicity—meaning it was significantly more toxic to cancer cells than to healthy ones. This property positions MOL as a promising alternative to traditional chemotherapy, which often damages both malignant

and non-cancerous cells. Overall, these results highlight the increasing relevance of natural plant compounds in the development of safer, more targeted cancer treatments. As the demand for eco-friendly and less harmful therapies grows, *Moringa oleifera* stands out as a potential key player in future cancer research and treatment strategies. The study not only reinforces the plant's medicinal value in the context of cancer but also points to its potential use in complementary and integrative health care. Continued research into the biological mechanisms behind MOL's effects may help broaden its therapeutic applications, paving the way for more holistic, plant-based approaches to managing and preventing cancer.[6]

Another study emphasized the powerful anticancer effects of the aqueous extract of *Moringa oleifera* (AEMO), which covers key bioactive compounds for example quinic acid, palmitic acid, octadecanoic acid, α-tocopherol (Vitamin E), and ɣ-sitosterol. In *in vivo* trials with mice affected by Ehrlich ascites carcinoma (EAC), AEMO significantly decreased tumor volume and weight, while also increasing the animals' lifespan. Importantly, AEMO did not cause significant damage to liver or kidney functions, nor did it affect hematological parameters, indicating its low toxicity. In *in vitro* studies, AEMO exhibited dose- and time-dependent cytotoxic effects on both EAC and HEp-2 cancer cell lines. It also promoted apoptosis in cancer cells by disrupting their mitochondrial membrane potential. These findings suggest that AEMO effectively inhibits tumor growth without harming normal bodily functions, making it a promising, safe, and natural option for cancer treatment.[7] The study highlighted the significant oncoprotective effects of *Moringa oleifera* leaf and bark extracts on MDA-MB-231 (breast cancer) and HCT-8 (colorectal cancer) cell lines. These extracts notably decreased cell survival, inhibited colony formation, and reduced cell motility by 70–90%. Additionally, treatment with the leaf and bark extracts resulted in a considerable increase in apoptotic cells, with a sevenfold upsurge in MDA-MB-231 cells and severalfold increases in HCT-8 cells. In contrast, seed extracts exhibited little to no anti-cancer activity, with minimal apoptosis observed. The cell cycle analysis revealed a G2/M phase halt, suggesting that the extracts are effective in halting cancer cell progression. GC-MS analysis identified several bioactive compounds, including eugenol, isopropyl isothiocyanate, D-allose, and hexadecanoic acid ethyl ester, all of which are known for their anti-cancer properties. These outcomes advocate that *Moringa oleifera* leaf and bark extracts, particularly those from Saudi Arabia, could serve as promising candidates for the treatment of breast and colorectal cancers. This report is the first to investigate the

oncoprotective effects of *Moringa* extracts from the region, providing valuable insights into their potential therapeutic applications.[8]

Anti-inflammatory Activity

The study analyzed twelve flavonoids in *Moringa oleifera* leaves from sub-Saharan Africa, including key compounds such as quercetin and kaempferol glucosides and glucoside malonates. To accurately quantify the flavonoids, acid hydrolysis was used to convert the conjugates into their aglycones, with recovery rates ranging from 92.6% to 107.5%. The total flavonoid content in *Moringa* leaves from Ghana, Senegal, and Zambia varied between 0.18% and 1.64% (g/dry weight), depending on the location and variety. Additionally, *Moringa* varieties with higher flavonoid content exhibited significant anti-inflammatory activity. An analysis of the thermal stability of flavonoid malonates revealed that these compounds were unstable when heated, breaking down into their respective flavonoid glycosides.[9] Another study scrutinised the inflammation lowering properties of a water-based *Moringa oleifera* root extract in rats. The results showed that a 750 mg/kg dose of the extract significantly reduced edema induced by carrageenin injection in the rat paw, with notable decreases observed at 1, 3, and 5 hours after injection. Increasing the dose to 1000 mg/kg did not enhance the effect at earlier time points and, in fact, worsened the edema at 5 hours. Indomethacin, a commonly used anti-inflammatory drug, also significantly reduced edema at all time intervals. These findings suggest that the 750 mg/kg dose of *Moringa oleifera* root extract has anti-inflammatory effects comparable to indomethacin, indicating its potential as a treatment for acute inflammation.[10]

In one more study, it was explored the therapeutic potential of the water-based extract of *Moringa oleifera* leaves, highlighting its significant antinociceptive (pain-relieving) and anti-inflammatory effects in laboratory animals. The extract exhibited dose-dependent pain-relieving properties across various pain models, such as the writhing, hot-plate, and formalin tests, as well as inflammation-suppressing action in the carrageenan-persuaded paw edema test. Notably, the study found that the pain-relieving effects were partially mediated through opioid receptors at the central level, demonstrated by naloxone's ability to reverse the effects in the hot-plate test. However, no such reversal occurred in the writhing test, suggesting a different mechanism at work. These findings align with the traditional medicinal use of *Moringa oleifera* for treating pain and inflammation, confirming its action through both central opioid-mediated pathways and peripheral non-opioid-mediated mechanisms. This study reinforces *Moringa oleifera's*

potential as a natural remedy for managing pain and inflammation, providing scientific backing to its folkloric uses.[11] The study found that the water-based extract of *Moringa oleifera* leaves (AEMO), when given at a dose of 200 mg/kg, showed significant anti-inflammatory effects in various experimental inflammation models in albino rats. The extract successfully reduced inflammation in models such as carrageenan-induced paw edema, cotton pellet-persuaded granuloma, and formaldehyde-persuaded paw edema. In all these models, the anti-inflammatory effects of the *Moringa oleifera* extract were similar to those of the standard anti-inflammatory drug dexamethasone. These results underscore the possible of *Moringa oleifera* as a natural and effective treatment for a range of inflammatory conditions.[12]

Antimicrobial Activity

The study found that the acetone extract of *Moringa oleifera* leaves revealed notable antibacterial activity against several bacterial strains, including *Escherichia coli*, *Enterobacter cloacae*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Micrococcus kristinae*. *M. kristinae* was particularly sensitive, with its growth inhibited at a concentration as low as 0.5 mg/ml. The extract was bactericidal against *E. coli* and *M. kristinae*, and bacteriostatic against *S. aureus*, *E. cloacae*, and *P. vulgaris*. Nevertheless, it exposed no activity against *Streptococcus faecalis*, *Bacillus pumilus*, *Klebsiella pneumoniae*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. Additionally, neither the acetone nor the aqueous extracts exhibited antifungal effects against *Candida albicans*, *Penicillium notatum*, *Aspergillus flavus*, or *Aspergillus niger*, even at higher concentrations. These findings underscore the antibacterial potential of *Moringa oleifera*'s acetone extract, suggesting it may be useful for treating bacterial infections.[13] The study highlighted the antimicrobial properties of *Moringa oleifera* seed extracts, which demonstrated effectiveness against both bacterial strains (including *Pasteurella multocida*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*) and fungal strains (*Fusarium solani* and *Rhizopus solani*). The extracts exhibited varying degrees of inhibition, with bacterial strains showing greater sensitivity likened to fungal strains. The antimicrobial effects were dose-dependent, and the extracts caused noticeable damage to fungal hyphae and apical branching. The minimum inhibitory concentrations (MIC) revealed that *Pasteurella multocida* and *Bacillus subtilis* were particularly sensitive to the extracts. Furthermore, the antimicrobial activity was found to be reduced by cations (Na+, K+, Mg2+, and Ca2+), with the most effective antimicrobial activity observed at temperatures ranging from 4°C to 37°C and at a neutral

pH of 7. This investigation supports the likely of *Moringa oleifera* seed extracts as a natural antimicrobial agent.[14]

The study investigated the antimicrobial effects of different extracts from *Moringa oleifera* root, including petroleum ether, ethyl acetate, chloroform, ethanol, and aqueous extracts, against several bacterial and fungal strains. The ethyl acetate extract unveiled the utmost substantial antibacterial activity, especially against *Pseudomonas aeruginosa*. The chloroform extract was ineffective against *Escherichia coli* and *Proteus mirabilis*. The water based extract showed the greatest inhibition of *Penicillium sp.* and was the only extract that inhibited *Aspergillus niger*. Phytochemical scrutiny discovered the presence of several bioactive compounds, comprising alkaloids, flavonoids, saponins, terpenoids, steroids, tannins, cardioglycosides, amino acids, and proteins, highlighting the potential therapeutic value of *Moringa oleifera* root extracts.[15]

Gastro-Protective Effects

The study found that *Moringa oleifera* leaf extract exhibited notable gastroprotective effects against aspirin-induced ulcers in rats. The lowest dose (200 mg/kg) provided minimal protection, resulting in significant mucosal damage and inflammation. The 400 mg/kg dose offered moderate protection, preserving some surface epithelial cells while still showing damage to deeper tissues. The highest dose (800 mg/kg) provided the most substantial protection, with significant preservation of the surface epithelium and enhanced mucus production, similar to the effects of cimetidine, a common anti-ulcer drug. These findings suggest that Moringa leaf extract contains active compounds that promote mucus production and offer potential as a safe and effective treatment for ulcers.[16] The study showed that Moringa oleifera leaf extract (MOAE) effectively alleviated functional constipation (FC) induced by loperamide in mice. MOAE regulated gastrointestinal hormones and neurotransmitters, as well as critical motility-related components within the enteric nervous system (ENS), interstitial cells of Cajal (ICCs), and smooth muscle cell (SMC) network. It also reduced intestinal inflammation, elevated levels of cecal short-chain fatty acids, enhanced the expression of colonic antimicrobial peptides, and strengthened the intestinal barrier. Furthermore, MOAE augmented fecal water content by suppressing the expression of colonic aquaporins (Aqp3 and Aqp4). Importantly, MOAE positively altered the gut microbiota by suppressing harmful, constipation-related bacteria and encouraging the growth of beneficial microbial

populations. These results suggest that MOAE promotes bowel movements through a combination of gut regulatory mechanisms and microbiota modulation.[17]

The study revealed that *Moringa oleifera* leaf extracts exhibit significant antiulcer effects in various experimental models of gastric and duodenal ulcers in rats. The extracts notably enhanced the healing of chronic gastric ulcers induced by acetic acid and effectively suppressed gastric acid secretion in pylorus-ligated animals. Additionally, the leaf extracts offered substantial protection against ulcers caused by ethanol, indomethacin, stress, and cysteamine, indicating both cytoprotective and antisecretory actions. In contrast, fruit extracts of *Moringa oleifera* did not show any significant antiulcer activity. Overall, the outcomes advocate that the therapeutic benefits of *Moringa oleifera* are primarily found in its leaves, supporting their potential use as a natural therapy for the prevention and management of gastric and duodenal ulcers.[18]

Antioxidant Effects

The study revealed that methanol extracts of *Moringa oleifera* pods (MOMtE) exhibit notable antidiabetic and antioxidant effects in a rat model of streptozotocin-induced diabetes. Treatment with MOMtE significantly improved crucial indicators of diabetes by lowering blood glucose and nitric oxide levels, while boosting serum insulin and protein levels. The extract also strengthened the antioxidant defense in the pancreas, as shown by increased antioxidant activity and decreased lipid peroxidation. Furthermore, tissue analysis showed that MOMtE helped repair pancreatic β-cell damage and restored normal islet structure. Overall, these results indicate that MOMtE supports blood sugar regulation and guards pancreatic health against oxidative stress, suggesting its promise as a natural remedy for diabetes and its related disorders.[19] The study revealed that *Moringa oleifera* possesses antioxidant compounds distributed across various plant parts, as identified in both ethanolic (E1) and saline (E2) extracts. Among the extracts, the ethanolic leaf tissue extract (E1d) exhibited the highest radical scavenging activity. Antioxidant presence was confirmed in all E1 and E2 extracts derived from flowers, inflorescence rachis, and leaf tissue, although the saline extracts reacted more slowly. Chromatographic analysis showed that the ethanolic extracts from flowers, inflorescence rachis, stem, and leaf tissue contained at least three flavonoids, while the saline extracts from flowers and leaf tissue contained at least two. These findings emphasize the strong antioxidant potential of *Moringa oleifera*, supporting its role as a valuable nutritional resource.[20]

Conclusion:

In conclusion, *Moringa oleifera* (MOL) shows great promise as a natural therapeutic option, especially for cancer treatment. Research indicates that MOL extracts, particularly from its leaves and bark, possess notable anticancer effects by promoting apoptosis, inhibiting tumor growth, and lowering reactive oxygen species in cancer cells. These extracts specifically target cancer cells while minimizing harm to healthy ones, making them a potentially safer alternative to conventional chemotherapy. Furthermore, MOL's antimicrobial, anti-inflammatory, and antioxidant properties enhance its therapeutic potential. As research advances, MOL's wide range of biological effects makes it a strong candidate for the development of eco-friendly, effective treatments for various health conditions.

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EXPLOITATION OF SALT'S POTENTIAL EXTREMOPHILES ON MICROPLASTICS

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Abstract:

Salt is the essential component in our daily life that is used for flavouring and preserving food, which helps in maintaining a balanced metabolic process and also plays a vital role in maintaining osmotic balance of cells. Regulated dietary intake of sodium helps in the reduction of hypertension and cardiovascular diseases. There are various types of salt used for human consumption, each with different mineral compositions and medicinal values. Salt which is mined from rocks and can be obtained from seawater were reported to have microplastics. Thus, the nutritional benefits and harmful contaminants of different types of salt need to be analysed effectively. The microplastics are tiny plastic particles usually in a range of 1 μm to 5 mm in size. They are formed due to the fragmentation of polymers and large plastic particles from industries, clothes and cosmetics. Due to their tiny size and lack of technology to quantify, they enter into salt easily from rocks and seawater causing microplastic pollution. This chapter analyses the salt types, their applications, and the screening of major pollutants in salt and their effect on human health.

Keywords: Salt, Health Impact, Microplastic.

1. Introduction:

Sodium chloride (NaCl), often referred to as "kitchen salt" or "table salt," is a refined salt that is used in cooking all around the world. Since ancient times, table salt has been used as a preservative and to flavor food. By boiling seawater, salt spring water, or salt rock mining, brine solution water is evaporated to produce salt [1]. Salt is linked to various diseases associated with lifestyle, including hypertension and cardiovascular conditions; however, it is also essential for specific metabolic processes and vital for cellular homeostasis [2]. The World Health Organization (WHO) recommends an amount of sodium intake in adults of about 2-5 grams per day [3]. Dietary sodium intake is associated with hypertension and cardiovascular diseases. Increased sodium intake leads to hypertension

and adverse effects on the heart, blood vessels, kidney, and brain whereas the decreased sodium intake reduces hypertension and heart problems [4].

Unrefined sea salts contain high-level mineral elements like Mg, K, and Ca, that are dissolved in seawater. The refined and unrefined salts obtained by rock mining contain a wide range of minerals such as Mg, Ca, Fe, S, N, and I, and thus have therapeutic benefits [4]. The trace minerals present in unrefined salt help in promoting human health, but there is a chance of toxic mineral element association, including aluminum (Al), chromium (Cr), mercury (Hg), lead (Pb) and Nickel (Ni) due to the contamination of collection sites [1].

The major pollutant analyzed even in refined salt is microplastics [MP]. The size of MPs, which are microscopic plastic particles, ranges from 1 μm to 5 mm. MPs are a major threat to the well-being of humans and the environment because of their small size, inability to be detected technologically, and potential harm to aquatic life and people. Large plastics and polymeric materials fragmenting is typically the cause of MP formation [5]. A significant portion of manufactured plastics wind up in the biosphere and contribute to environmental contamination and pollution, since plastic waste accounts for 60–80% (270,000 metric tons of polymer fragments) of all coastal debris [6].

Humans are reported to consume between 40,000 and 50,000 MPs annually, which can have a range of adverse effects on health [5]. India is the third largest manufacturer of salt in the world, with around 24 million metric tons of raw salt produced annually, of which 20% is mostly exported to other nations worldwide. In India, Tamil Nadu comes in second place behind Gujarat based on the rate of salt production [6]. There have been numerous reports from different regions of India regarding the presence of microplastics in salts from salt pans and commercially packaged salts, with diverse polymer outcomes [6]. This chapter investigates the type of contaminants in the salt such as microplastic and their applications in various fields.

2. Types of Salt:

2.1. Sea Salt:

The most common process for making salt is solar evaporation, which requires greater temperatures. Shallow ponds, where the sun and wind evaporate the water, are where salt is originally collected. Salt can be harvested manually or with the use of a machine.

This process is used to make the largest portion of salt [2]. Sodium chloride, the primary salt element and a necessary component for maintaining the osmotic balance of

cells, makes up the majority of salt [1]. The sea salt mineral composition varies with location and process of drying [2]. Salt-based preservation is a century-old technique that prolongs food's shelf life and prevents food from spoiling while preserving its flavor and texture. Salt prevents the natural ageing and discolouration caused by enzymatic browning reactions, slows the growth of microorganisms, and preserves food [2].

2.2. Himalayan Salt:

2.2.1 Black salt:

Black salt is a sulphurous, strong-smelling kiln-fired halite that is used in South Asia. It's also referred to as "Himalayan black salt" since they were mined within the region of the Himalayas [7]. It is made by melting and roasting raw Himalayan rock salt with a small amount of the fruit pulp of Amla (*Phyllanthus emblica*), Harad (*Terminalia chebula*), and Behera (*Terminalia bellerica*) as well as the bark of babul (*Acacia nilotica*). Triphala, or "three fruits," refers to the three myrobalans that are utilized collectively [8]. It is mostly composed of sodium chloride and trace amounts of sodium impurities (sulphate, sodium bisulphate, sodium sulphide, and iron sulphide). Iron sulphide gives black salt its dark violet colour, sodium chloride gives it its salty flavor. Black salt's slightly savoury flavour and unique odour is caused by a variety of compounds, with sulphide being the most significant contributor. The acidic bisulphates/bisulphites contribute a moderately sour taste [7]. Another element that contributes to the smell of spoilt milk and rotten eggs is sulphide. The salt is aged, stored, and cooled after firing before being sold [2]. The finished product appears black in crystal form, it is frequently processed into a fine powder. Black salt has long been utilized in Ayurvedic medicine and South Asian cuisine [8]

2.2.2 Pink salt:

Himalayan pink salt is the world's purest kind of salt. Nearly 84 naturally occurring minerals and elements that are present in the human body are present in it [2]. Refined and unrefined salts, which have purity levels of 99% and 96%, respectively, are the two types of salt that are mostly used. Some critical trace minerals, including Mg, Ca, Fe, S, N, and I, are found in both of these forms [4]. Compared to many other salts, it has a stronger flavor due to its mineral concentration. As a result, it is utilized as a finishing and cooking salt. For hours, it maintains the temperature. The many nutritional and therapeutic benefits of Himalayan rock salts have made them extremely valuable to customers [4]. It is obtained from the Himalayan mountains of India and Pakistan [2]. Salt is deposited by sedimentary rocks in the northwest Himalayas. Following mining from Pakistan's mines, the world's

second-largest producer of rock salt, the deposited salt is used all over the world. In addition, Himalayan rock salt is sourced directly from the source and distributed by local traders in India and many other Himalayan nations [4].

3. Health impacts of salt:

Blood pressure and salt intake are consistently correlated, according to a large body of research. Although too much sodium (salt) in the diet causes fluid retention and a consequent increase in blood pressure, sodium is necessary for controlling blood volume, blood pressure, osmotic equilibrium, and pH levels. This represents for both human and experimental animal models [9].

Salt has a significant role in maintaining the fluid equilibrium of the entire body. A high salt intake results in thirst and increased fluid intake, which raises the volume of blood overall since the fluid is then held in the intravascular compartment. Fluid retention brought on by dysregulation of these systems could be made worse by excessive salt consumption and lessened by consuming less salt [2].

High blood pressure due to hypertension increases the mortality rate of cardiovascular diseases, mainly due to the occurrence of stroke [9]. The higher the amount of salt taken, the more calcium the body loses through urination. Inadequate blood calcium levels can cause calcium to leak out of the bones. Therefore, a diet heavy in sodium has an extra unintended consequence, which is osteoporosis, a condition that thins bones [2].

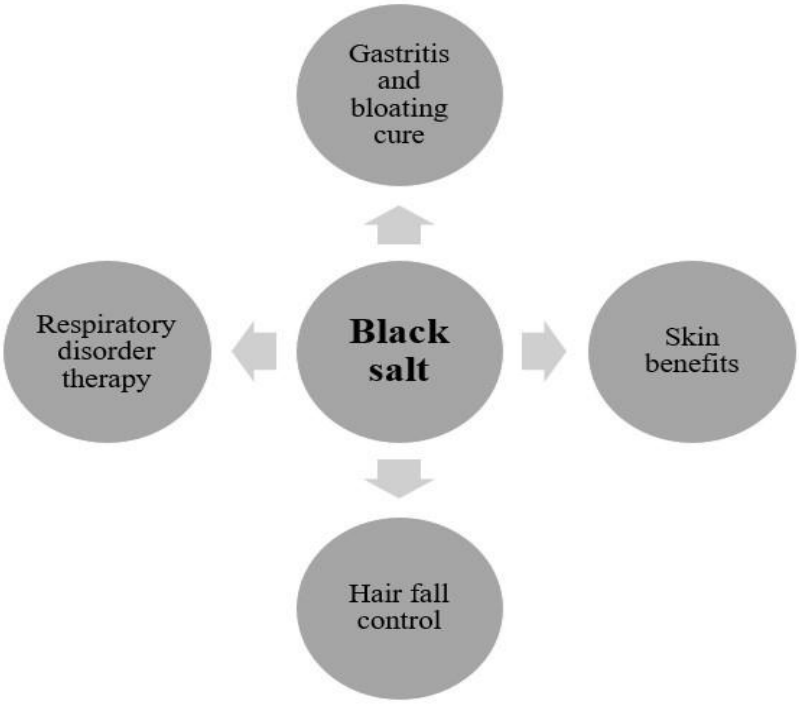
Salt and high salt food intake is associated with stomach cancer due to the salt association with *Helicobacter pylori* infection [10]. Table salt, a refined form of sea salt is usually added with iodine, which helps in reducing iodine deficiency diseases in humans [1].

Pink salt contains iron and calcium which helps in increasing the hemoglobin count and bone strength respectively. Moreover, they contain less sodium than usual sea salt, and also due to the presence of a variety of minerals such as calcium, magnesium, zinc, iron, and iodine, the pink salt provides many nutritional benefits [3,4]

Black salt is used as an ayurvedic medicine and is also utilized as an ingredient in many products for gastritis, stomach disease-related treatment, and boating because it enhances digestion without aggravating gastritis or creating stomach irritation [2,8]. As a skin-healing agent, Indian black salt can be added to lukewarm water during a bath to help relieve sprains, athlete's foot, cracked feet, and swollen feet. By using inhalers containing

black salt, respiratory issues such as the common cold and more severe conditions including allergies, sinuses, and asthma can be prevented [7].

H_2S levels are high in black salt. H_2S has become a viable option for cardiovascular disease prevention since reasonable levels of H_2S can influence mitochondrial function and prolong the lifespan of cardiac cells [8,11].



4. Screening of Pollutants:

Various studies show terrestrial and marine salts contain microplastics [12]. The salt samples collected from different countries and different sites were analyzed and have shown microplastic presence.

4.1. Microplastic Screening:

To eliminate the organic matter content, 100 ml of a 30% H_2O_2 solution was added to the salt and left in a spinning agitator. One litre of distilled water that had been prefiltered using nitrocellulose filter paper with pore sizes of 0.45 µm was used to dissolve the 200 g of salt samples. Dust and other residues (sediment particles) were eliminated from the salt samples by centrifuging the prepared salt solutions at 1900 rpm [12]. The solution is filtered through a cellulose membrane with a vacuum pump and the filtrates are then transferred to a clean petri dish and dried at room temperature. The filter paper is then used for analysis and identification of particles by SEM and FTIR.[14].

4.2. Mineral Screening:

Five grams of salt were dissolved in one ml of nitric acid (HNO3, 65% v/v) and 100 ml of ultrapure water for each sample, which was then allowed to rest for three days. The sample was prepared in closed vessels to reduce the issue of selenium volatility. Following that, 0.45 μm PTFE (Polytetrafluorethylene) filters were used to filter each sample and are used for analysis by ICP-MS [1].

4.3. Heavy Metal Screening:

An atomic Absorption spectrophotometer (AAS) is used to analyse the elements by dissolving 2g of salt samples into 3ml nitric acid this was followed by the addition of 1ml concentrated hydrochloric acid Distilled water was used to dilute the solution to 100 ml. The concentration of each element at particular wavelengths was measured using a cathode hallow lamp [15].

5. Characterization of Screened Pollutants:

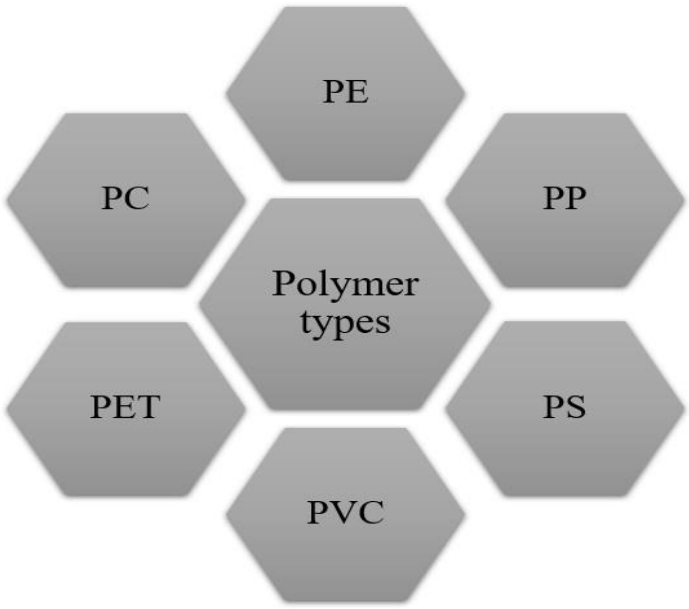
5.1.SEM Analysis:

SEM (Scanning Electron Microscope) microphotographs show that the fragment-type MPs had a rough surface with uneven edges, whereas the fibre-type MPs had flat, smooth, cylindrical surfaces. On the other hand, granular surfaces were adhered to some amorphous materials by microbead-type MPs. Sheet-type MPs had flat, layered, and uneven surfaces, while foam-type MPs showed numerous tiny breaks with surface unevenness [5].

5.2. FTIR:

The Fourier Transform Infrared Spectroscope was used to identify the polymers in a selected set of microplastics based on their colors and shapes. The absorbance in the range of 4000 to 400 cm^{-1} was used to record the spectra. The IR spectrum analysis was carried out by compressing all of the filtrate containing microplastics into pellets using KBr and a stainlesssteel dish (1 cm^2) and then using FT-IR, which emitted infrared radiation, to determine the spectrum values or wavelength number. The percentage of each polymer type was derived by grouping similar spectra from all samples [14].

Polyethene (PE), polyester (PES), polyvinyl chloride (PVC), polypropylene (PP), cellulose acetate, polyethylene terephthalate (PET), polyacrylonitrile (PAN), polycarbonate (PC), polymethyl methacrylate (PMMA), polystyrene, nylon, polytetrafluoroethylene, high-density polyethylene (HDPE), and acrylonitrile butadiene styrene (ABS) were the polymer types found in the majority of the salt samples through FT-IR identification. Polyethylene displayed dominance [6].



5.3. ICP-MS:

The single quadrupole inductively coupled plasma-mass spectrometer (ICP-MS), which was equipped with a PFA cyclonic spray chamber and driven by a 27 MHz RF solid-state generator, was used to determine the contents of Al, Ca, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn. ICP-MS analysis uses each mineral's isotopes to analyze various minerals in salt [1].

5.4. AAS:

A direct mercury analyzer, which is based on thermal decomposition amalgamation-atomic absorption spectrophotometry (TDA-AAS), was also used to determine the amount of mercury present in salt samples. Because it allows for direct sample analysis without the requirement for pre-treatment, the DMA-80 is a more flexible analytical tool than ICP-MS. Approximately 100 mg of each homogenized sample were first dried for three minutes at 230°C, and then they were thermally decomposed for three minutes at 650 °C. Working at its normal wavelength of 253.7 allowed for the determination of the Hg concentration [1].

6. Halophilic Microorganism:

6.1. Bacteria:

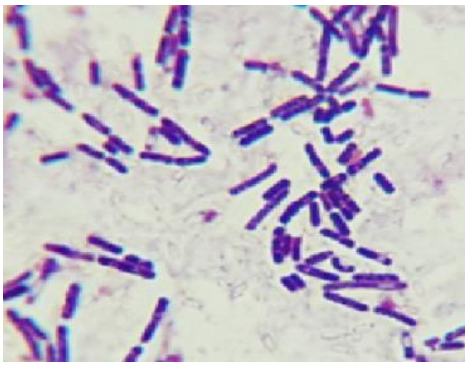
Most of the studies regarding microplastic degrading halophilic bacteria show that the *Bacillus* sp., and *Pseudomonas* sp., were effective degraders till date due to their ability to produce hydrolytic enzymes. The *Bacillus* species including *Bacillus subtilis, Bacillus cereus* and *Bacillus gotheilii* and the *Pseudomonas* species including *Pseudomonas aeruginosa* and *Pseudomonas putida* [16, 17].

Table 1: Biochemical characteristics of bacterial isolates

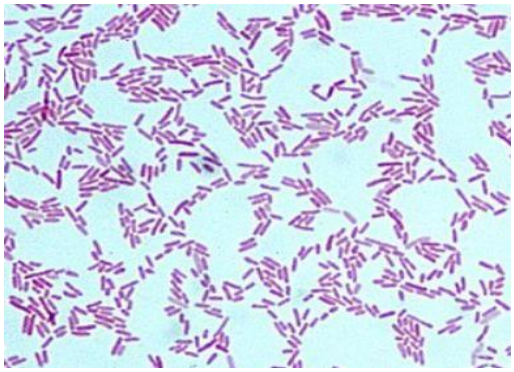
S. No.	Biochemical Characteristics	*Bacillus subtilis*	*Pseudomonas eruginosa*
1.	Capsule	Most strains are non capsulated, but some might contain polyglutamic capsule.	Non-Capsulated
2.	Shape	Rod	Rod
3.	Gram Staining	Gram-Positive	Gram-Negative
4.	Catalase	Positive (+)	Positive (+)
5.	Oxidase	Variable	Positive (+)
6.	Citrate	Positive (+)	Positive (+)
7.	Methyl Red (MR)	Negative (-)	Negative (-)
8.	Voges Proskauer (VR)	Positive (+)	Negative (-)
9.	OF (Oxidative-Fermentative)	Facultative Heterofermentative	Oxidative
10.	Coagulase	Positive (+)	Negative (-)
11.	DNase	Negative (-)	Negative (-)
12.	Urease	Negative (-)	Negative (-)
13.	Gas	Negative (-)	Negative (-)
14.	H2S	Negative (-)	Negative (-)
15.	Haemolysis	β-haemolytic	β -haemolytic
16.	Motility	Motile with peritrichous flagella	Motile with single flagella
17.	Nitrate Reduction	Positive (+)	Positive (+)
18.	Gelatin Hydrolysis	Positive (+)	Positive (+)
19.	Pigment Production	Positive (+)	Positive(+) (Yellow-green/Blue)
20.	Indole	Negative (-)	Negative (-)
21.	TSIA (Triple Sugar Iron Agar)	Alkali/Alkali (Red/ Red)	Alkali/Alkali (Red/ Red)
22.	Spore	Endospore-forming	Non-sporing

Bacillus is a gram-positive, rod-shaped bacteria and a member of the phylum Bacillota. *Bacillus* species can be either obligate aerobes which are dependent on oxygen, or facultative anaerobes which can survive in the absence of oxygen.

Pseudomonas is a genus of Gram-negative, rod-shaped, and polar-flagellated bacteria measuring 0.5 to 0.8μm belonging to the family Pseudomonadaceae. It is aerobic-facultative anaerobic bacterium.



Gram-stained *Bacillus* under 100x magnification

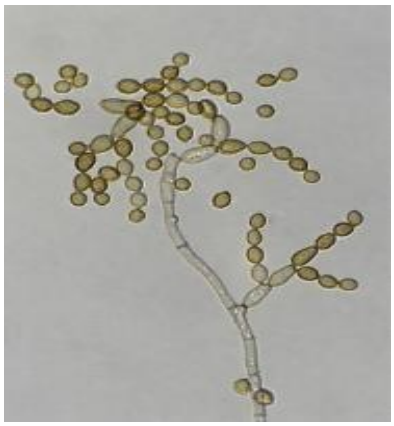


Gram-stained *Pseudomonas* under 100x magnification

6.2. Fungi:

Recent studies on microplastic degradation through halotolerant fungi show that hydrolytic enzyme-producing fungi such as *Cladosporium* sp., which includes *Cladosporium halotolerans* [18,19].

Cladosporium halotolerans is a fungus found in hypersaline environments. It has globoid conidia. They can also be isolated from dolphin skin.



Fungi under 30x magnification

Conclusion:

Because of the possible harm to human health posed by MPs, there is a growing scientific interest in human MP exposure and an increasing number of investigations of

MPs in edible salt products. This variability was connected to every operation, including QA/QC, MP identification, and sample treatment. However, variability largely resulted from various MP identification techniques related to particle selection and minimal MP cutoff size. Environmental samples like salt contain a range of MPs with various sizes, shapes, colours, and polymers. For method validation, this range of MPs ought to be accessible as reference standard materials.

To obtain reliable implications regarding human MP intake and MP source, compiling different salt MP datasets for comparative analysis, MP data should be corrected for the measured/targeted minimum MP size. Some studies also show that the MP contamination in mined salts would have arisen from the manufacturing process or packaging and storage

operations. Since salt is one of the main components of food, methods for decreasing or getting rid of MP contamination in commercial salts are essential for living better and healthier lives.

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COMPUTATIONAL CHEMISTRY IN DRUG DESIGN: TOOLS, TRENDS, AND TRANSFORMATIVE APPLICATIONS

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Abstract:

The paper highlights the effectiveness of computer-aided drug discovery (CADD) in reducing costs, time, and animal testing in drug development. It focuses on molecular docking for predicting ligand-target interactions and QSAR techniques for linking chemical structures to biological activities. The study reviews their principles, advantages, limitations, challenges, and applications in disease treatment. It also explores future big data and AI opportunities to enhance drug discovery processes.

Keywords: Computational Chemistry, Molecular Modeling, Molecular Docking, Molecular Dynamics Simulations, In Silico Drug Design.

Introduction:

Medicine, a science focused on healing and health promotion, has evolved significantly from prehistoric times to the present. It encompasses illness prevention, diagnosis, treatment, and the use of plant-based and synthetic medications. Defined as "the art of illness prevention," modern medicine plays a critical role in combating infectious diseases, managing emergencies, and controlling chronic conditions.

Drug development begins with identifying an unmet clinical need, often based on the hypothesis that targeting a specific protein or pathway may have therapeutic effects. This process involves extensive academic, clinical, and commercial research, requiring 12–15 years and over $1 billion to bring a new pharmaceutical from concept to market. Key preclinical stages include identifying targets and optimizing compounds with desirable medicinal properties.

Modern advancements focus on preventive and therapeutic measures, including vaccines for hypertension, diabetes, and cancer, alongside holistic approaches like meditation, yoga, and spirituality. These aim to enhance longevity, promote lifestyle changes, and support healthy aging, contributing to a life of greater happiness and well-being.

Modern drug discovery combines traditional medicinal chemistry with ligand-based (LBDD) and structure-based (SBDD) approaches, essential for lead optimization and developing new chemical entities. Since the 1970s, information technology (IT) has become integral to the process, enabling advancements in enzyme kinetics, X-ray crystallography, and protein structure prediction.

Historically, drug discovery followed a linear, siloed approach, relying on large-scale chemical library screenings against limited biological targets. While advancements like high-throughput screening (HTS) and combinatorial chemistry improved efficiency, the anticipated productivity gains have not been fully achieved.

Leading pharmaceutical companies and research organizations leverage CADD to accelerate drug development, reduce costs, and minimize late-stage failures. Key CADD methods include QSAR, pharmacophore modeling, molecular docking, statistical learning, and quantum mechanics for identifying novel inhibitors.

CADD integrates with wet-lab techniques to investigate drug resistance, discover new antibiotic targets, and develop novel antibiotics. Current focuses include:

- Predictive tools for assessing ADME/Tox liabilities,
- Enhanced data design and management systems,
- Programs for generating large libraries of pharmacologically relevant compounds, and
- Algorithms for evaluating selectivity and potency of lead candidates. 9

The study explores essential tools and services aiding the discovery of innovative drug candidates.

CADD, also known as **in silico screening**, is a powerful tool applied across multiple stages of drug discovery and development. It facilitates the design, testing, and manufacturing of molecules optimized for improved therapeutic outcomes. This review focuses on computational methodologies, including novel approaches and practical applications in drug development.

CADD has contributed to the creation of clinically approved drugs and encompasses techniques such as homology modeling, molecular docking, pharmacophore creation, multi-target drug design, quantitative structure–activity relationship (QSAR), and conformation generation. The field, including subdomains like computational molecule design (CAMD) and computer-assisted rational drug design, is rapidly gaining recognition and integration into modern drug research and development processes.

Classification of computer-aided methods for drug discovery

1. Quantitative structure activity relationship (QSAR)

Quantitative Structure–Activity Relationship (QSAR) is a computational modeling technique used in drug design and discovery. It involves creating mathematical models to predict the biological activity or physicochemical properties of chemical compounds based on their chemical structure.

The core principle of QSAR is:

"Similar chemical structures tend to exhibit similar biological activities."

QSAR Works as follows

1. **Data Collection**

   Start with a dataset of compounds with known chemical structures and experimentally measured biological activities (e.g., IC_{50}, Ki, EC_{50}).

2. **Descriptor Calculation**

   Molecular descriptors are numerical values that represent molecular properties. These include:

   - **Constitutional**: number of atoms, types of atoms.
   - **Topological**: connectivity and shape.
   - **Electronic**: dipole moment, electron distribution.
   - **Geometric**: molecular surface area, volume.
   - **Hydrophobic**: logP (partition coefficient), hydrophilic-lipophilic balance.

3. **Model Building**

   Statistical or machine learning techniques (e.g., linear regression, decision trees, random forests, support vector machines, neural networks) are used to correlate descriptors with activity.

4. **Validation**

   The model is validated using cross-validation or external datasets to check for **predictive power** and **robustness**.

5. **Prediction**

   The validated model can then be used to predict the biological activity of **new, untested compounds**.

2. Docking

Docking studies are computational simulations that predict how a small molecule (ligand) binds to a target macromolecule (typically a protein). The goal is to identify the

most stable binding pose, estimate the binding affinity, and understand interactions (like hydrogen bonding, hydrophobic contacts, etc.) between the ligand and the target site.

Docking is a virtual counterpart to experimental binding assays, helping researchers:

- Screen compounds before synthesis
- Guide lead optimization
- Reduce cost and time in drug discovery

Components of Docking

Ligand: A small molecule, often a drug candidate, that will be docked into a **binding site** on the target protein.

Receptor (Target Protein): A biological macromolecule (commonly an enzyme or receptor) with a known 3D structure—usually obtained from:

X-ray crystallography

NMR spectroscopy Cryo-EM

Homology modeling (if experimental structure is unavailable)

Binding Site:

- The specific region on the receptor where interaction occurs. It can be known from experimental data
- Predicted using tools like **CASTp**, **SiteMap**, or **FTMap**

Types of Docking

Rigid Docking

- Both ligand and receptor are treated as rigid.
- Fast but less accurate.

Flexible Docking

- Ligand is flexible; sometimes receptor side chains are too.
- More realistic and accurate, though computationally intensive.

Docking Workflow

Preparation

- Clean the protein: remove water, co-crystallized ligands, add hydrogens.
- Define the binding site (coordinates or active site residues).
- Prepare ligand: energy minimization, ionization states, tautomers.

Docking Algorithm

- The program generates multiple poses (conformations) of the ligand.
- These poses are placed within the binding site.

Scoring

Each pose is evaluated using a **scoring function** to estimate binding affinity (lower energy better binding).

Types of scoring functions:

- **Force-field-based** (e.g., AMBER, CHARMM)
- **Empirical**
- **Knowledge-based**
- **Machine learning-based** (emerging trend)

Analysis

Top poses are analyzed for key interactions:

- Hydrogen bonds
- π-π stacking
- Salt bridges
- Hydrophobic contacts

Applications of Docking Studies

- Virtual screening of compound libraries
- Hit identification in early drug discovery
- Binding mode analysis for known inhibitors
- Structure–Activity Relationship (SAR) understanding
- Lead optimization

Table 1: Software for docking studies

Software	**Features**	**License**
AutoDock / AutoDock Vina	Widely used, good for academic users	Free/Open source
Schrödinger Glide	High-accuracy, commercial-grade docking	Paid
MOE (Molecular Operating Environment)	Integrated suite for modeling and docking	Paid
Gold (by CCDC)	Flexible docking, genetic algorithm	Paid
SwissDock	Web-based, convenient	Free
DockThor, PatchDock, ClusPro	Specialized tools (protein–protein, blind docking)	Free

3. Lead optimization

Lead Optimization is the stage in drug development where a "lead compound"—a molecule with desired biological activity—is chemically modified to enhance its drug-like properties.

The goal is to refine and improve the compound so that it becomes a viable clinical candidate with:

- Higher efficacy
- Better selectivity
- Improved pharmacokinetics (PK)
- Reduced toxicity

Objectives of Lead Optimization

- Increase Potency: Improve the compound's activity (lower IC_{50}/Ki values).
- Enhance Selectivity: Minimize off-target effects to avoid side effects.
- Improve ADMET: Ensure the compound has good Absorption, Distribution, Metabolism, Excretion, and low Toxicity.
- Optimize Pharmacokinetics (PK): Suitable half-life, bioavailability, and clearance rates.
- Improve Physicochemical Properties: Solubility, lipophilicity (LogP), pKa, and permeability.

Strategies in Lead Optimization

1. Structure–Activity Relationship (SAR) Analysis
   - Correlate structural changes with biological activity.
   - Identify key functional groups and scaffolds contributing to binding.
2. Bioisosteric Replacement
   - Replace functional groups with bioisosteres (similar in shape/charge) to improve properties without loss of activity.
3. Molecular Modeling & Docking
   - Use docking and QSAR to predict better binding interactions and guide rational modifications.
4. Metabolic Stability Studies
   - Modify metabolically unstable regions to increase plasma half-life.
   - Avoid sites prone to CYP450 metabolism.

5. Optimization of Solubility and Permeability
   - Modify polar groups, molecular weight, or lipophilicity (LogP) to balance solubility and membrane penetration.
6. Reducing Toxicity
   - Eliminate structural alerts (toxicophores) identified by in silico toxicity prediction or in vitro screening.

Table 2: Example of Lead Optimization Process

Iteration	Chemical Change	Result
Lead 1	–OH to $–OCH_3$	Increased potency
Lead 2	Added fluorine on aromatic ring	Increased metabolic stability
Lead 3	Modified amide to urea	Improved water solubility
Lead 4	Removed basic amine	Reduced off-target activity

4. ADME estimation

ADME stands for:

- Absorption
- Distribution
- Metabolism
- Excretion

These four processes describe the fate of a drug inside the body after administration and determine how much of the drug reaches its target site in an active form.

Understanding ADME is essential in lead optimization, dose selection, formulation development, and toxicity prediction.

A – Absorption

Absorption refers to the process by which a drug moves from its site of administration (e.g., oral, intravenous, intramuscular) into the bloodstream.

Key Factors Affecting Absorption:

- Solubility: Poorly soluble drugs have lower absorption.
- Permeability: Drugs must cross biological membranes (e.g., intestinal wall).
- pH & pKa: Ionization state influences membrane crossing.
- First-pass metabolism: Liver can degrade drugs before systemic circulation (common in oral drugs).
- Transporters: e.g., P-glycoprotein (efflux pump) can reduce absorption.

Common Models/Tests:

- Caco-2 cell permeability

- LogP/LogD (lipophilicity)
- Solubility assays
- Bioavailability studies

D – Distribution

Distribution refers to how a drug spreads through the body's compartments and tissues after entering the bloodstream.

Influencing Factors:

- Plasma protein binding: Drugs bound to proteins (e.g., albumin) are inactive.
- Tissue permeability: Ability to penetrate tissues/organs.
- Blood–brain barrier (BBB): Restricts many drugs from entering the CNS.
- Volume of Distribution (Vd): A theoretical value indicating drug distribution; high Vd suggests extensive tissue binding.

Considerations:

- Lipophilic drugs tend to distribute widely.
- Polar drugs often remain in the bloodstream or extracellular fluid.

M – Metabolism

Metabolism is the chemical modification of a drug by the body, primarily in the liver.

Two Phases:

1. Phase I (Functionalization Reactions):
    - Introduce or expose functional groups (–OH, $–NH_2$)
    - Enzymes: Cytochrome P450s (CYP450)
    - Reactions: Oxidation, reduction, hydrolysis
2. Phase II (Conjugation Reactions):
    - Add polar groups to increase water solubility for excretion
    - Reactions: Glucuronidation, sulfation, acetylation, methylation

Key Concerns:

- Metabolite toxicity
- Drug-drug interactions (e.g., CYP3A4 inhibitors like grapefruit juice)
- Poor metabolism → accumulation or poor efficacy

E – Excretion

Excretion is the removal of drugs or their metabolites from the body, mainly through:

Routes:

- Renal (kidneys → urine): Most common

- Hepatic (liver → bile → feces)
- Lungs: Volatile compounds (e.g., anesthetics)
- Sweat, saliva, breast milk (minor)

Key Metrics:

- Clearance (CL): Volume of plasma cleared per unit time
- Half-life (t½): Time it takes for drug concentration to reduce by 50%
- Total body clearance = Renal CL + Hepatic CL + Others

5. Homology modelling

Homology modeling (also known as comparative modeling) is a computational method used to predict the 3D structure of a protein when its experimental structure (via X-ray crystallography, NMR, or Cryo-EM) is not available.

It works on the principle that proteins with similar amino acid sequences tend to have similar 3D structures. So, if a target protein has a known homolog (template) with a solved structure, the target's structure can be modeled based on that template.

Steps of Homology Modeling

Template Identification

- Use BLAST, HHblits, or HMMER to find homologous proteins with known 3D structures (usually in the PDB).
- Look for high sequence identity (>30% is preferred).

Sequence Alignment

- Align the target sequence with the selected template.
- Quality of alignment is critical—misaligned residues lead to bad models.
- Tools: Clustal Omega, MAFFT, T-Coffee, or software-specific aligners.

Model Building

- Based on the alignment, the software builds the backbone and side chains of the target structure by copying coordinates from the template.
- Loops and gaps are modeled using:

Model Refinement

- Energy minimization and steric clash resolution.
- Tools use **molecular mechanics force fields** (e.g., AMBER, CHARMM) to optimize geometry.

Model Validation

- Assess model quality using:
    - **Ramachandran plot** (via PROCHECK)

- **Verify3D**, **ERRAT**
- **ProSA**, **MolProbity**

Table 3: Popular Tools & Servers for Homology Modeling

Tool/Server	Features	Access
SWISS-MODEL	User-friendly, automatic modeling	https://swissmodel.expasy.org
Modeller	Python-based, powerful for batch modeling	Free (academic use)
Phyre2	Fold recognition + homology modeling	http://www.sbg.bio.ic.ac.uk/phyre2
I-TASSER	Combines threading and ab initio	https://zhanggroup.org/I-TASSER/
Robetta	Deep learning and hybrid modeling	http://robetta.bakerlab.org

6. Structure based drug discovery

Structure-Based Drug Discovery (SBDD) is a rational drug design approach that uses the 3D structure of a biological target (usually a protein) to design or optimize compounds (ligands) that can bind effectively and modulate its function.

Workflow of SBDD

Target Structure Preparation

- Retrieve structure from **Protein Data Bank (PDB)**
- Remove non-essential molecules (e.g., water, ions)
- Add missing hydrogens and fix bond orders
- Energy minimization

Binding Site Identification

- Identify known active sites or predict new pockets using tools like:
  - CASTp
  - SiteMap (Schrödinger)
  - DoGSiteScorer

Ligand Design & Virtual Screening

- Design ligands **de novo** or select from chemical libraries
- Use **molecular docking** to simulate ligand binding
- Score and rank compounds based on binding affinity

Lead Optimization

- Improve potency, selectivity, and ADMET properties
- Use **Structure-Activity Relationship (SAR)** data

Experimental Validation

- Synthesize top compounds
- Test in **biochemical assays** and **cell-based systems**

Table 4: Key Tools & Software in SBDD

Task	**Tools**
Protein prep	UCSF Chimera, PyMOL, Discovery Studio
Pocket detection	SiteMap, FTMap, CASTp
Docking	AutoDock, Glide (Schrödinger), GOLD, MOE
Visualization	PyMOL, Chimera, Discovery Studio
Binding affinity	MM-GBSA, MM-PBSA, FEP+
Dynamics	GROMACS, AMBER, Desmond
Ligand generation	LigBuilder, SPROUT, MOE

Types of SBDD Approaches

De Novo Drug Design

- Build novel ligands from scratch inside the binding site
- Algorithms grow molecules fragment-by-fragment to optimize fit

Molecular Docking

- Fit existing small molecules into the target site
- Predict binding poses, energies, and interactions

Pharmacophore Modeling

- Identify the essential features required for binding
- Design or screen compounds that match this pharmacophore

Molecular Dynamics (MD) Simulations

- Assess the stability of the protein–ligand complex over time
- Study flexibility and conformational changes

7. Molecular dynamics

Molecular Dynamics (MD) is a computer simulation technique that models the physical movements of atoms and molecules over time. It allows scientists to observe how biomolecules like proteins, DNA, and ligands behave in a dynamic environment—far beyond the static pictures provided by crystallography or docking.

MD Simulation Workflow

Structure Preparation

Import protein or complex (e.g., from PDB)

Add hydrogens, correct missing atoms or residues

System Setup

- Define force field
- Solvate the system in a water box
- Add counter-ions (e.g., Na^+/Cl^-) to neutralize charge

Energy Minimization

- Remove any **steric clashes** or bad contacts
- Ensure system is in a low-energy state

Equilibration

- Gradually **heat** the system (e.g., from 0 K to 300 K)
- Stabilize **pressure and temperature** using thermostats/barostats

Production Run

- Perform the actual simulation (e.g., 100 ns)
- Collect **trajectories** (atomic coordinates over time)

Trajectory Analysis

- **RMSD** (Root Mean Square Deviation): Structural stability
- **RMSF** (Fluctuation): Flexibility of residues
- **Radius of gyration**: Protein compactness
- **Hydrogen bonds**, **SASA**, **MM-PBSA/MM-GBSA** for binding energy

Table 5: Popular Software for MD Simulations

Software	Highlights
GROMACS	Fast, open-source, widely used
AMBER	Strong in protein–ligand systems
NAMD	Scalable for large systems
Desmond	Integrated with Schrödinger tools
CHARMM	Comprehensive but complex
OpenMM	GPU-accelerated and flexible

8. Docking method in drug discovery

Molecular docking is a computer simulation technique used to **predict the preferred orientation** of a **small molecule (ligand)** when it binds to a **target protein (receptor)**. The goal is to estimate:

- How well the ligand fits the active/binding site of the protein (geometry)
- The **binding affinity** (energy of interaction)
- The **key interactions** (hydrogen bonds, hydrophobic contacts, etc.)

Workflow of Molecular Docking

Preparation of Target Protein

- Obtain from **Protein Data Bank (PDB)**
- Clean the structure: remove water, ligands, and ions (unless relevant)
- Add hydrogens and correct protonation states
- Define the **binding site** (known active site or predicted pocket)

Preparation of Ligand Molecules

- Draw or import compound structures
- Optimize geometry, assign partial charges
- Convert to suitable format (e.g., PDBQT for AutoDock)

Docking

- Ligands are placed into the binding pocket in various **poses (conformations)**
- The software scores each pose based on **binding energy**

Scoring & Ranking

- **Scoring functions** calculate how well each ligand binds to the receptor.
- Top-ranked ligands are **selected for further analysis or synthesis**.

Table 6: Popular Docking Software

Tool	**Description**	**License**
AutoDock / AutoDock Vina	Open-source, widely used, flexible docking	Free
Glide (Schrödinger)	High accuracy, commercial	Proprietary
GOLD	Genetic algorithm-based, reliable	Commercial
MOE	Full suite for modeling, docking, QSAR	Commercial
SwissDock	Web-based, based on AutoDock	Free (web)
RosettaLigand	Flexibility in docking both ligand and protein	Free (academic)

Types of Docking

1. Rigid Docking
   - Both ligand and receptor are treated as rigid.
   - Fast but less realistic.
2. Flexible Ligand Docking
   - Ligand can rotate bonds and adjust conformation.
   - More accurate, commonly used.
3. Flexible Docking (Ligand + Receptor)
   - Accounts for flexibility in both the protein and the ligand.

- Most realistic, but computationally expensive.

Scoring functions estimate the binding free energy based on:

- Hydrogen bonds
- Electrostatic interactions
- Hydrophobic contacts
- van der Waals forces
- Desolvation and entropy

There are three main types:

1. Force-field based (e.g., AutoDock)
2. Empirical (e.g., GlideScore)
3. Knowledge-based/statistical (e.g., PMF)

9. QSAR for drug discovery

QSAR (Quantitative Structure–Activity Relationship) is a computational modeling approach that correlates the **chemical structure** of compounds with their **biological activity** using mathematical models.

The fundamental idea:

"Similar molecules often have similar biological activities."

QSAR helps predict how active or effective a new or untested compound might be—**without** needing to synthesize or test it in a lab.

QSAR Works: The Basic Concept

1. **Collect Data**

   Gather a dataset of compounds with known structures and biological activities (e.g., IC_{50}, EC_{50}).

2. **Calculate Descriptors**

   Convert chemical structures into **numerical values** (descriptors) that capture:

   - **Physicochemical** properties (e.g., logP, MW, H-bond donors)
   - **Topological** or **electronic** properties
   - **3D conformational** features

3. **Model Building**

   Use statistical or machine learning methods to relate descriptors to biological activity.

   Common algorithms:

   - Linear regression
   - Partial Least Squares (PLS)

- Random Forest
- Support Vector Machines (SVM)
- Neural Networks

4. **Validation**

   Assess the model's accuracy using **cross-validation**, **external test sets**, or **Y-randomization**.

5. **Prediction**

   Use the validated model to **predict activities** of new or hypothetical molecules.

Table 7: Types of Descriptors Used in QSAR

Descriptor Type	**Examples**
Constitutional	Molecular weight, atom count
Topological	Connectivity indices, shape
Geometrical	Molecular volume, surface area
Electronic	Dipole moment, HOMO-LUMO gap
Hydrophobic	logP, hydrophobic surface area

Types of QSAR Models

2D-QSAR

- Based on **2D structure** (no 3D conformation)
- Uses simple descriptors like logP, molecular weight, etc.
- Fast and interpretable

3D-QSAR

- Incorporates **3D spatial information**
- Key techniques:
  - **CoMFA (Comparative Molecular Field Analysis)**
  - **CoMSIA (Comparative Molecular Similarity Indices Analysis)**

Table 8: Software Tools for QSAR Modeling

Tool	**Type**	**Use**
PaDEL-Descriptor	Open-source	Descriptor calculation
KNIME	Open-source	Workflow design for ML & QSAR
QSAR Toolbox	Free (OECD)	Regulatory QSAR modeling
MOE	Commercial	2D/3D QSAR and visualization
Schrödinger QikProp	Commercial	ADME/QSAR modeling

Conclusion:

Computational chemistry has emerged as a cornerstone of modern drug design, revolutionizing the way therapeutic agents are discovered, optimized, and validated. Through powerful tools such as molecular docking, QSAR modeling, molecular dynamics simulations, and structure-based drug design, researchers can now explore vast chemical spaces and biological targets with unprecedented precision and speed.

By integrating physics-based models, data-driven algorithms, and advances in machine learning, computational methods enable more informed decision-making at every stage of the drug discovery pipeline—from target identification to lead optimization and ADME profiling. These approaches not only enhance efficiency and reduce costs but also contribute to reducing late-stage failures, accelerating the path from concept to clinic.

As technology continues to evolve, the fusion of artificial intelligence, cloud computing, and quantum chemistry is poised to further transform computational drug discovery. The future lies in a seamless synergy between in silico modeling and experimental validation, paving the way for personalized, efficient, and safer therapeutics. In this context, computational chemistry is not just a supporting tool but a driving force in the next era of pharmaceutical innovation.

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3D CELL CULTURE: ADVANCEMENTS AND LIMITATIONS

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Abstract:

Three-dimensional (3D) cell culture technologies have transformed the practice of in vitro biology by providing more physiologically relevant models that closely mimic the structural and functional complexity of native tissues. In contrast to traditional two-dimensional (2D) monolayer cultures, 3D systems present an environment that better facilitates natural cell-cell and cell-matrix interactions, spatial organization, and biochemical gradients, resulting in more faithful modeling of cellular behavior in vivo. This review offers an exhaustive overview of existing 3D cell culture methods, which consist of scaffold-based methods (using natural or synthetic biomaterials), scaffold-free systems (e.g., spheroids and organoids), and novel platforms such as 3D bioprinting and microfluidic organ-on-chip technologies. The Author critically analyzes the strengths and weaknesses of each approach, emphasizing their potential in various biomedical applications such as cancer biology, stem cell research, regenerative medicine, drug screening, and toxicology.

Keywords: 3D Cell Culture, Organoids, Microfluidic, Scaffolding

Introduction:

The power to culture cells in vitro has been a pillar of biomedical research for many years, facilitating seminal breakthroughs across the range of molecular biology to pharmacology. Yet conventional two-dimensional (2D) cell culture systems, in which cells are grown as monolayers on plane plastic or glass substrates, are incapable of mimicking the intricate structure, dynamic microenvironment, and cellular interactions found in living tissue [1]. These constraints have led to a large translational gap between in vitro results and in vivo outcomes, especially in fields like cancer research, drug discovery, and tissue engineering. To address these issues, three-dimensional (3D) cell culture systems have become potent alternatives that provide a more physiologically relevant environment for cell growth and function [2]. Cells are able to communicate with their environment in all

directions in 3D cultures and create structures more similar to the native tissue architecture and sustaining gradients of nutrients, oxygen, and signaling molecules important for correct cellular behavior [3]. These systems encompass a wide variety of technologies, including scaffold-based cultures with the use of natural or synthetic extracellular matrices, scaffold-free spheroid forming methods, organoids from stem cells or patient biopsies, and newer platforms such as 3D bioprinting and microfluidic organ-on-chip devices. Together, these models have allowed better examination of mechanisms of disease, development of tissues, and drug action.

As the technology advances, 3D cell culture systems become more and more incorporated into preclinical research protocols, providing increased predictive capacity, enhanced reproducibility, and decreased reliance on animal models [4-5]. This chapter is intended to provide a comprehensive description of the predominant forms of 3D cell culture platforms, compare their relative strengths and weaknesses, and discuss their development, ranging from biochemical assays for disease modeling to imaging-based studies. Through an integration of current advances and mapping out future pathways, this review emphasizes the vital role of 3D cell culture in the gap between bench and bedside translation.

Recent Advancement in 3D Culture Technology:

Over the past few years, the field of 3D cell culture has witnessed tremendous development, fueled by the requirement for more relevant and stable in vitro models capable of filling the gap between traditional 2D systems and the intricate in vivo setting. These developments have considerably improved the physiological significance, reproducibility, and scalability of 3D cultures, rendering them essential reagents in contemporary biomedical research.

Organoids and Patient-Derived Models

Organoids are one of the greatest advances in 3D cell culture technology, being miniaturized three-dimensional structures that replicate the cellular organization, architecture, and, in most instances, the function of their tissue of origin [6]. Organoids are generated from pluripotent stem cells (PSCs) or adult stem/progenitor cells and have been generated to replicate a range of organs, such as the intestine, liver, pancreas, brain, lung, and kidney. Their capacity for in vitro self-organization and tissue-specific differentiation render them robust systems for the modeling of organ formation, disease etiology, and host-pathogen interactions [7]. Arguably the most promising use of organoid technology is

the potential to generate patient-derived organoids (PDOs), which can be grown directly from primary tissue biopsies obtained from single patients. These models preserve the genetic, molecular, and histopathological characteristics of the donor tissue, allowing for very personalized studies of disease development and response to therapy. In cancer research, PDOs are now crucial tools for simulating tumor heterogeneity and drug resistance, and are being used ever more routinely in precision medicine pipelines to determine patient-specific treatment regimens [8]. In addition, organoids derived from patients with hereditary genetic diseases can be used to test gene editing approaches and therapeutic interventions within a patient-specific setting.

3D Bioprinting Technologies:

3D bioprinting has quickly become a state-of-the-art technology in the area of tissue engineering and regenerative medicine, allowing for the accurate construction of three-dimensional, tissue-like constructs via layer-by-layer deposition of bioinks [9]. These bioinks—consisting of living cells, biomaterials, and growth factors are formulated to replicate the native extracellular matrix and facilitate cellular viability, differentiation, and function. New advancements in bioprinting technologies, such as extrusion-based, inkjet, laser-assisted, and stereolithographic bioprinting, have greatly improved the resolution, complexity, and fidelity of printed structures [10]. Through spatial control over a variety of cell types and matrix components, 3D bioprinting makes it possible to fabricate heterogeneous tissue models that better mimic the architecture and cellular microenvironment of native organs [11]. This has significant implications for drug screening, disease modeling, and regenerative therapies. For instance, bioprinted tumor models with stromal and vascular elements provide a more complete platform for anti-cancer drug testing and the analysis of tumor-stroma interactions.

Microfluidic and Organ-on-Chip Systems:

Microfluidic and organ-on-chip (OoC) platforms are a new and growing mature technology in the field of 3D cell culture, set to mimic dynamic physiological conditions in human tissues and organs in an engineered, miniature platform [12]. These devices combine micro-scale channels, chambers, and porous membranes to mimic critical biomechanical stimuli like fluid shear stress, pressure gradients, and cyclic stretching, all of which are important for cellular function and tissue response in vivo [13]. By seeding living cells into these microenvironments, organ-on-chip devices can replicate specific tissue interfaces like the alveolar-capillary barrier of the lung or the endothelial lining of the

blood vessels with unprecedented fidelity. Recent advances have made it possible to create multi-organ chips or "body-on-a-chip" systems, in which connected microfluidic chambers mimic systemic interactions between several tissues, to better study intricate physiological and pathological processes [14]. Such platforms are especially useful for drug discovery and toxicology since they enable real-time observation of cellular responses to drugs under dynamic flow, frequently yielding more predictive outcomes than conventional static cultures or animal models.

Advanced Scaffolding and Hydrogel Materials:

Advancements in scaffold and hydrogel materials have played a central role in increasing the structural and functional fidelity of 3D cell culture systems. Scaffolds give the physical structure that is crucial for cell adhesion, proliferation, migration, and differentiation, and replicates the native extracellular matrix (ECM) closely [15]. New breakthroughs have centered on designing scaffolds with mechanistically tunable properties, biodegradability, and bioactivity to accommodate varying cell types and tissue models. Both natural biomaterials like collagen, gelatin, alginate, hyaluronic acid, and fibrin, and synthetic polymers like polyethylene glycol (PEG), poly (lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL) are popular. Natural ones provide better biocompatibility and cell-interactive properties, while synthetic scaffolds offer better mechanical strength and degradation kinetics control [16]. Hybrid scaffolds, which are a combination of both, are being designed more and more to provide optimal environments for tissue-specific functions.

Advantages of 3D Cell culture:

The transition from conventional two-dimensional (2D) cell culture models to three-dimensional (3D) systems has a number of important benefits that greatly improve the physiological significance and predictive capability of in vitro research. Perhaps the most significant advantage of 3D cell culture is that it can more closely approximate the in vivo setting by giving cells a more natural, spatially organized structure [17]. In 3D cultures, cells can generate intricate tissue-like structures that mimic the complex cell-cell and cell-matrix interactions of native tissues. These interactions are essential for the preservation of cellular functions like differentiation, migration, and signal transduction, which are generally lost in 2D models. Consequently, 3D systems have better cellular behavior, more precise gene expression profiles, and better drug responses, giving a more accurate model for disease mechanism, drug toxicity, and therapeutic effect studies [18].

Another important benefit of 3D cultures is that they can more accurately represent cellular heterogeneity, an aspect that is important in disease modeling, especially in cancer research. Tumor organoids or spheroids, for instance, better reflect the heterogeneity of solid tumors, such as areas of necrosis, hypoxia, and drug resistance that are not always present in 2D cultures [19]. Such added complexity makes it possible to more accurately test anticancer drugs and predict therapeutic outcomes. Furthermore, 3D models are better suited to examine cell migration and invasion, processes that are critical in the study of metastasis, wound healing, and tissue regeneration. 3D cell culture systems also provide a higher predictive value for drug discovery and screening [20].

Limitations of 3D Cell Culture:

While 3D cell culture systems have tremendous benefits, there are still several limitations and challenges that make them difficult to use on a large scale in both research and clinical settings. One of the major limitations is the scalability and reproducibility of 3D cultures [21]. While small-scale 3D models, including spheroids or organoids, have been useful in research, scaling these systems up into high-throughput screening or large-scale production is challenging. Most 3D models are also not standardized with different protocols, biomaterial compositions, and culture conditions, making it difficult to get reproducible results from distinct laboratories or experiments [22]. The absence of vascularization in a large number of 3D culture systems is also a main challenge. In the absence of a viable vasculature, cells in the interior of a 3D construct cannot be properly supplied with oxygen, nutrients, and waste removal, resulting in cellular necrosis and inadequate mimicry of tissue homeostasis [23]. Additionally, the tissue microenvironment complexity in 3D cultures can introduce heterogeneity, and it becomes challenging to make distinct conclusions from experiments. Lastly, cost and technical know-how are persistent hurdles [24]. The equipment and materials needed for sophisticated 3D culture methods, including microfluidic devices, 3D printers, or specialized bioreactors, may be costly and need highly trained staff to handle. This complicates the implementation of 3D cell culture models on a large scale by most research laboratories, especially those with limited budgets. Additionally, the time and labor-intensive process of establishing and cultivating 3D cultures can slow research and increase expense, especially in high-throughput drug discovery and personalized medicine applications [25].

Conclusion and Future Perspectives:

Overall, 3D cell culture systems are a great step forward for human biology modeling, providing an improved and more physiologically relevant substitute to conventional 2D cell cultures. They are revolutionizing studies in a wide range of areas, such as drug discovery, disease modeling, cancer research, and regenerative medicine, through the ability to better recapitulate tissue complexity, cellular interactions, and in vivo-like environments [26]. Advances in technologies such as organoids, bioprinting, organ-on-chip devices, and advanced scaffolding materials have led to more consistent models that closely resemble human physiology. Though the enormous strides have been taken, problems persist, especially scalability, standardization, vascularization, and technical proficiency [27-28]. To achieve the full potential of 3D cell culture, however, it will be important to develop standardized protocols, scale down processes efficiently, and make complex technologies less expensive. It will take academia, industry, and regulatory agencies working together to advance universally applicable, reproducible, and cost-efficient 3D culture systems. Eventually, as technological advancements keep advancing, 3D cell culture models will become a vital tool not just for research but also for clinical purposes, possibly revolutionizing the way we comprehend human biology, detect diseases, and create treatments.

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SYNTHESIS AND SPECTROSCOPIC STUDY OF A THIOSEMICARBAZONE LIGAND AND ITS AU (III) COMPLEX

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Abstract:

Thiosemicarbazones are well-known for their broad spectrum of biological activities and strong metal-chelating ability, making them valuable in both pharmaceutical and analytical applications. In this study, a novel ligand, 2-chloroquinoline-3-carbaldehyde thiosemicarbazone ((2-Chloro-QAT)), was synthesized and characterized using UV-Visible spectroscopy, elemental analysis, infrared spectroscopy, and X-ray diffraction techniques. The antimicrobial potential of the ligand was evaluated against *Klebsiella pneumoniae*, revealing promising inhibitory activity. Further, the complexation of 2-Chloro-QAT with Au (III) in solution was investigated spectrophotometrically. Key parameters such as absorption maxima (λ_{max}), optimum pH, reagent concentration, and complex stability were determined. The study also assessed adherence to Beer's law, Sandell's sensitivity, stoichiometry, dissociation constant, and the effect of interfering ions. Results support the ligand's dual functionality—as a bioactive agent and a sensitive reagent for the detection of Au(III). This work highlights the potential of 2-Chloro-QAT in coordination chemistry and its applicability in both medicinal and analytical domains.

Keywords: Thiosemicarbazone, 2-chloroquinoline, Spectrophotometry, Gold (III) Complex, Antimicrobial Activity, Analytical Reagent, Metal-Ligand Stoichiometry

1. Introduction:

Thiosemicarbazones represent a versatile class of organic compounds known for their wide-ranging pharmacological and chemical applications. Owing to their ability to coordinate with metal ions through sulphur and nitrogen donor atoms, these ligands have shown significant potential in medicinal chemistry [1,2]. Numerous studies have documented their broad-spectrum biological activities, including antibacterial, antifungal, antitumor and anticancer properties [3–5]. Their ability to inhibit key enzymes and disrupt cellular processes has made them attractive candidates for drug development against multidrug-resistant pathogens and various forms of cancer [6].

Beyond their biomedical relevance, thiosemicarbazones serve as effective analytical reagents due to their chelating ability and strong chromogenic responses upon metal complexation [7]. This makes them particularly useful in qualitative and quantitative analysis of transition metals through spectrophotometric techniques [8]. Among the various metal ions explored, gold(III) ions (Au^{3+}) have drawn special attention. Gold compounds possess notable anti-inflammatory and anticancer properties and exhibit interesting coordination chemistry [9,10]. The high affinity of Au(III) for donor atoms such as sulphur makes it an ideal for complex formation with thiosemicarbazone ligands. Additionally, its spectroscopic response enhances the accuracy and sensitivity of detection methods, justifying its selection in the present analytical study [11].

In this work, we report the synthesis and characterization of a novel ligand, 2-chloroquinoline-3-carbaldehyde thiosemicarbazone (2-Chloro-QAT). The compound was synthesized and characterized using UV-Visible spectroscopy (to determine λ_{max}), infrared (IR) spectroscopy, elemental analysis, and X-ray diffraction (XRD). Its antibacterial activity was evaluated against *Klebsiella pneumoniae*, a Gram-negative bacterium associated with clinical infections [12].

Furthermore, the complexation behaviour of 2-Chloro-QAT with Au(III) ions in solution was systematically studied using spectrophotometric techniques. The complex was characterized based on its absorption properties (λ_{max}), optimal pH for complex formation, effect of reagent concentration and overall stability. Analytical performance parameters such as validity of Beer's law, Sandell's sensitivity, stoichiometry of the complex, dissociation constant and the influence of diverse ions were also studied. This dual approach [combining biological evaluation with analytical application] underscores the multifunctional potential of thiosemicarbazones and their metal complexes [13].

2. Experimental Details

2.1. Synthesis and Characterization of 2-Chloroquinoline-3-Carbaldehyde Thiosemicarbazone (2-Chloro-QAT)

All reagents and solvents used in this study were of analytical grade and procured from Merck Ltd. and used without further purification. Buffer solutions required for spectrophotometric studies were prepared following standard procedures [14].

Instrumentation:

- UV-Visible spectrophotometric measurements were carried out using an ELICO model 171 spectrophotometer.

- Infrared (IR) spectra were recorded using a Perkin Elmer 221 spectrophotometer [15]
- X-ray diffraction (XRD) analysis was performed on a PW-3710 diffractometer.

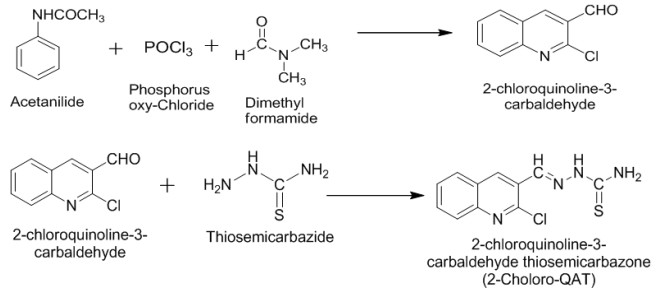
Synthesis of 2-Chloroquinoline-3-carbaldehyde (Intermediate):

The intermediate, 2-chloroquinoline-3-carbaldehyde, was synthesized via a Vilsmeier–Haack reaction. In this method, acetanilide was treated with phosphorus oxychloride ($POCl_3$) in the presence of dimethylformamide (DMF) as a solvent. The reaction yields the aldehyde functionalized quinoline derivative, which was subsequently isolated and purified.

Synthesis of 2-Chloro-QAT:

The target ligand, 2-chloroquinoline-3-carbaldehyde thiosemicarbazone (2-Chloro-QAT), was obtained by refluxing equimolar amounts of the synthesized aldehyde and thiosemicarbazide in a minimal quantity of ethanol. The reaction mixture was heated under reflux conditions until completion. The resulting product was filtered, washed, and recrystallized from ethanol to afford a yellow crystalline solid. The compound had a sharp melting point of 225 °C and a calculated molecular weight of 264.73 g/mol. This purified ligand was then subjected to further analytical and spectrophotometric studies.

Reactions



Characterization of 2-Chloroquinoline-3-carbaldehyde Thiosemicarbazone (2-Chloro-QAT)

The synthesized ligand, 2-Chloro-QAT, was characterized using various physicochemical and biological techniques to confirm its structure and investigate its properties.

Elemental Analysis:

Elemental composition was determined following standard protocols for CHNS analysis [18]to verify the purity and consistency of the synthesized ligand with its molecular formula.

Table 1: Elemental analysis

Sr. No.	Chemical Analysis	Percentage Found	Expected Percentage
1	Carbon	49.80	49.90
2	Hydrogen	03.20	03.40
3	Nitrogen	21.80	21.17
4	Sulphur	12.00	12.10
5	Chloride	13.20	13.42

Antimicrobial Activity:

The antibacterial potential of 2-Chloro-QAT was evaluated against the Gram-negative bacterium *Klebsiella pneumoniae*. The disc diffusion method was employed for this study, and the effectiveness of the ligand was assessed by measuring the diameter of the inhibition zones around the discs.

UV-Visible Spectroscopy:

The UV-Visible absorption spectrum of 2-Chloro-QAT was recorded across various wavelengths within the UV-Visible range. The maximum absorption (λ_{max}) was identified to provide insight into the electronic transitions and confirm the presence of the conjugated system.

Infrared (IR) Spectroscopy:

IR spectral analysis was performed in the range of 4000–700 cm^{-1}. The characteristic absorption bands corresponding to functional groups in the ligand were identified (as presented in Table 2), providing evidence of successful formation of the thiosemicarbazone moiety. The IR spectrum is shown in Figure 1.

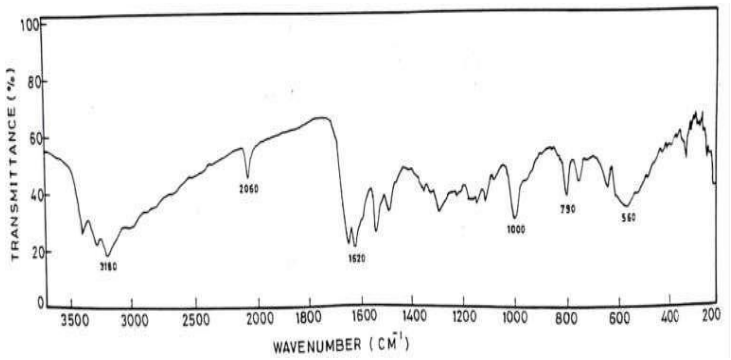


Fig. 1: IR Spectra of 2-Chloroquinoline-3-Carbaldehyde Thiosemicarbazone

Table 2: Frequencies and Corresponding Functional Groups

Sr. No.	Frequencies (cm^{-1})	Expected Elements / Functional Groups
1	3400	Simple H-bonded $–NH_2$, $–NH_3$
2	3380	–C=N stretch
3	3290	–OH, =C–H_2, –CHO (weakly)
4	2200	–N–C=S stretch
5	1700	Aldehyde >C=O stretch
6	1600	Pyridine, Quinoline rings, aromatic skeletal vibration
7	1540	–C=S, –NH (medium intensity)
8	1480	–C=S, –NH, Pyridine
9	1280	–C=S (strong intensity)
10	1140	Other olefins ≡C–H (medium)
11	1110	Other olefins ≡C–H (medium)
12	800	Five adjacent aromatic –C–H
13	750	≡C–Cl (alkyl/aryl halide stretch)

X-ray Diffraction (XRD) Analysis:

To further confirm the crystalline nature and structural arrangement of the synthesized compound, XRD analysis was conducted using a PW 3710 diffractometer equipped with a Cu anode (λ = 1.54 Å). The diffraction pattern, shown in Figure 2, provided data on the phase purity and crystallinity of the ligand.

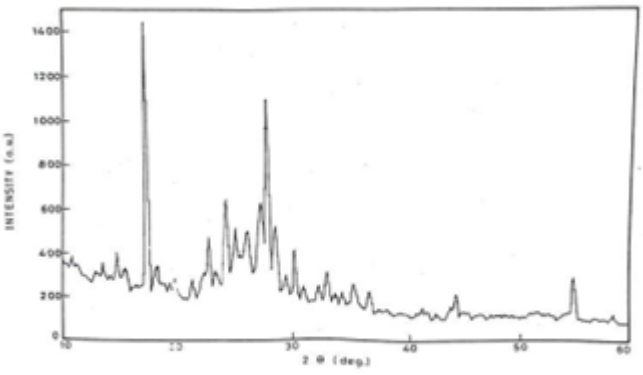


Fig. 2: X-RD of 2-Chloroquinoline-3-carbaldehyde Thiosemicarbazone

This combination of spectral, structural and biological analyses confirms the successful synthesis of 2-Chloro-QAT and supports its potential applications in both coordination chemistry and antimicrobial studies.

2.2. Spectrophotometric Study of the Au(III)–2-Chloro-QAT Complex

A detailed spectrophotometric investigation was carried out to study the interaction between Au(III) and the ligand 2-chloroquinoline-3-carbaldehyde thiosemicarbazone (2-Chloro-QAT). Equimolar solutions of Au(III) and the ligand were prepared at a concentration of 5.05×10^{-4} M. A working solution was obtained by mixing 0.5 mL of Au(III), 0.5 mL of ligand, and 1.0 mL of pH 9 buffer (prepared using boric acid, borax, and NaCl), followed by dilution to 10 mL with a DMF-water mixture [1:1]. The resulting complex solution was subjected to UV-Visible spectrophotometric analysis, and the λ_{max} was observed at 400 nm. From the absorbance data, the molar absorptivity (ε) was calculated.

To examine the stability of the complex over time, absorbance was measured at intervals of 24, 48, and 72 hours. The results indicated consistent absorbance, confirming complex stability under the given conditions.

The effect of pH on complex formation was studied by mixing 0.5 mL each of Au(III) and ligand, with 1.0 mL of buffer solutions of varying pH (1–10). Each mixture was diluted to 10 mL with a 1:1 DMF–water solution, and the absorbance at 400 nm was recorded. A graph of absorbance versus pH was plotted, revealing pH 9 as the optimum pH for complexation.

For evaluating the effect of reagent concentration, the volume of Au(III) was kept constant at 0.5 mL, while the ligand volume was varied from 0.1 to 1.0 mL. A fixed volume of buffer (1 mL, pH 9) was added, and the final volume was adjusted to 10 mL using DMF–water (1:1). The absorbance at 400 nm was monitored to determine the ideal ligand-to-metal ratio and it was found that 0.8 mL reagent was suitable.

The stability of the complex was further assessed over a period of three days, confirming no significant degradation in absorbance, which indicates a stable complex.

To verify the validity of Beer's law, different concentrations of Au(III) were used [0.1 – 0.8 mL] while maintaining constant volumes of ligand (1.0 mL) and buffer (1.0 mL, pH 9). Each solution was diluted to 10 mL with DMF–water and the absorbance at 400 nm was recorded. A linear calibration curve was obtained. Sandell's sensitivity was also calculated [17]

The stoichiometry of the complex was evaluated using both Job's method of continuous variation [16] and the mole ratio method. In Job's method, equimolar solutions of Au(III) and 2-Chloro-QAT (5.05×10^{-5} M) were mixed in various proportions with 1.0

mL buffer (pH 9) and diluted to 10 mL with DMF–water. Absorbance at 400 nm was recorded, and the stoichiometric ratio was determined from the peak of the Job's plot. In the mole ratio method the Au(III) was kept constant, while varying the ligand volume. The absorbance results were used to generate a plot confirming the metal-to-ligand stoichiometry.

The dissociation constant was calculated from plot and Mathematical equation, while stability constant of the complex was determined by inversing dissociation constant.
To assess the effect of diverse ions, equimolar solutions of Au(III) and 2-Chloro-QAT (5.05×10^{-4} M) were mixed with 1.0 mL of buffer (pH 9) and 1.0 mL of the interfering ion solution. The mixtures were diluted to 10 mL and absorbance at 400 nm was measured. The tolerance limit for each ion was determined based on the percentage deviation from the standard absorbance, thereby establishing the selectivity of the method.

3. Results and Discussion

3.1 Synthesis and Characterization of 2-Chloroquinoline-3-carbaldehyde Thiosemicarbazone (2-Chloro-QAT)

The synthesis of 2-Chloro-QAT was achieved through a two-step process. Initially, the intermediate 2-chloroquinoline-3-carbaldehyde was synthesized via a Vilsmeier–Haack reaction, involving the treatment of acetanilide with $POCl_3$ in DMF. The resultant quinoline-aldehyde was purified and subsequently condensed with thiosemicarbazide in ethanol under reflux conditions to yield the target ligand as a yellow crystalline solid. The ligand exhibited a sharp melting point at 225 °C and its molecular weight was determined to be 264.73 g/mol.

Characterization through UV-Visible spectrophotometry revealed a distinct absorption peak (λ_{max}) at 260 nm, confirming the presence of a conjugated system. The molar absorptivity was calculated to be 1.297×10^4 L mol^{-1} cm^{-1}, indicating strong absorption in the UV range. The infrared (IR) spectrum showed characteristic bands corresponding to N–H, C=N, and C=S stretching vibrations, consistent with the formation of the thiosemicarbazone moiety. X-ray diffraction (XRD) analysis suggested the ligand crystallizes in an orthorhombic system, as confirmed by the diffraction pattern. Elemental analysis (C, H, N, S, Cl) was in good agreement with the calculated values, supporting the proposed molecular formula. Additionally, preliminary biological screening demonstrated antimicrobial activity against *Klebsiella pneumoniae*, highlighting its potential as a bioactive compound.

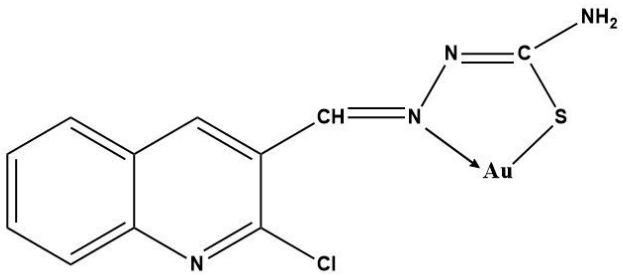
3.2. Spectrophotometric Studies of the Au(III)–2-Chloro-QAT Complex

The coordination behaviour of 2-Chloro-QAT with Au(III) was explored through UV-Visible spectrophotometric methods. A distinct charge transfer band was observed at 400 nm, indicative of complex formation[19]. The complexation was carried out in a buffered medium (pH 9) using a 1:1 DMF-water solvent system. Optimal absorbance was achieved when equimolar concentrations (5.05×10^{-4} M) of Au(III) and ligand were employed.

The effect of pH on complex stability was systematically studied across the pH range 1–10. Maximum complexation occurred at pH 9, suggesting that deprotonation of the ligand enhances its chelating ability. The influence of reagent concentration was also evaluated; maintaining a constant volume of Au(III) while varying the ligand revealed that 0.8 mL of ligand provided the highest absorbance, supporting a likely 1:1 stoichiometry.

Stability of the complex was assessed over a 72-hour period, during which no significant change in absorbance was noted, confirming its consistent stability. Beer's law was validated across a concentration range of 0.1–0.8 mL Au (III), with a linear calibration curve obtained and Sandell's sensitivity was calculated, further establishing the analytical potential of the method.

Stoichiometric analysis using Job's method of continuous variation and the mole ratio method both indicated a 1:1 metal-to-ligand ratio. The dissociation constant and stability constant were calculated from the respective absorbance plots, with a high stability constant reinforcing the strong binding affinity of 2-Chloro-QAT towards Au (III).



To evaluate selectivity, common interfering ions were introduced during complexation. The method demonstrated high tolerance levels for most ions, confirming its applicability in diverse matrices. These findings suggest that the Au (III)–2-Chloro-QAT complex is both stable and selective, rendering the ligand a promising candidate for metal ion detection and coordination studies.

Conclusion:

In this study, a novel ligand, 2-chloroquinoline-3-carbaldehyde thiosemicarbazone (2-Chloro-QAT), was successfully synthesized via a straightforward two-step protocol involving a Vilsmeier–Haack reaction followed by condensation with thiosemicarbazide. The structure of the ligand was confirmed through elemental analysis, IR spectroscopy, X-ray diffraction, and UV-Vis spectrophotometry. The ligand exhibited a distinct absorption in the UV region and demonstrated antimicrobial activity, suggesting potential biological relevance.

The spectrophotometric investigation of its interaction with Au (III) revealed stable and well-defined complex formation under alkaline conditions (optimum pH 9), with a 1:1 metal-to-ligand stoichiometry. The complex exhibited strong absorbance at 400 nm, high molar absorptivity, and remarkable temporal stability, indicating the ligand's strong affinity and selectivity for Au (III). The method developed was found to be sensitive and tolerant to common interfering ions, demonstrating its applicability in analytical or environmental settings.

While the current study focused primarily on the spectrophotometric characterization, structural elucidation of the metal complex via advanced techniques such as NMR or single-crystal X-ray crystallography could further validate the coordination mode. Moreover, the biological potential of the ligand and its metal complex warrants deeper pharmacological evaluation.

The findings from this work not only contribute to the growing interest in thiosemicarbazone-based metal chelators but also open avenues for their application in analytical chemistry, coordination chemistry and possibly medicinal chemistry, particularly in the development of metal-based antimicrobial or anticancer agents.

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WOMEN'S MENOPAUSE - PROBLEMS AND SOLUTIONS

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Introduction:

Some women in their old age try to look as beautiful as they used to. Sometimes they are not successful in doing so. She was a fifty-year-old educated woman. Although her youth has faded, she dressed like a twenty-five-year-old girl. Her lips were painted with lipstick. Her face was decorated with makeup. But her nature was no longer as playful and happy as before. She had become very irritable. She used to get annoyed. They had a twenty-two-year-old daughter. The daughter used to say that her mother should not be so sarcastic and make everyone laugh. Now she is old. She should behave like a sensible, mature woman, and dress like one. Her mother was not in the mood to listen to this. She would sit in front of the mirror for hours and look at her appearance. Sometimes she would even cry. She also had some physical ailments. Her chest would throb. Even when walking, she would feel short of breath. She felt nauseous. She would not eat because of her loss of appetite. She had weakness. She felt dizzy. Sometimes hot steam would suddenly come out of her ears and mouth. Her head and limbs ached. She also had insomnia. Her husband took her to many doctors. She was treated. She would feel better from time to time. But the same problem would start again. She was mentally exhausted. They were afraid that she would go crazy. After a thorough investigation, it was found that the woman had not been receiving proper treatment at home for the past few months. The daughter used to understand her, but would always fight with her. Her husband even He was very upset. He thought she was sexually incapable. Her face was starting to wrinkle. Her breasts were starting to sag and she found sex painful.

There was only one reason for this! The woman had stopped menstruating. Due to the ignorance of this period and the various symptoms that came with it, the woman began to feel disgusted. Her physical attractiveness had decreased and her sexual life was over: she felt that she would not be able to satisfy her husband. What is special is that this woman was known in society as a beautiful woman in her previous life and she was proud of it. But as her youth began to decline, her mind began to fear the idea of old age and when her menopause approached, she completely collapsed. Because she was ignorant about the

changes that had taken place or were taking place in her mind and body due to menopause, and she was confused and started struggling to look more and more beautiful as before.

What is Menopause?

The permanent cessation of menstruation, that is, the permanent cessation of a woman's reproduction or fertility, is called menopause or ritusamapti. During menopause, menstruation gradually stops. Menstruation begins to become irregular. Just as a girl's menstruation comes irregularly during her period, her period also becomes irregular during menopause. The gap between two periods increases. Bleeding decreases. But it is sometimes more and sometimes less. Then menstruation stops. If menstruation does not come for a year, it is considered that ritusamapti has occurred. Still, it is necessary to get a medical examination to make sure that there is no other reason behind the cessation of menstruation. Also, even if a woman's period is prolonged during menopause, it should be ensured that she is not pregnant. Because a woman's fertility is not completely stopped during the pre-menopause period.

In a woman's menstrual cycle, each month, an egg is released from the ovary One egg matures. If this egg does not meet the sperm of the man, the increased layers of the uterine lining break and fall out through the vagina. Blood vessels also break in this lining and bleeding occurs. We call this bleeding menstruation. When a woman reaches menopause, the ovaries do not produce estrogen, so the process of releasing the egg does not take place; therefore, the monthly process of the uterine lining growing every month and breaking and falling off and causing bleeding stops. Another reason for menopause is that due to the effect of the hormone estrogen, the amount of estrogen that starts the menstrual cycle of a woman decreases gradually when a woman turns forty and then finally becomes negligible. As a result, menstruation stops permanently.

According to experts, a woman's menstruation can stop between the ages of 40 and 45, and sometimes between the ages of 45 and 47, and in fact, at any time up to the age of 55. If menstruation stops before the age of forty, we cannot call it natural menopause. It may also be related to disorders of the genitals or gonads. But there may be exceptions to this. This means that it is difficult to say at what age menopause will occur.

For a few months after a girl's menstruation begins, the hormones released from the 'pituitary gland' are sometimes less and sometimes more, so the hormones released from the woman's ovaries are also released in irregular amounts. Therefore, her menstruation remains irregular until the age of sixteen to seventeen. Similarly, when a woman is 43 to

45, which means that menopause is approaching, her menstruation also becomes irregular. Because even then, less hormones are produced. This means that just as a girl's menstruation is uncertain during adolescence; the same can be true during a woman's menopause. Therefore, it is difficult to say exactly at what age a woman's menstruation will stop. However, it can stop up to a maximum of 55 years and usually by 45 years.

Reasons for Early Menopause:

Women who are iron deficient are those women who are anemia Women who are anemic may stop menstruating early. Girls from poor households who do very hard work, work in cold weather, work beyond their physical strength, and those who have a disease like tuberculosis; their periods also stop early. Girls who do not get married early and those who have this problem, may miss out on the joy of having children because they cannot tell exactly when their periods will stop early after they get married late. But there is no reason to panic. Diseases like tuberculosis or anemia can definitely be cured with medical treatment.

Some women do not follow birth control. They allow many children to be born in a row. Due to this, they have to bear their children for years, and there is also mental stress. Due to this, menstruation can also stop early. It has also been seen In some families that those whose periods start late, their periods stop early, and those whose periods start early, their periods stop late. But there are exceptions to this too. The reason for this is the irregularity and uncertainty of menstruation and its cessation. Repeated abortions also cause a woman’s menstruation to stop early.

Reasons for Late Menstruation:

Women who are healthy and strong, who stay away from mental stress, do physical labor, but do not overdo it, try to stay slim without getting obese, breastfeed their children properly, whose thyroid glands are not dysfunctional, and who keep a proper, regular and longer distance between their two children may have their menstruation stop late. There is no reason to be disappointed if menstruation stops early or late, because the uncertainty or irregularity of menopause is natural.

Now, if it is not possible to say exactly at what age menstruation will stop in menopause, can we at least say when it will stop permanently? Yes, of course. A woman can have a period for six months to a year. If a woman has not had her period for a year, it is believed that her period has stopped. However, the reason for this cessation of menstruation should be checked with the help of a doctor to see if there is any physical

disorder. If there is no such thing, then it is definitely believed that she has gone through menopause.

Symptoms of Menopause:

Menopause does not affect everyone, only twenty percent of women can experience this problem. That too, two to three years after the cessation of menstruation, very few women in our country experience such problems. Some women start experiencing physical and mental problems some time before menopause and they continue for some time after menopause.

During menopause, the function of the female ovaries slows down and stops. Due to this, the hormones estrogen and progesterone decrease. Due to this, the blood supply to the skin of the head, lips, cheeks, earlobes, nose, chin, forehead, neck, chest, etc. increases. The blood vessels in the skin suddenly contract and dilate. Due to this, the skin of that organ becomes red and hot steam comes out of that organ, heat is felt there. This is called 'Hot Flushes'. But this problem is cured with treatment. Such hot steam comes out of the body once or several times or even every hour during the day or night. The woman becomes restless and restless. But then she sweats a lot. Sometimes she feels cold and weak. 'Hot flushes' cause headaches, chest palpitations, shortness of breath, feeling like she has a fever, dizziness, difficulty sleeping, limbs twitch, pain in the grave, restlessness and anxiety and makes the nature irritable.

If the nuisance of 'hot flushes' is getting worse, it is beneficial to seek medical advice. You should see a gynecologist and show your condition. On their advice, you should use the medicine 'Estrogen'. If 'hot flushes' are a general nuisance, keep your mind calm. Don't panic. Wear loose, cotton clothes as you sweat. Sweating reduces the amount of water in the body. For this, drink plenty of water, buttermilk, lemon, Drink plenty of fluids such as syrup.

During menopause, the fat under the skin decreases. The skin becomes dry. Wrinkles appear on the face. Hair starts to look white. The voice changes. Hair grows on the face, especially on the cheeks and upper lip. Weight increases and obesity occurs. The stomach, waist, hips, thighs, buttocks look puffy and full. A woman looks like she has lost weight, but some women do not get fat. Those parts become thinner. During menopause, some women's blood pressure increases while others decrease. Heart attacks or heart problems occur due to the narrowing of the blood vessels supplying blood to the heart. Due to the loss of calcium salts and phosphorus through urine, the strength of the bones

decreases. They become brittle. They can break even with a simple shock. But this does not always happen. Still, be careful while walking or working on wet surfaces. Muscle strength also decreases during menopause. Because the body's protein metabolism is increased.

During menopause, women's internal organs are also affected. The uterus and cervix become smaller. The lining of the uterus becomes thinner. The blood vessels in it disappear. The size of the ovaries becomes about half of its previous size. The skin of the vagina becomes dry. Because horizontal wrinkles are formed in the contracted part of the narrowed vagina and vaginal discharge is also reduced. Due to this, inflammation also occurs there. The external genitalia become itchy. Sexual desire decreases. Therefore, to keep the vagina moist, one should consume plenty of fluids. Discomforts such as indigestion, constipation, loss of appetite, frequent urination, vomiting, and nausea are also seen during menopause. For this, it is also necessary to consume plenty of fluids.

During menopause, a woman's mood also changes. The nature becomes irritable, touchy, depression, anger and restlessness occur. Happiness does not seem like it. Happiness and contentment decrease. A little madness also occurs.

It happens. Sometimes hysteria-like disorders also occur. The mind cannot focus on one place. The mind cannot concentrate. Forgetfulness also occurs. Tingling in the hands and feet. Boredom and fatigue occur.

Remedies for Menopause Problems:

When menopause occurs, a woman must visit a gynecologist and get her medical check-up done regularly. Blood pressure should be checked, blood tests should be done. Sometimes during this period, due to hormonal imbalance, there is heavy bleeding during menstruation. Sometimes it lasts for a long time. Whether this heavy bleeding is due to menopause or some other disorder of the genitals; this should be examined by a specialist. Whether it is cervical cancer or not, this can also be diagnosed on the basis of a 'Pap smear' examination. If there is bleeding due to menopause, then 'drug therapy' and 'curating' are useful to control it. But if the bleeding is excessive and persistent, a hysterectomy is an emergency surgery to remove the uterus.

In a woman who has gone through menopause, the amount of the hormone 'estrogen' decreases; it becomes almost negligible. The production of the hormone 'estrogen' does not start again naturally. Experts believe that taking estrogen pills and injections as per medical advice can reduce the troublesome symptoms of menopause. However, estrogen medications should not be taken hastily, without a medical

examination, because taking too much 'estrogen' can be harmful and taking too little has no effect.

Some experts believe that women experiencing menopause symptoms do not need to take 'estrogen'. If the woman focuses on her favorite work, her relatives try to understand her, and the woman is given scientific information about these symptoms, the discomfort decreases or does not occur at all once she gets used to the changes. According to these experts, women who take estrogen have a higher incidence of uterine and breast cancer. But according to some experts, the idea that taking estrogen causes cancer is wrong. According to them, those who have cancer or are likely to get it can develop cancer if they take estrogen. According to them, estrogen is a boon that gives women their previous happy life. The youth that a woman gets gives her femininity.

It is because of this estrogen that the rate of heart attacks in women is found to be low. According to these experts, if a medical examination is done after the end of menstruation and the right amount of estrogen is taken, breast or uterine cancer does not occur. According to them, if women who have experienced menopause symptoms take the right amount of estrogen, their health will change within a month. Wrinkles on the face disappear. Hair loss on the face disappears. The breasts become round and plump. The uterus returns to its previous shape. The length and position of the narrowed vagina becomes the same as before. The vagina is moist as before. Therefore, sexual activity and intercourse do not feel painful. Sexual satisfaction can be obtained. It feels like old age is going away.

Feeling like youth has been achieved, the mood becomes cheerful. The face looks happy and cheerful. According to these experts, after such a favorable change due to the effect of 'estrogen', it is necessary for husband and wife to have intercourse from time to time to keep their sexual organs functional. Also, if there is a problem of vaginal bleeding while taking 'estrogen', one should see a specialist. Also, it is necessary to get yourself checked regularly by a doctor while taking 'estrogen'. Even if 'estrogen' treatment is not being continued, it is necessary to get other symptoms or problems that occur during the menopause period due to menopause or some other disease, it is also necessary to get it checked through a medical examination.

In fact, no woman becomes old immediately after menstruation stops. Her sexual desire does not decrease immediately, on the contrary Some women may also experience increased libido. Due to reduced family responsibilities, the certainty of not having children

in the future, and the realization that there is no one else close to them except their husbands in their old age, the bond of affection, love, mental, and emotional attachment to each other becomes stronger. A more passionate relationship can also develop between husband and wife. They can live a more free and happy love life even after menopause. Therefore, we no longer have the ability to reproduce like before and we will not be able to support our husbands in love life as before; this fear should not be harbored after menopause.

Why do women experience changes in their minds and bodies after menopause? If we know this, we will be able to protect ourselves from the disappointment, sadness, loneliness, sensitivity, hysteria, etc. that may arise due to misunderstanding and ignorance, and with renewed vigor, we can try to make our love life satisfying, enjoyable, and healthy. So, we should not assume that our libido has disappeared because our menstrual cycle has stopped. Libido and sexual satisfaction depend on the mind, not because of the reduced estrogen in menopause. Even if libido decreases, we should not feel bad about it. Because in the post-menopausal period, what is more important and necessary than sexual satisfaction is the affection, love and respect we receive from our loved ones.

A woman does go through menopause. But if we want to eliminate the problems of future menopause, women should take good care of their health from the very beginning, that is, from the time they are in their youth. And they should try to stay strong both physically and mentally. That is, there is no special problem during menopause. And it is not felt.

Instead of making a fuss about the problems that occur during menopause, we should get used to it by thinking that the problem is now normal. Therefore, the problem is felt less. The second reason is that sometimes, even after treatment, the problems during menopause do not disappear completely. During menopause, women should eat a healthy diet. The diet should include substitute foods, pulses, cereals, fish, egg whites, skimmed milk, iron, vitamin tablets (as per the doctor's advice). Sweets, oil, ghee, butter, curd, sugar, jaggery, nuts should be consumed in very small quantities. Sometimes a menopausal woman feels that even her grown-up children do not understand her. At such times, her husband feels very close.

At such times, the husband should treat his wife with love and sympathy. But that sympathy should not be perceived as a begging for mercy by the woman. Since a menopausal woman's mind is sensitive, she should not do any act or behavior that will hurt

her self-esteem and cause her mental distress. This should also be kept in mind for other relatives of the woman's family. Do not use words like "I am getting old" or "I am not smart enough". A menopausal woman should not blame herself for her own problems. And she should not be arrogant and expect undue help from others. And she has the right to do so. She should not be in this delusion. She should also take a role of reconciliation. She should also try to maintain her physical and mental health herself. Postmenopausal women should engage themselves in their favorite hobbies.

They should connect with the environment in which they feel happy and free. Some people may want to go to the library and read books, some may want to take a walk in the garden with their children and grandchildren. They may find peace in going to the temple and listening to kirtan. Some may want to ease their sorrow by chatting with friends. Some may want to do social work, some may find happiness by staying at home with their children, telling them stories, playing with them, and making them happy.

They can maintain their interest by enjoying good and interesting programs that are being broadcasted on television and radio at home. Even watching movies or plays with their husbands or family occasionally will keep their minds entertained. If you engage your mind in all these things that you like, your mind and body remain refreshed. And the pain of menopause goes away somewhere. Because the mind is not aware of it and even if it does happen, it does not feel intense.

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ISOLATION OF PSYCHROTROPHIC BACTERIA IN MILK FROM DAIRIES OF AURANGABAD

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Abstract:

In this study, psychrotrophic bacteria were isolated from milk samples from buffalo, cows, and curd obtained from five dairies in Aurangabad, namely Bharat milk dairy, Quality Milk dairy, Seema Milk Dairy, Janta Milk dairy and Ajanta Milk Dairy in different seasons such as winter, summer and rainy. All the samples were assigned codes to study further. Total 25 psychrotrophic bacteria were isolated from buffalo and cow milk, out of which nine isolates obtained from sample BBM [Bharat Buffalo Milk], two from SBM [Seema Buffalo Milk], five from QCM [Quality Cow Milk], four from JBM [Janta Buffalo Milk], two from JCM [Janta Cow Milk], three from ABM [Aurangabad Buffalo Milk]. Morphological and biochemical characters of psychrotrophic bacterial isolates were studied.

Keywords: Psychrotrophic Bacteria, Milk, Dairies in Aurangabad

Introduction:

According to Atherton and Newlander, (1987) milk is a whole clean fresh lacteal secretion which is free from colostrum obtained from some animals such as buffalo, cow, goat, sheep, camel etc. having not less than 8.25 % milk solids and 3.25% of milk fat. It is a well-known fact that milk is not a homogeneous fluid. Similarly, milk is also not a pure colloidal system. It could be even observed that in some milk products, the fat globules exist as hydrophobic colloids along with air bubbles, and crystals of fat and lactose. Milk contains on an average 3.3% proteins with nine essential amino acids necessary for human beings (Hardings, 1999). On the basis of physicochemical properties of milk, milk proteins are categorized into caseins and constitute 82% of milk proteins. There are three sub classes of casein protein namely α- casein β- casein K- casein. These subclasses show different amino acid composition, functional properties and genetic variability. Casein is a phosphorous containing protein and precipitate at 4.6 pH. Another milk protein is serum (whey) protein present in solution form in milk without phosphorous, but contain sulfur

containing amino acid. It is present 18% of the total milk protein with 20%, 50% α and β lactoglobulin respectively. The detailed functioning of serum (whey) proteins are not known but β lactoglobulin is considered as a carrier of vitamin A, while α lactoglobulin is involved in lactose synthesis in memory gland. Similarly, lactoferin and transferrin helps in iron absorption. Whey proteins are poor digestible in intestine as compare to caseins (Yadav *et al.,* 1993).

Microorganism of Raw Milk

Milk considered as an ideal medium for microbial growth mainly bacteria. A heterogenous population of bacteria such as mesophilic, thermophilic, psychrophilic found in milk and forms a milk micro flora.

Mesophilic Bacteria

Microorganisms which grow optimally at 30^0C - 37^0C are referred as mesophilic. These bacteria introduced into the milk through insanitary conditions of milking equipment, milker and handler, farm water supplies, transport and storage (Yadav *et al.,* 1993). Mesophilic bacteria generally found in milk *are Acinetobacter viscosymbioticum, A. lipopyticum, A. viscolactis, Bacillus cerus, B. subtilis, Corynebacterium diptheriae, C. pyogenes, C. bovis, E. coli, Enterobacter aerogens, Flavobacterium maloloris, F. arborescens, klebsiella pneumonia, lactobacillus casei, L. plantarum, L. acidophilus, Micrococcus flavum, M. varians, M. luteus, Pseudomonas fluorescens, Pseudomonas aeroginosa, Pseudomonas fragi, Staphylococcus aureus, S. epdermidis, Candida, Saccharomyces, Penicillium, Aspergillus, Rhizopus* (Yadav *et al.,* 1993).

Thermophilic Bacteria

Thermophilic bacteria are those which can survive and grow at pasteurization temperature. These bacteria can grow at temperature ranges from 55^0C -70^0C. Beside this few facultative thermophile may grow at 37^0C or lower by SPC (Standard plate Count Method) which is an important method for enumeration of bacteria. Thermophilic bacteria of raw milk are aerobic or facultative anaerobic spore forming rods such as *Bacillus stearothermophilus, Bacillus thermoacidurans, Bacillus calidolactis, Bacillus coagulans.* Major source of thermophilic bacterial contamination of milk and milk products are uncleaned utensils. (Yadav *et al.,* 1993).

Psychrotrophic Bacteria

Psychrotrophic bacteria differ from psychrophilic bacteria, as psychrophiles have optimum growth temperature of 15^0C or lower and 20^0C as a maximum growth

temperature. Minimum temperature at which the psychrophiles can grow is 0^0C or lower. (Kushner, 1978). Bacteria which can grow at a temperature of 7^0C or lower but their optimum growth temperature was higher in mesophilic range, called as **psychrotrophs**. Their optimal metabolic activity is expressed at temperatures between 20^0C and 30^0C. However, they can grow and multiply at low temperatures through an enrichment of polyunsaturated fatty acid in their membrane lipids. In other words, the altered cell membrane secures sufficient permeability for membrane fluidity and transport activity of metabolites necessary for growth and reproduction of bacteria at low temperatures (Schinik, 1999). Studies reveals that psychrotrophic bacteria have been isolated from different cold environments such as Antarctic regions, deep oceans, also from environments where seasonally cold conditions were observed shows their ability to resist high temperature.

These bacteria are able to produce cold active enzymes with higher catalytic activities at lower temperatures compare to mesophilic and thermophilic temperature. Recently biotechnology and dairy science focused on applications of psychrophiles, psychrotrophs and cold active enzymes. Different biochemical reactions carried out by milk micro flora leads to flavor deterioration and milk spoilage. These reaction leads to breakdown of lactose, protein, fat and makes the milk not suitable for drinking. Microorganisms brings the fermentation of milk constituents like conversion of milk to curd due to lactic acid formation which is considered as normal fermentation whereas roppiness, gassiness, proteolysis, lipolysis are unusual fermentation reactions.

Material and Methods:

Sampling Sites

In this research work samples of milk and curd were collected from different dairies of Aurangabad city. It was observed maximum people in Aurangabad city purchased milk and milk products from there nearby dairies while returning from their job or shops. Dairy owners purchase the milk from milkman that are coming from nearby rural areas. This purchased milk were stored by dairy personnel in refrigerator. Temperature in refrigerator is maintained below 70^0C. It is needless to say that leftover milk in refrigerator was get mixed with fresh milk. It allows psychrotrophic bacteria to proliferate in refrigerators. Variation was found in cleaning of dairy and different milk utensils, refrigerator etc. By considering above observations five dairies were selected for sampling of milk and curd. Dairies were selected on the basis of total sale of milk and milk products daily, maintenance

of hygiene, no of employees working, location of dairies and average no. of 200 customers who purchased daily milk and milk products from particular dairy. Name of dairies were Bharat milk dairy, Quality Milk dairy, Seema Milk Dairy, Janta Milk dairy and Ajanta Milk Dairy. Sample of milk and curd was collected in different seasons such as winter, summer and rainy.

Isolation of Psychrotrophic Bacteria from Milk Sample

Isolation of psychrotrophic bacteria was carried out from the milk sample (buffalo/cow) and curd sample (sweet/sour). A loop full suspension of selected dilutions (10^{-6}, 10^{-8}, 10^{-10}) of each sample was streaked by four quadrant method on sterile milk agar plates and incubated for 10 days at 7^0C temperature for the growth of psychrotrophic bacteria. After incubation colonies on milk agar plates were observed and different colonies were selected on the basis of colony characters, morphological characters and biochemical characters. Selected bacterial colonies were checked for its purity and transferred on milk agar slants after proper labeling for further study. After growth on milk agar slants, slants were kept in refrigerator and repeatedly sub cultured at regular intervals.

Biochemical Characteristics

Fermentation of Different Sugars

All the isolates were tested for their ability to ferment different types of sugars in sterile peptone broth with 1% carbohydrate, and 0.01% of phenol red indicator solution described by Cruickshank *et al.,* (1975). Different sugars tested against selected isolates are as follows.

- Monosaccharides: These are the carbohydrates having one sugar. Monosaccharides further divided into different types on the basis of no of carbon present in sugar such as triose (3C), tetrose (4C), pentose (5C), hexose (6C), and heptose (7C).
- Pentoses (5C): L-Arabinose, L-Rhamnose, D-Xylose, D-Ribose.
- Hexoses (6C): D-Glucose, D-Galactose, D-Fructose, Mannose.
- Disaccharides: These are the sugars which contain two sugars linked by glycosidic linkage namely Sucrose, Lactose, and Maltose.
- Trisaccharides: These are the sugars which contain three sugars linked by glycosidic linkage namely Raffinose. Polyhydric Alcohols: Sorbitol, Mannitol.

Result and Discussion:

Sample Collection and Coding

Psychrotrophic bacteria were isolated from buffalo and cow milk and investigated in this study. The sour and sweet curd samples were also used in this study. All the samples were collected from different dairies of Aurangabad namely Bharat milk dairy, Quality Milk dairy, Seema Milk Dairy, Janta Milk dairy and Ajanta Milk Dairy in different seasons such as winter, summer and rainy. All the samples were assigned codes to study further. The buffalo milk samples were coded BBM (Bharat buffalo milk), QBM [Quality buffalo milk], SBM [Seema buffalo milk], ABM [Ajanta buffalo milk] and JBM [Janta buffalo milk]. These samples were further separated by seasonal variation shown in Table 1.

Table 1: Codes of Buffalo milk samples in different seasons.

S. No.	Codes Assigned	Winter(w)	Summer(s)	Rainy(r)
1	BBM	BBMw	BBMs	BBMr
2	QBM	QBMw	QBMs	QBMr
3	SBM	SBMw	SBMs	SBMr
4	ABM	ABMw	ABMs	ABMr
5	JBM	JBMw	JBMs	JBMr

Table 1 shows each milk sample further separated by seasonal variation i.e. BBMw [Bharat buffalo milk winter season], BBMs [Bharat buffalo milk summer season], BBMr [Bharat buffalo milk rainy season]. Other milk samples also assigned codes accordingly.

Cow milk samples were coded BCM [Bharat cow milk], QCM [Quality cow milk], SCM [Seema cow milk], ACM [Ajanta cow milk] and JCM [Janta cow milk]. Seasonal variation of these samples shown in Table 2.

Table 2: Codes of Cow milk samples in different seasons.

S. No.	Codes Assigned	Winter(w)	Summer(s)	Rainy(r)
1	BCM	BCMw	BCMs	BCMr
2	QCM	QCMw	QCMs	QCMr
3	SCM	SCMw	SCMs	SCMr
4	ACM	ACMw	ACMs	ACMr
5	JCM	JCMw	JCMs	JCMr

Table 1 and 2 shows each milk sample further separated by seasonal variation i.e. BCMw [Bharat cow milk winter season], BCMs [Bharat cow milk summer season], BCMr [Bharat cow milk rainy season]. Other milk samples also assigned codes accordingly.

Isolation of Psychrotrophic Bacteria

Total 25 psychrotrophic bacteria were isolated from milk samples. Two types of milk ie buffalo milk and cow milk used for the isolation of psychrotrophic bacteria. All the psychrotrophic bacterial isolates were assign codes and shown in Table 3 and 4. These tables showed code of samples, number of isolates and respective sample name.

Table 3 and 4 shows that total twenty-five samples were isolated from buffalo and cow milk from selected dairies of Aurangabad. Nine isolates obtained from sample BBM [Bharat Buffalo Milk] namely BBM-1,BBM-2, BBM-3, BBM-4, BBM-5, BBM-6, BBM-7, BBM-8, BBM-9, two from SBM [Seema Buffalo Milk] sample namely SBM-1, SBM-2, five from QCM [Quality Cow Milk] sample namely QCM-1, QCM-2, QCM-3, QCM-4, QCM-5, four from JBM [Janta Buffalo Milk] namely JBM-1, JBM-2, JBM-3, JBM-4, two from JCM [Janta Cow Milk] namely JCM-1, JCM-2, three from ABM [Aurangabad Buffalo Milk] sample namely ABM-1, ABM-2, ABM-3.

Table 3: Buffalo milk samples

Codes of Samples	**No. of Isolates**	**Codes of Isolates**
BBM	09	BBM-1, BBM-2, BBM-3, BBM-4, BBM-5, BBM-6, BBM-7, BBM-8, BBM-9
SBM	02	SBM-1, SBM-2
JBM	04	JBM-1, JBM-2, JBM-3, JBM-4
ABM	03	ABM-1, ABM-2, ABM-3

Table 4: Cow milk samples

Codes of Samples	**No. of Isolates**	**Codes of Isolates**
QCM	05	QCM-1, QCM-2, QCM-3, QCM-4, QCM-5
JCM	02	JCM-1, JCM-2

Cultural Characteristics of the Bacterial Isolates

Table 5: Colony Characters of psychrotrophic bacterial isolates.

Sr. No	Code of isolates	Size in [mm]	Shape	Colour	Margin	Opacity	Elevation	Consistency
1	BBM-1	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
2	BBM-2	1.0	Circular	Yellow	Entire	Opaque	Convex	Mucoid
3	BBM-3	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
4	BBM-4	1.0	Circular	Yellow	Entire	Opaque	Convex	Mucoid
5	BBM-5	1.0	Circular	Yellow	Entire	Opaque	Convex	Mucoid
6	BBM-6	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
7	BBM-7	1.0	Circular	Off-white	Entire	Opaque	Convex	Mucoid
8	BBM-8	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
9	BBM-9	1.0	Circular	Colourless	Entire	Opaque	Convex	Mucoid
10	SBM-1	1.5	Circular	White	Entire	Opaque	Convex	Mucoid
11	SBM-2	1.0	Circular	White	Entire	Non opaque	Convex	Mucoid
12	JBM-1	1.0	Circular	Milky white	Entire	Opaque	Convex	Mucoid
13	JBM-2	1.0	Circular	Golden yellow	Entire	Opaque	Convex	Mucoid
14	JBM-3	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
15	JBM-4	1.0	Circular	Colourless	Entire	Opaque	Convex	Mucoid
16	ABM-1	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
17	ABM-2	1.5	Circular	White	Entire	Opaque	Convex	Mucoid
18	ABM-3	1.5	Circular	White	Entire	Non Opaque	Convex	Mucoid
19	QCM-1	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
20	QCM-2	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
21	QCM-3	1.0	Circular	Off white	Entire	Semi translucent	Convex	Mucoid
22	QCM-4	1.0	Circular	Off white	Entire	Opaque	Convex	Mucoid
23	QCM-5	1.0	Circular	White	Entire	Opaque	Convex	Mucoid
24	JCM-1	1.0	Circular	Golden yellow	Entire	Non Opaque	Convex	Mucoid
25	JCM-2	1.5	Circular	Milky white	Entire	Opaque	Convex	Smooth

Table 6: Physicochemical properties of bacterial isolates.

Sr. No	Code of isolates	Gram's nature	Motility Motility	Morphology	Spore production	PHB Granules
1	BBM-1	Gram positive	Motile	Rods	- ve	- ve
2	BBM-2	Gram positive	Non motile	Cocci	- ve	- ve
3	BBM-3	Gram positive	Non motile	Short Rods	- ve	- ve
4	BBM-4	Gram positive	Non motile	Rods	- ve	- ve
5	BBM-5	Gram positive	Non motile	Cocci	- ve	- ve
6	BBM-6	Gram positive	Non motile	Rods	+ ve	+ ve
7	BBM-7	Gram negative	Motile	Rods	- ve	- ve
8	BBM-8	Gram positive	Non motile	Cocci in pair	- ve	- ve
9	BBM-9	Gram positive	Non motile	Rods	- ve	- ve
10	SBM-1	Gram positive	Non motile	Rods	- ve	- ve
11	SBM-2	Gram negative	Motile	Rods	- ve	- ve
12	JBM-1	Gram positive	Non motile	Cocci in bunch	- ve	- ve
13	JBM-2	Gram positive	Non motile	Rods	+ ve	+ ve
14	JBM-3	Gram positive	Non motile	Cocci	- ve	- ve
15	JBM-4	Gram positive	Non motile	Rods	- ve	- ve
16	ABM-1	Gram negative	Motile	Rods	- ve	- ve
17	ABM-2	Gram positive	Motile	Cocci	- ve	+ ve
18	ABM-3	Gram positive	Motile	Rods	- ve	- ve
19	QCM-1	Gram positive	Non motile	Cocci	- ve	- ve
20	QCM-2	Gram positive	Non motile	Rods	+ ve	+ ve
21	QCM-3	Gram positive	Motile	Rods	- ve	- ve
22	QCM-4	Gram positive	Non motile	Cocci	- ve	- ve
23	QCM-5	Gram positive	Non motile	Rods	- ve	- ve
24	JCM-1	Gram positive	Non motile	Rods	- ve	- ve
25	JCM-2	Gram positive	Motile	Rods	- ve	- ve

Table 5 shows Colony characters of all 25 psychrotrophic bacterial isolates obtained from milk and curd sample of different dairies of Aurangabad were noted down. The average size of colony was observed is 1mm in diameter in twenty two isolates namely BBM-1, BBM-2, BBM-3, BBM-4, BBM-5, BBM-6, BBM-7, BBM-8, BBM-9, SBM-2, JBM-1, JBM-2, JBM-3, JBM-4, ABM-1 ABM-3, QCM-1, QCM-2, QCM-3, QCM-4, QCM-5, JCM-1. The three isolates namely SBM-1, ABM-2 AND JCM-2 observed 1.5 mm. The shape of all the psychrotrophic bacterial isolates were circular with entire margin. The colour of the colonies were white, off white, yellow, golden yellow or orange red. Twelve isolates produced white colonies namely BBM-1, BBM-2, BBM3, BBM-6, BBM-8, SBM-1, SBM-2, ABM-1, ABM-2, QCM-1, QCM-2, QCM-5, three isolates produced off white and milky white colonies namely QCM-3, QCM-4. Six isolates produced yellow and golden yellow colonies namely BBM-4, BBM-5, ABM-3, BBM-7, JBM-2, JCM-1. Two colonies produced colourless colonies namely BBM-9, JBM-4. Elevation of the colonies shows that twenty one isolates produced convex type of elevation and four of the isolates produced flat elevation. Consistency and opacity of isolates shows that twenty two colonies with opaque and three non-opaque while one isolate shows semi translucent type of consistency.

Gram's nature, morphology, motility, spore formation and PHB granules production by all twenty five isolates were shown in Table 6.

All the 25 psychrotrophic bacterial isolates were Gram stained and their Gram's nature, motility and morphology were noted down. Isolates were also checked whether they are spore former or not and able to produce PHB granules. Table 4 shows that three of the psychrotrophic bacterial isolates were Gram negative and motile namely BBM-7, SBM-2, ABM-1. Remaining isolates were Gram positive non motile. All the isolates were observed under oil immersion lens in compound microscope after Gram staining and morphology was observed. Six isolates showed cocci shaped bacteria namely BBM2, BBM-8, JBM-1, ABM-2, QCM-1, QCM-4, and nineteen rod shaped. Sporulation and PHB granules production were also checked for the isolates. BBM-6, JBM-2, QCM-2, showed positive spore formation and PHB granules production.

Biochemical Characteristics

Fermentation of Different Sugars by Isolates

All the isolates were checked for their ability to ferment different types of sugars. Utilization of different sugars and production of acid and gas helps in identification of isolates as many of the bacteria able to ferment some key sugars. Sugars used for the

fermentation by psychrotrophic bacterial isolates namely glucose, lactose, mannitol, sucrose, arabinose, maltose, galactose, xylose, ribose, raffinose, rhamnnose, fructose, sorbitol, mannose.

Table 7: Fermentation of sugars by bacterial isolates.

Sr. No	Code of isolates	Glucose	Ribose	Mannitol	Lactose	Sucrose	Xylose	Raffinose
1	BBM-1	+ ve	- ve	- ve	- ve	- ve	- ve	- ve
2	BBM-2	+ ve	- ve	+ ve	+ ve	- ve	- ve	+ ve
3	BBM-3	+ ve	- ve	- ve	+ ve	- ve	- ve	- ve
4	BBM-4	+ ve	- ve	- ve	+ ve	- ve	- ve	- ve
5	BBM-5	+ ve	- ve	+ ve	+ ve	- ve	- ve	+ ve
6	BBM-6	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
7	BBM-7	+ ve	+ve	- ve	+ ve	- ve	- ve	- ve
8	BBM-8	+ ve	- ve	- ve	- ve	+ ve	+ ve	- ve
9	BBM-9	+ ve	+ ve	- ve	+ ve	+ ve	- ve	- ve
10	SBM-1	+ ve	+ ve	- ve	+ ve	- ve	- ve	- ve
11	SBM-2	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve
12	JBM-1	+ ve	- ve	- ve	+ ve	+ ve	- ve	- ve
13	JBM-2	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
14	JBM-3	+ ve	- ve	- ve	+ ve	+ ve	- ve	- ve
15	JBM-4	+ ve	+ ve	- ve	+ ve	+ ve	- ve	- ve
16	ABM-1	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve
17	ABM-2	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve
18	ABM-3	+ ve	+ ve	- ve	+ ve	+ ve	- ve	- ve
19	QCM-1	+ ve	- ve	+ ve	+ ve	+ ve	- ve	- ve
20	QCM-2	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
21	QCM-3	+ ve	+ ve	- ve	- ve	- ve	+ ve	- ve
22	QCM-4	+ ve	+ ve	- ve	+ ve	+ ve	- ve	+ ve
23	QCM-5	+ ve	+ ve	- ve	- ve	+ ve	- ve	- ve
24	JCM-1	+ ve	+ ve	- ve	- ve	+ ve	+ ve	- ve
25	JCM-2	+ ve	+ ve	- ve	(+) ve	+ ve	- ve	- ve

+ ve [Acid production], - ve [Negative test], (+) [Acid and gas production]

Table 8: Fermentation of sugars by bacterial isolates.

Sr. No	Code of isolates	Arabinose	Galactose	Mannose	Sorbitol	Rhamnose	Fructose	Maltose
1	BBM-1	- ve	- ve	- ve	- ve	- ve	- ve	- ve
2	BBM-2	+ ve	- ve	- ve	- ve	+ ve	- ve	- ve
3	BBM-3	- ve	- ve	- ve	- ve	+ ve	- ve	+ ve
4	BBM-4	- ve	- ve	- ve	- ve	- ve	+ ve	- ve
5	BBM-5	+ ve	- ve	- ve	+ ve	+ ve	- ve	- ve
6	BBM-6	- ve	- ve	- ve	- ve	- ve	- ve	- ve
7	BBM-7	+ ve	- ve	+ ve	- ve	- ve	- ve	- ve
8	BBM-8	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve
9	BBM-9	- ve	+ ve	- ve	- ve	- ve	- ve	- ve
10	SBM-1	- ve	- ve	- ve	- ve	- ve	- ve	- ve
11	SBM-2	+ ve	- ve	+ ve	- ve	- ve	- ve	- ve
12	JBM-1	- ve	+ ve	- ve	+ ve	- ve	- ve	- ve
13	JBM-2	- ve	+ ve	- ve	- ve	- ve	- ve	+ ve
14	JBM-3	- ve	+ ve	- ve	+ ve	- ve	- ve	+ ve
15	JBM-4	- ve	+ ve	- ve	- ve	- ve	- ve	- ve
16	ABM-1	+ ve	- ve	+ ve	- ve	- ve	- ve	- ve
17	ABM-2	- ve	+ ve	- ve	+ ve	- ve	+ ve	- ve
18	ABM-3	- ve	- ve	+ ve	- ve	- ve	- ve	- ve
19	QCM-1	- ve	+ ve	+ ve	- ve	- ve	+ ve	+ ve
20	QCM-2	- ve	+ ve	+ ve	- ve	- ve	- ve	+ ve
21	QCM-3	+ ve	- ve	+ ve	- ve	- ve	- ve	- ve
22	QCM-4	- ve	+ ve	+ ve	- ve	- ve	+ ve	+ ve
23	QCM-5	+ ve	- ve	- ve	- ve	- ve	- ve	- ve
24	JCM-1	- ve	- ve	+ ve	- ve	- ve	- ve	- ve
25	JCM-2	- ve	- ve	+ ve	- ve	- ve	- ve	- ve

Where + ve [Acid production], - ve [Negative test], (+) [Acid and gas production]

Tables 7 and 8 shows the fermentation of different sugars. Most of the isolates produce acid on utilization of glucose, only four do not produced acid from glucose. Fourteen isolates namely BBM-6, BBM-9, SBM-1, SBM-2, JBM-1, JBM-2, JBM-4, ABM-1, ABM-2, QCM-2, QCM-4, QCM-5, JCM-1, JCM-2, produced acid from ribose sugar, while remaining isolates did not fermented ribose. Three isolates produced acid from mannitol sugar namely BBM-6, JBM-2, QCM-2. Fifteen isolates fermented the lactose sugar and produced acid namely BBM-3, BBM-4, BBM-5, BBM-6, BBM-9, SBM-1, SBM-2, JBM-1, JBM-2, JBM-4, ABM-1, ABM-2, QCM-2, QCM-3, QCM-4 and two isolates produced acid and gas from lactose sugar. Sucrose sugar was fermented by thirteen isolates and produced only acid no gas formation was observed. These isolates were namely BBM-6, BBM-8, BBM-9, JBM-2, JBM-3, JBM-4, ABM-1, ABM-2, QCM-2, QCM-4, QCM-5, JCM-1, JCM-2. Four isolates fermented arabinose sugar and produced acid namely BBM-7, SBM-2, ABM-1, QCM-5 while other isolates did not produced acid. Mannose was fermented by isolates JBM-3, ABM-3, QCM-2, JCM-1, and produced acid only. Sorbitol is fermented by isolates namely BBM-5, BBM-8, ABM-1, ABM-2. The isolates which fermented fructose and sorbitol sugar produced acid only while remaining isolates did not produced acid. Six isolates fermented raffinose and produced acid namely BBM-3,. Nine isolates fermented maltose sugar and produced acid namely BBM-3, BBM-5, JBM-1, JBM-2, QCM-2.

Conclusion:

The present study reveals that dairies needs proper cleaning of utensils used for milk storage under refrigeration. Studies on Psychrotrophic bacteria suggest that these bacteria can grow in refrigerated milk and changes the nutritional properties of milk.

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CHRONIC MYELOID LEUKEMIA: MECHANISMS, DIAGNOSIS, AND THERAPEUTIC STRATEGIES

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Abstract:

Chronic myeloid leukemia (CML) is a prevalent form of leukemia, representing around 15% of new adult leukemia diagnoses. It is characterized by malignant transformation of white blood cells and progresses through three distinct stages: the chronic phase, the accelerated phase, and the blast phase. A defining feature of CML is the presence of the Philadelphia chromosome, which results in continuous activation of tyrosine kinase via the BCR-ABL1 fusion protein. This persistent signaling plays a crucial role in the disease's development. The advent of tyrosine kinase inhibitors (TKIs) has dramatically improved the prognosis for individuals with CML. Progression to the blast phase has become exceedingly rare, and patients often achieve a life expectancy similar to that of the general population.At present, four TKIs—imatinib, dasatinib, bosutinib, and nilotinib—have been approved by the U.S. Food and Drug Administration (FDA) as first-line therapies for patients newly diagnosed with chronic-phase CML. Additionally, ponatinib and asciminib are authorized for use in cases involving drug resistance or more advanced stages of the disease. Although TKI therapy is largely effective, a minority of patients develop resistance, typically due to point mutations within the BCR-ABL1 gene. Management of resistant cases requires selecting an appropriate second- or third-generation TKI based on the specific mutation identified. Emerging research indicates that patients achieving a deep and sustained molecular response may be able to discontinue TKI therapy while maintaining long-term remission. This chapter seeks to offer a comprehensive overview of CML, covering its underlying mechanisms, diagnostic strategies, and available treatment options.

Keywords: Chronic Myeloid Leukemia, Pathophysiology, Tyrosine Kinase Inhibitor, Philadelphia Chromosome, Imatinib

Introduction:

Chronic myeloid leukemia (CML) is a type of myeloproliferative neoplasm marked by the excessive growth of granulocytes, mainly in adults. It accounts for approximately 15% of all newly diagnosed leukemia cases, with an incidence rate of around 1.0–1.5 per 100,000 people each year.[1] Since the introduction of imatinib in 2000, the annual mortality rate for CML has significantly declined from 10–20% to just 1–2%. [2]

Chronic myeloid leukemia (CML) is characterized by the uncontrolled proliferation of myeloid cells in the bone marrow and their buildup in the bloodstream, notably involving mature granulocytes and their precursors. A marked increase in basophils is a key clinical feature. CML was the first cancer linked to a specific genetic abnormality — the Philadelphia chromosome — identified in 1960 by Peter Nowell and David Hungerford in Philadelphia, Pennsylvania. [3]

At the core of CML pathophysiology is the Philadelphia chromosome, formed by a translocation between chromosomes 9 and 22, resulting in the creation of the BCR-ABL1 oncogene. This rearrangement fuses the ABL gene from chromosome 9 with part of the BCR ("breakpoint cluster region") gene from chromosome 22. The abnormal fusion gene produces a protein, typically weighing p210 and occasionally p185. This fusion protein functions as a tyrosine kinase, as the ABL portion contains a domain capable of adding phosphate groups to tyrosine residues. [4-6] Tyrosine kinase is constitutively activated by this fusion gene, giving mutant hematopoietic stem cells a proliferative advantage over normal ones. [7-8]

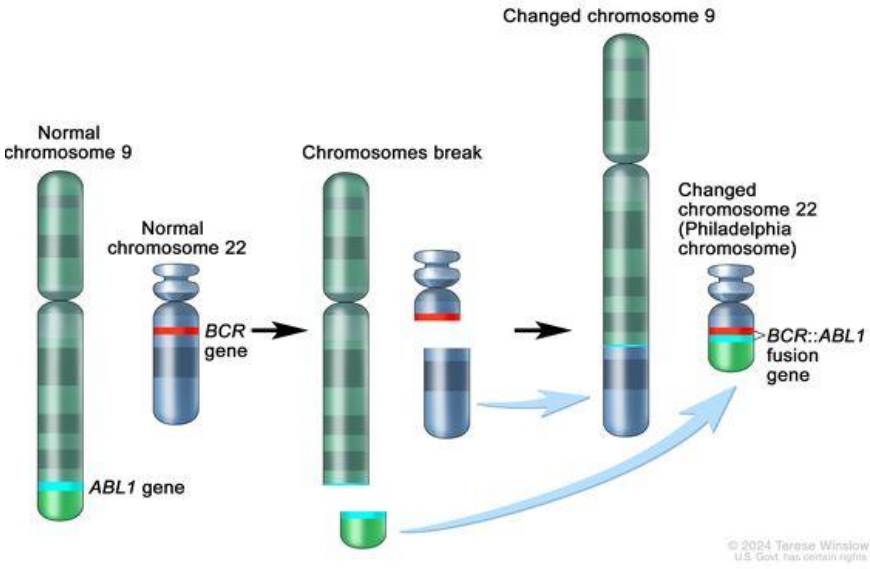

Fig. 1: Translocation to form Philadelphia chromosome. [9]

Classification of CML:

According to clinical features, CML develops in three stages. It starts in the chronic phase, moves into an accelerated phase over a number of years, and ends in a terminal phase known as blast crisis if treatment is not received. By the time of diagnosis, some patients might already be experiencing blast crisis or the accelerated phase.[10] While the blast phase is linked to a worse prognosis and resembles acute leukemia, the chronic phase is the most prevalent at diagnosis.

Table 1: Different phases of CML

Phase	Blast Cell Percentage in Blood/Bone Marrow	Key Features & Symptoms	Clinical Notes & Prognosis
Chronic phase	< 10% blasts	Often mild or no symptoms; fatigue, anemia	Most patients (~85%) diagnosed in this phase; responds well to treatment; slow disease progression
Accelerated phase	10–19% blasts	Fever, weight loss, poor appetite, worsening anemia, enlarged spleen, new chromosomal abnormalities	Disease progresses faster; less responsive to therapy; signs include abnormal blood counts (e.g., basophils ≥20%, low platelets not due to treatment)
Blast phase	≥ 20% blasts	Resembles acute leukemia; fever, severe fatigue, spread of blasts to other organs	Most aggressive phase; difficult to treat; poor prognosis; blast cells can be myeloid (~70-80%) or lymphoid (~20-30%)

1. Chronic Phase

The leukemia cells proliferate slowly during this initial and most stable phase of CML. The chronic period is when the majority of patients receives their diagnoses. Tests of the bone marrow and blood at this stage of CML reveal fewer than 10% of immature white blood cells (blasts) and primarily mature, functional blood cells. If there are symptoms,

they are typically minor or nonexistent and could include abnormal platelet counts or anemia. During this stage, patients usually react favorably to traditional therapy.

2. Accelerated Phase

This is the second and intermediate phase of CML. During this stage, the disease worsens and there are more immature blast cells (10–19%) in the bone marrow or blood. Additional anomalies include new chromosomal alterations and basophil counts ≥20%. Fatigue, weight loss, fever, and an enlarged spleen are some of the symptoms that become more noticeable. Compared to the chronic phase, the disease responds less well to treatment during this period.

3. Blast Phase (Blast Crisis or Acute Phase)

This is the most aggressive stage of CML and resembles acute leukemia. During this stage, 20% or more blasts were discovered in the bone marrow or blood, and occasionally leukemia cells spread to other organs. Serious symptoms include fever, exhaustion, weight loss, enlarged spleen, bleeding, infections, and bone pain are frequently experienced by patients. The blast phase is more difficult to treat and may need stem cell transplantation and intense chemotherapy.[11] Table 1 provides a summary of the stages of CML.

Symptoms of CML:

The symptoms of CML often develop gradually and can be vague, sometimes resembling other conditions. Many people with CML may initially have no symptoms and are diagnosed through routine blood tests. Commonly symptoms of CML are fatigue and weakness (due to anemia lead to the crowding out of healthy red blood cells by leukemia cells); night sweats and fever (often related to infections due to low levels of normal white blood cells); unexplained weight loss (as the disease can consume the body's energy, leading to weight loss even without changes in diet); bone pain (leukemia cells spreading to the surface of bones or joints can cause discomfort or pain); enlarged spleen (felt as fullness or pain below the ribs on the left side, sometimes causing early satiety); shortness of breath (due to anemia and reduced oxygen-carrying capacity of the blood); bruising or bleeding easily (resulting from low platelet counts, which can cause nosebleeds, bleeding gums, or easy bruising); swollen lymph nodes (enlargement of lymph glands in the neck, armpits, or groin); headaches and blurred vision (may occur if very high white blood cell counts clog small blood vessels in the brain or eyes).

Less common symptoms: painful, prolonged erections (priapism), swollen or painful joints, and other rare symptoms due to blood vessel blockage by excess white cells. These

symptoms arise because the abnormal leukemia cells crowd out normal blood cells in the bone marrow and accumulate in organs such as the spleen and lymph nodes. [12]

Diagnosis of CML:

The diagnosis of CML involves a combination of blood tests, bone marrow examination, and genetic testing to confirm the presence of leukemia and identify its characteristic genetic abnormalities.

1. Complete blood count (CBC) with differential count

In CML, there is typically a very high white blood cell count, often with many immature cells (blasts). Red blood cells may be low, and platelet counts can be high or low depending on disease severity.

2. Bone marrow aspiration and biopsy

Samples of bone marrow are taken, usually from the hip bone, to evaluate the marrow's cellular makeup and confirm the diagnosis. This helps determine the phase of CML and guides treatment decisions, including whether stem cell transplantation is needed.

3. Genetic and molecular testing

Cytogenetic analysis (Karyotyping): Examines chromosomes in dividing cells from blood or bone marrow to detect the Philadelphia chromosome (Ph), a shortened chromosome 22 caused by translocation between chromosomes 9 and 22, which is present in most CML cases.

Fluorescence In Situ Hybridization (FISH): Uses fluorescent probes to detect the BCR-ABL gene fusion on chromosomes in non-dividing cells, providing faster results than conventional cytogenetics.

Polymerase chain reaction (PCR): A highly sensitive test that detects and quantifies BCR-ABL gene transcripts in blood or marrow, confirming diagnosis and monitoring treatment response. PCR can detect very low levels of disease.

4. Additional Tests

Imaging tests like CT or MRI scans are not required for diagnosis but may be used to assess organ enlargement (e.g., spleen) or investigate symptoms. Physical examination includes checking for an enlarged spleen or liver and lymph node swelling. [13]

Treatment of CML:

The introduction of tyrosine kinase inhibitors (TKIs) has revolutionized CML treatment, significantly improving patient survival and quality of life. TKIs, such as imatinib

and its successors, have enabled many patients to achieve deep molecular responses, potentially leading to treatment-free remission. [14-15]

This review aims to provide an overview of CML, covering its pathophysiology, clinical manifestations, treatment options, and recent advancements in management strategies. It will explore the role of TKIs, the challenges associated with long-term treatment, and the potential for treatment-free remission, highlighting the current state of CML management and future directions in this field.

Treatment to CML:

CML treatment depends on the disease phase, patient health, and response to therapy. The main treatment approaches include targeted therapy, chemotherapy, immunotherapy, and stem cell transplantation. The only curative treatment for CML is a bone marrow transplant or an allergenic stem cell transplant. The other treatment is with tyrosine kinase inhibitors.

Key Treatments for CML:

1. Tyrosine kinase inhibitors (TKIs) – standard first-line therapy

TKIs target the BCR-ABL fusion protein that drives CML cell growth. TKIs are highly effective in the chronic phase, controlling the disease for long periods and enabling many patients to live normal lifespan. Common TKIs include imatinib**,** dasatinib**,** nilotinib**,** bosutinib**,** and asciminib**.** For patients with the T315I mutation resistant to most TKIs, ponatinib and asciminib are effective options.

CML treatments with highlighting characteristics, indications, and key points based on recent clinical evidence and guidelines are summarized in Table 2 and Table 3.

2. Chemotherapy

Chemotherapy is used mainly in accelerated or blast phases or when TKIs are ineffective. Chemotherapy can also serve as palliative therapy to relieve symptoms in advanced stages. Drugs such as fludarabine, idarubicin, and cytarabine may be used, often in combination with TKIs to control aggressive disease.

3. Stem cell (bone marrow) transplantation

This is the only potentially curative treatment for CML. It is usually reserved for patients who do not respond well to TKIs or those in advanced phases. It involves high-dose chemotherapy followed by infusion of healthy donor stem cells to restore normal blood cell production.

Table 2: Summary of approved TKI for CML

TKI Name (Brand Name)	Genera-tion	Approved Use in CML Phases	Dose (mg)	Typical Indications	Key Features	Ref.
Imatinib (Gleevec)	1st	Chronic, accelerated, and blast phases	400 daily	1st line therapy for newly diagnosed CML; also used after interferon failure	First TKI approved; well-established efficacy and safety; good long-term survival; mild to moderate side effects common (fluid retention, edema)	16
Dasatinib (Sprycel)	2nd	Chronic, accelerated, and blast phases	50–100 daily	First-line or second-line after imatinib resistance or intolerance	More potent than imatinib; faster molecular response; risk of pleural effusion and pulmonary hypertension	17
Nilotinib (Tasigna)	2nd	Chronic, and accelerated phases	300–400 twice daily	First-line or second-line after imatinib failure or intolerance	Faster deep molecular responses; stricter fasting requirements; cardiovascular risks require monitoring	18
Bosutinib (Bosulif)	2nd	Chronic, accelerated, and blast phases	400–500 daily	Second-line or later when imatinib, dasatinib, nilotinib not suitable	Effective in resistant/intolerant patients; gastrointestinal side effects	19
Ponatinib (Iclusig)	3rd	Accelerated, blast phases and chronic phase with T315I mutation	45 daily	Resistant/intolerant to other TKIs or with T315I mutation	Potent against T315I mutation; risk of arterial occlusive events	20
Asciminib (Scemblix)	3rd (allosteric inhibitor)	Chronic phase after ≥2 prior TKIs or with T315I mutation	40 daily	Third-line therapy; alternative for resistant cases	Novel mechanism targeting myristoyl pocket; better toxicity profile; effective in resistant CML; approved recently	21

Table 3: Different treatment options of CML

Treatment Type	Drugs	Indications / Use	Key Features & Notes	Ref.
Tyrosine Kinase Inhibitors (TKIs)	Imatinib Nilotinib Dasatinib Bosutinib Ponatinib Asciminib	First-line for chronic phase; later lines for resistant/intolerant cases; ponatinib and asciminib for T315I mutation	Target BCR-ABL fusion protein; highly effective in chronic phase; newer TKIs and higher-dose imatinib show faster responses; ponatinib most effective for T315I mutation; treatment adherence critical; some side effects and discontinuation possible	15, 22, 24
Chemotherapy	Cytarabine, Fludarabine, Idarubicin	Accelerated/blast phases or TKI-resistant disease	Used in advanced phases or combined with TKIs; controls aggressive disease; more toxic than TKIs; palliative in some cases	23
Stem Cell Transplantation	Allogeneic Hematopoietic Cell Transplant	Patients with TKI resistance, advanced phases, or relapse	Only potentially curative option; high risk of complications; reserved for selected patients; requires donor match	23
Immunotherapy	Interferon-alpha (IFN-α)	Alternative or adjunct when TKIs contraindicated or ineffective	Stimulates immune response; less commonly used since TKIs; may be combined with TKIs or chemotherapy	22
Supportive & Other Therapies	Leukapheresis (to reduce high WBC), Donor Lymphocyte Infusion (post-transplant)	Emergency management of leukostasis; boost immune response after transplant	Temporary measures to manage symptoms or enhance graft-versus-leukemia effect	23

4. Immunotherapy (Interferon Alpha)

Immunotherapy used less frequently since the advent of TKIs but may be considered if TKIs are contraindicated or ineffective. It works by stimulating the immune system to attack leukemia cells.

5. Other Treatments

Donor lymphocyte infusion (DLI) may be used after stem cell transplant to boost immune response against leukemia cells.

Treatment by Phase

Chronic Phase: Primarily treated with TKIs; most patients respond well.

Accelerated Phase: TKIs (like bosutinib, imatinib) often combined with consideration of stem cell transplant.

Blast Phase: Intensive treatment with TKIs, chemotherapy, and stem cell transplant if possible; prognosis is poorer.

Conclusion:

Nowadays, CML is regarded as a paradigm for targeted cancer treatment. The goals of ongoing research are to increase the percentage of patients who can safely stop treatment and sustain remission, enhance outcomes in advanced phases, and overcome TKI resistance. In order to maximize patient survival and quality of life, the future of CML management depends on further understanding the biology of the illness and improving tailored treatment plans.

TKIs provide efficient disease management and are the mainstay of CML treatment, particularly during the chronic period. Stem cell transplantation and chemotherapy are only used in patients that are advanced or resistant. When necessary, immunotherapy and other supportive therapies may be applied. The illness stage, genetic alterations, patient health, and therapy response all influence the decision and modifications of treatment. Since TKI is more effective than earlier treatments, it is presently the frontline treatment for CML. It is hoped that more people may experience remission without treatment in the future.

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RECENT INSIGHTS INTO THE MANAGEMENT AND EMERGING THERAPIES FOR MULTIPLE SCLEROSIS

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Abstract:

Multiple sclerosis (MS) is a condition of the central nervous system (CNS) that is characterized by chronicity, autoimmune disease, and inflammation. It affects millions of people all over the world. This chapter offers a thorough examination of MS, emphasizing its statistics, root cause, and contemporary management approaches. Epidemiological research indicates disparate prevalence rates across locations, with a greater incidence noted in women and persons of Northern European ancestry. The etiology of MS is multifaceted, encompassing environmental, genetic, and immunological factors. Contemporary management options seek to alter disease progression, diminish relapses, and alleviate symptoms, predominantly via disease-modifying medications (DMTs). Notwithstanding the advancements in pharmaceutical interventions, there persists a considerable necessity for more efficacious medicines to tackle the progressive characteristics of MS. This chapter examines the novel therapeutics for MS, encompassing Bruton's tyrosine kinase inhibitors, monoclonal antibodies, neuroprotective measures, and remyelination techniques. These innovative strategies provide optimism for improved management of the illness, neuroprotection, and the restoration of neurological function in individuals with MS. This chapter elucidates potential future approaches in managing MS and existing research initiatives aimed at enhancing patient outcomes through a comprehensive analysis of various medicines.

Keywords: Multiple Sclerosis, Autoimmune Disease, Disease-Modifying Therapies, Neuroprotection, Remyelination, Novel Therapeutics

Introduction to MS:

MS is the predominant non-traumatic neurological disorder among young individuals. It has become an autoimmune demyelinating and neurodegenerative disorder of the central nervous system. Multiple sclerosis targets the myelinated axons within the CNS, resulting in the degradation of both myelin and axons to differing extents. The

predominant autoimmune disorder leading to nontraumatic neurological deficits in young people is MS. Inflammation accompanied by demyelination, astroglial proliferation (gliosis), and neurodegeneration are the two pathological characteristics of MS.[1] MS an autoimmune neurodegenerative disease that affects the CNS, which includes the brain and spinal cord. Auto- reactive immune cells, such as T cells, target and damage the lipid- rich myelin layer that surrounds axons in the CNS. This demyelination impairs the conduction of electrical impulses along nerve fibers, leading to neurologic dysfunction. immune cells penetrate the blood-brain barrier (BBB) to access the CNS. The BBB normally acts as a protective barrier, regulating the passage of substances between the bloodstream and the brain. However, in MS, the BBB may become compromised, allowing immune cells to infiltrate the CNS and contribute to tissue damage.[2]

MS is on the rise in developed and developing nations, but its cause is unknown.[3] MS is complex, and numerous genes modestly influence disease vulnerability, along with well-defined environmental factors such vitamin D or UVB exposure to radiation, Epstein–Barr virus (EBV) infection, being obese, and smoking.[4] On the clinical level, MS can relapse or progress. Relapsing MS, with discrete bouts of neurological impairment accompanied by partial, complete, or no remission, is most prevalent. Relapses normally reduce, but a progressive worsening often follows, ending in uninterrupted advancement.[5] Inflammation is related with relapses and neurodegeneration with development, but both diseases are present in most individuals across the illness continuum.[2]

MS is an inflammatory condition that typically manifests between the ages of 20 and 40. In the United States, a minimum of 350,000 individuals are afflicted with MS. It results in significant handicap due to impairments in sensory, motor, autonomic, and cognition functions. Fewer than 10% of patients with MS exhibit progression from onset, classified as primary progressive MS (PPMS).[6] Relapsing-remitting multiple sclerosis (RR-MS) is the most prevalent form, occurring in 85%–90% of cases, and it affects women approximately twice as frequently as males. The majority of people with relapsing-remitting multiple sclerosis (RR-MS) subsequently progress to secondary progressive multiple sclerosis (SP-MS). Approximately 10%–15% of individuals exhibit a gradual beginning of disease and continuous progression, referred to as primary progressive (PP)-MS.[7]

The existence of MS may only be confirmed through clinical and/or radiographic evidence of lesions in the CNS that are dispersed in space (DIS) and time (DIT). Before the advent of MRI, the identification of DIS and DIT relied solely on clinical observations.[8] MS

has traditionally been categorized as an organ-specific, T-cell mediated autoimmune disorder. The efficacy of B-cell focused therapies contests the conventional T-cell autoimmune paradigm.[9] It is conventionally seen as a biphasic disease, wherein initial inflammation leads to relapsing–remitting forms, while subsequent neurodegeneration results in non-relapsing progression, namely secondary and primary progressive MS.[10] Contemporary management options concentrate on addressing acute episodes, alleviating symptoms, and diminishing biological activity via disease-modifying medications.[2]

Types of MS

MS is a complicated and intricate neurologic condition that manifests in multiple diverse forms, all of which have unique clinical characteristics and a unique history of the disease. To correctly understand the various types of MS and alter treatment plans for each patient, it is essential to classify the disease into several types. Relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), primary progressive multiple sclerosis (PPMS), and clinically isolated syndrome (CIS) progressive relapsing multiple sclerosis (PRMS) are the main forms of MS. Individuals and professionals face diverse barriers while dealing with the distinct patterns of recurrence, remission, and disability progression that characterise all types.[11]

About 85% of MS patients usually have relapsing-remitting disease (RRMS) with well-defined sequential bouts of neurologic impairment and remission. [12] In 10 years, about 50% of RRMS patients develop secondary progressive MS (SPMS), which causes irreversible neurologic damage. By 25 years, 90% do.[13] Primary progressive MS (PPMS) affects 10% of MS patients and causes a gradual deterioration in neurological functioning with no healing. Progressive relapsing MS (PRMS), the fourth clinical disease course, affects 5% of MS sufferers and is distinguished by persistent neurologic loss and well-defined acute attacks regardless of recovery.[14]

Epidemiology of MS

The prevalence of MS is rising on a worldwide scale. MS is more common in regions with higher latitudes. The MS International Federation reported that the global average rate for MS has risen from 30 cases per 100,000 in 2008 to 33 cases per 100,000 in 2013.[15] Although women now account for a greater number of MS cases, this was not always the case. Gender parity was nearly universal in case series encompassing the early 20th century.[4] The gender ratio has kept rising ever since, and in most industrialized nations it stands nearly 3:1 (male to female). Countries bordering the equator have the lowest

prevalence, whereas those in North America, Western Europe, New Zealand, and Australasia have the greatest (>100 cases per 100,000 population). In the East, it is very rare (with a prevalence rate of fewer than 5 per 100,000), and in Africa and South America, it seems to be quite rare as well.[16]

The prevalence of RRMS is approximately three times higher in women than in males, with an average age of onset around 30 years. In contrast, PPMS has equal rates of occurrence in both sexes, with an average age of onset around 40 years.[17] Before the important studies started appearing across the early 1980s, the incidence of MS among Indians was expected to be approximately 1 in 100,000. This was a significant change from earlier thinking about the disease in Country.[18] The estimated incidence of MS in a previous study was at 1.33 per 100,000 people. According to earlier research, there were an estimated 3.2 MS cases per year in southern India, compared to 4.15 MS cases in the north. This suggests that MS is possibly more widespread in the north of the country.[19]

Etiology of MS

A complicated interaction between immune system failure, environmental variables, and genetic predisposition results in MS. The precise cause of MS remains unknown; however, researchers have identified some risk factors for the condition.[20]

The exact heritability of MS is unclear since defining heredity in a polygenic disease may be difficult. Researchers have shown that there is a strong genetic component to MS risk, with most studies estimating a 25% risk based on coherence rates among monozygotic twins.[21] The rates of dizygotic twins are comparable to those of non-twin siblings, whereas the rates of female monozygotic twins are much greater than those of male monozygotic twins. Even with extensive genome-wide association studies (GWASs), approximately 20–30% of the apparent heritability of MS can currently be explained. The remaining 70–80% may not yet be known, but it is thought to reflect how a person's lifelong exposure to their environment affects how their genes are expressed or methylated, which in turn affects how heritable they are regarded to be.[22]

Current Management of MS:

Demyelination and neuronal degeneration are symptoms of MS, which is a chronic autoimmune disorder that affects the brain and spinal cord. Among its many symptoms are changes in vision, lethargy, weak muscles, and cognitive decline. MS exhibits a highly varied disease trajectory, complicating therapeutic efforts.[23] Relapse prevention, disease development aversion, and symptom relief to improve quality of life are the therapy goals.

The principal objectives of MS treatment are to alleviate symptoms, diminish the frequency and intensity of relapses, and inhibit or decelerate disease progression. This necessitates a comprehensive strategy integrating disease-modifying drugs (DMTs), symptom treatment, and supportive care.[24]

Disease-Modifying Therapies (DMTs):

Recently, there have been a plethora of oral and monoclonal antibody therapies for MS. At the present time, fifteen disease-modifying treatments are available for relapsing MS. For relapse MS, there are now five authorized disease-modifying therapies (DMTs), including three different glatiramer acetate formulations and five different interferon beta formulations. The initial medicine ocrelizumab, which treats primary progressive MS, has just been licensed.[25] People with dormant MS are not to do anything. In a Disease that occurs only in one patient, Individuals presenting with a clinically isolated disease are encouraged to utilize interferon beta or glatiramer acetate according to the ECTRIMS/EAN and AAN guidelines. Brain magnetic resonance imaging (MRI) abnormalities and the onset of MS-like neurological symptoms indicate an increased chance of developing the disease.[26] Defusing the situation before it escalates is the top priority of management. Please refer to the appendix on bmj.com for information on the indications, which differ according on the licensing by regulatory agencies. Patients with active relapse MS should have access to disease-modifying treatments, according to global recommendations.[27]

Several disease-modifying therapies (DMTs) target different aspects of lymphocyte quantity, proliferation, trafficking, and cytokine synthesis. These include alemtuzumab, ocrelizumab, cladribine, teriflunomide, mitoxantrone, fingolimod, natalizumab, and interferon beta and glatiramer acetate. Due to our poor understanding of the disease's pathophysiology, the mechanisms of action of disease-modifying treatments (DMTs) are still not fully understood. By modulating the dysregulated immune system in MS, disease-modifying therapies (DMTs) mitigate central nervous system inflammation, hence reducing relapses and the formation of new inflammatory lesions.[28]

Symptom Management

Patients with MS can greatly benefit from symptom management in terms of both health and quality of life. Physicians prioritize slowing disease progression and preventing disability, often neglecting symptom control. Clinicians view MS as characterized by immunological dysfunction, relapses, and disability, linked to imaging-visible brain lesions.[29]

Efficient symptom treatment for MS/MS-related complications is essential due to the disease's very variable and frequently progressing characteristics, which can impact several bodily systems. Up to 80% of patients experience tiredness, making it among the most prevalent complaints.[30] It may manifest autonomously from physical exertion and is frequently characterized as overwhelming. Management solutions encompass lifestyle modifications, including energy conservation methods, planned rest intervals, and moderate aerobic activity. Pharmacological interventions, including amantadine, modafinil, and l-carnitine, are frequently employed, however their effectiveness differs among individuals.[31]

Spasticity and muscular rigidity are prevalent, especially in the lower extremities, resulting in pain, compromised movement, and contractures. First-line therapies include of baclofen, tizanidine, and dantrolene, which diminish muscle tone by targeting the spinal cord or muscle fibers. [32] Botulinum toxin injections or intrathecal baclofen pumps are sometimes employed for resistant spasticity. Physical therapy, consistent stretching, and swimming activities are highly advised as non-pharmacological supplements to enhance flexibility and mobility.[33]

Neuropathic pain, characterized by burning, tingling, or stabbing sensations, is typically managed with anticonvulsants like gabapentin or pregabalin, as well as tricyclic antidepressants such as amitriptyline. Carbamazepine may be advantageous in instances of central pain or trigeminal neuralgia.[34] Simultaneously, bladder and bowel dysfunction frequently necessitate tailored treatment strategies. Symptoms of overactive bladder, such as urgency and frequency, are managed with anticholinergics (e.g., oxybutynin, solifenacin), although intermittent catheterization may be required for urine retention. Dietary adjustments and stool softeners are beneficial for constipation, but incontinence may necessitate behavioral treatment and pharmacological interventions.[35]

Cognitive and emotional symptoms, such as memory impairment, diminished processing speed, depression, and anxiety, are increasingly acknowledged as substantial factors contributing to disability in MS.[36] Cognitive rehabilitation, mindfulness therapy, and digital brain training tools are emerging methodologies. Although certain research has examined donepezil and modafinil for cognitive enhancement, the evidence stays ambiguous. Depression and anxiety are treated with a combination of selective serotonin reuptake inhibitors (SSRIs) and psychological therapy. A multidisciplinary strategy that includes neurologists, mental health professionals, urologists, physiotherapists, and

occupational therapists is frequently crucial for providing comprehensive care and enhancing patient living conditions.[37]

Emerging Therapies for the Management of MS

MS is a chronic autoimmune condition characterized by inflammation, demyelination, and neurodegeneration within the CNS.Although existing disease-modifying therapies (DMTs) have enhanced patient outcomes, there persists a necessity for medicines that can arrest disease development, facilitate remyelination, and reinstate neurological function. Recent studies have concentrated on creating innovative treatments aimed at different facets of MS pathophysiology.[38]

Bruton's tyrosine kinase (BTK) inhibitors have surfaced as viable oral treatments for MS. Evobrutinib, a selective BTK inhibitor, has shown effectiveness in diminishing disease activity in relapse MS. In a Phase II trial, evobrutinib demonstrated a substantial decrease in gadolinium-enhancing lesions relative to placebo, accompanied by an acceptable safety profile.[39] Fenebrutinib, an additional BTK inhibitor, is presently in Phase III trials and has demonstrated promise in decreasing relapse rates and postponing disease progression in both relapsing and primary progressive MS.[40]

Monoclonal antibodies directed at specific immunological components have demonstrated potential. Ublituximab, a monoclonal antibody targeting CD20, has shown effectiveness in decreasing annualized recurrence rates and MRI lesion activity in relapsing MS.[41] Temelimab, which targets the envelope protein of human endogenous retrovirus type W (HERV-W), aims to reduce inflammation of the brain and promote remyelination. Preliminary experiments have demonstrated its ability to diminish brain damage and enhance cognitive performance.[42]

Neuroprotective treatments are being investigated to tackle the progressive facets of MS. Elezanumab, a monoclonal antibody targeting repulsive guidance molecule A (RGMa), has demonstrated potential in facilitating neuroregeneration and neuroprotection in preclinical studies. Phase I studies have demonstrated its safety and tolerability in humans, facilitating subsequent clinical development.[43]

Remyelination medicines represent a significant focus of ongoing research. Agents that target oligodendrocyte progenitor cells (OPCs) seek to improve remyelination and rehabilitate neurological function. Clemastine fumarate, an antihistamine, has demonstrated limited effectiveness in facilitating remyelination in optic neuritis linked to

MS. Additional drugs, including opicinumab, are under investigation for their capacity to promote oligodendrocyte precursor cell development and remyelination.[44]

Stem cell therapies present a promising approach for immune system restoration and neuro-regeneration. Autologous hematopoietic stem cell transplantation (AHSCT) has demonstrated effectiveness in very active relapse MS, resulting in extended remission for certain patients. AHSCT entails hazards linked to immunosuppression and necessitates meticulous patient selection.[45]

Recent research indicates that the modification of gut microbiota may affect the activity of MS. Dietary therapy, probiotics, and fecal microbiota transplantation are under investigation as supplementary therapies. For experimental models of MS, a ketogenic diet has shown promise in modifying immune responses and alleviating disease severity. Human studies are now being conducted to evaluate the effectiveness of these therapies.[46]

In conclusion, the domain of MS therapy is swiftly advancing, with numerous novel medications addressing diverse facets of disease pathogenesis. The effectiveness and safety of these novel medications must be confirmed through continuing studies and clinical trials before they can be used in clinical practice to improve patient outcomes.

Conclusion:

In conclusion, MS is a difficult and complicated neurological disorder that significantly impacts the existence of millions globally. Despite advancements in the comprehension of its statistics, etiology, and pathophysiology, considerable obstacles persist in delivering efficient therapies for all variants of MS, especially the progressive forms. Contemporary therapeutic options predominantly include disease-modifying medications, symptomatic interventions, and supportive care; nonetheless, these methodologies exhibit limits, especially in decelerating disease development or enhancing neuroprotection.

Novel therapeutics, such as advanced biologics, neuroprotective compounds, and remyelination approaches, exhibit significant potential for enhancing disease outcomes. Investigations into targeted therapeutics persist in advancing, providing novel pathways for the treatment of MS with enhanced precision and diminished adverse effects. Nevertheless, sustained efforts are necessary to comprehensively elucidate the causes of MS and to ascertain tailored treatment strategies that more effectively accommodate the disease's varied characteristics. The future of MS management necessitates a holistic

approach that integrates pharmacological treatments, rehabilitation, and individualized care to enhance the standard of life for individuals impacted by the condition.

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MATHEMATICAL MODELING IN PHARMACEUTICAL AND HEALTH SCIENCES: APPLICATIONS AND IMPACT

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Introduction:

Mathematical modeling is a cornerstone of scientific research, especially in pharmaceutical and health sciences. By providing quantitative frameworks, mathematical models help to understand complex biological systems, predict outcomes, and optimize drug development and healthcare strategies. These models are designed to simulate real-world phenomena, making them essential tools in areas like pharmacokinetics, drug delivery, disease modeling, and health policy analysis.

Foundations of Mathematical Modeling in Pharma and Health Science

Mathematical models in pharmaceutical and health sciences are grounded in principles from various branches of mathematics, such as calculus, differential equations, and statistics. These models attempt to represent real biological processes with mathematical expressions to predict and analyze the behavior of drugs within the body, disease progression, and the impact of healthcare interventions. At its core, the goal of mathematical modeling is to simplify complex biological processes into quantifiable relationships that can be analyzed computationally (Keener & Sneyd, 2009).

In pharmacology, one of the most widely used models is the pharmacokinetic (PK) model, which describes how a drug is absorbed, distributed, metabolized, and excreted by the body (ADME). Similarly, pharmacodynamic (PD) models describe the relationship between drug concentration at the site of action and the resulting therapeutic effect. These models help optimize drug dosages and schedules to maximize therapeutic benefits while minimizing side effects (Gabrielsson & Weiner, 2012).

Mathematical Modeling in Drug Development

Mathematical models are invaluable in drug development, particularly in early-stage research. They help researchers predict how a drug will behave in the human body and how it might interact with different biological targets. Pharmacokinetic-pharmacodynamic (PK-PD) models are key to drug discovery, providing insights into drug absorption,

distribution, metabolism, and excretion, as well as how these processes correlate with therapeutic effects (Gabrielsson & Weiner, 2012).

For example, mathematical modeling can predict the optimal dosage of a drug that would achieve the desired therapeutic effect while avoiding toxic side effects. This predictive capability not only accelerates the development process but also reduces the likelihood of failure in later clinical trials, ultimately saving time and costs (Benson *et al.,* 2020).

In addition to traditional compartmental models, newer approaches like physiologically-based pharmacokinetic (PBPK) models are gaining popularity. These models provide more detailed simulations of drug behavior by considering factors like organ-specific blood flow rates and tissue characteristics, making them more accurate and applicable across diverse populations (Jones *et al.,* 2015).

Role of Mathematical Models in Personalized Medicine

One of the most promising and transformative applications of mathematical modeling in healthcare is in the field of personalized medicine. Personalized medicine aims to tailor medical treatments to the individual characteristics of each patient, based on factors such as their genetic makeup, lifestyle, and environmental influences. Mathematical models play a critical role in analyzing complex datasets from these diverse sources to predict how a patient will respond to a particular drug, enabling more precise and effective treatment strategies.

A key area where mathematical models shine is in the integration of pharmacogenomics—the study of how genes influence a person's response to drugs. For instance, genetic variations in genes such as CYP2C9, which affects the metabolism of warfarin (a commonly used blood thinner), can lead to differing drug responses among patients. Some individuals may metabolize the drug too quickly, while others may do so too slowly, increasing the risk of adverse effects like bleeding or clotting. By incorporating this genetic information into mathematical models, clinicians can adjust the warfarin dosage for each patient, optimizing the therapeutic outcome and minimizing the risk of side effects (Gage *et al.,* 2008).

In recent years, the integration of machine learning techniques with traditional pharmacokinetic models has further improved the precision of personalized medicine. These hybrid models can process vast amounts of patient data, uncovering hidden patterns and predicting how individuals will respond to various treatments. By doing so, they can

recommend the most suitable drug dosage and scheduling, ensuring more effective and individualized care (Mayr *et al.,* 2016). The combination of personalized data and mathematical modeling holds significant promise for advancing targeted therapies, ultimately revolutionizing the way medical treatments are delivered.

Mathematical Modeling for Public Health and Policy

Mathematical models are invaluable tools not only in drug development but also in shaping public health policy and management. In the realm of infectious disease control, epidemiological models such as the SIR (Susceptible-Infected-Recovered) model have been instrumental in predicting disease spread and guiding public health interventions. For instance, during the COVID-19 pandemic, SIR models helped forecast the course of the outbreak, assess the impact of preventive measures like vaccination and quarantine, and assist policymakers in deciding when and how to implement these strategies (Ferguson *et al.,* 2020).

Beyond infectious diseases, mathematical models are equally crucial in managing non-communicable diseases (NCDs), such as diabetes, heart disease, and cancer. Models that simulate the progression of these chronic conditions allow healthcare providers to make more informed decisions about prevention, diagnosis, and treatment. For example, diabetes progression models help doctors understand the impact of various treatment strategies on long-term health outcomes (Clarke *et al.,* 2004).

Additionally, resource allocation models like linear programming are essential for managing limited healthcare resources, particularly in crisis situations. For example, during the opioid epidemic or healthcare shortages, these models help optimize the distribution of essential resources, ensuring they reach the populations most in need (Townsend *et al.,* 2020). Thus, mathematical modeling is pivotal in improving both individual patient outcomes and overall public health efficiency.

Conclusion:

In conclusion, mathematical modeling is an essential tool in pharmaceutical and health sciences, with applications ranging from drug development and personalized medicine to public health and policy planning. These models offer invaluable insights into drug behavior, disease dynamics, and healthcare management, making them indispensable in modern scientific research and healthcare decision-making. As the field continues to evolve, the integration of advanced computational techniques and large datasets promises

to further enhance the accuracy and applicability of mathematical models, improving outcomes for both individual patients and public health at large.

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FENUGREEK AND HUMAN HEALTH: FROM KITCHEN SPICE TO NATURAL MEDICINE

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Abstract:

This chapter explores the extensive therapeutic potential of *Trigonella foenum-graecum* (fenugreek) seeds, focusing on their anti-inflammatory, antioxidant, and blood sugar lowering effects, alongside their roles in cancer treatment, antimicrobial action, and gastrointestinal protection. Fenugreek seeds are rich in bioactive compounds such as triglycerides, fatty acids, flavonoid-C-glycosides, and saccharides, contributing to their wide-ranging health benefits. The seeds' antioxidant and anti-inflammatory properties were demonstrated through experimental models, including rat arthritis models, where mucilage and petroleum ether extracts significantly reduced inflammation markers and enhanced antioxidant enzyme activity. Additionally, fractions containing alkaloids and flavonoids enhanced the anti-inflammatory effects, offering new insights into their potential for pain relief and inflammation suppression. In metabolic health, fenugreek has shown substantial anti-diabetic and cholesterol-lowering effects. The seeds' saponins and high fiber content aid in blood sugar regulation by slowing gastric emptying and inhibiting carbohydrate-digesting enzymes, while improving lipid profiles and reducing LDL cholesterol. A key active compound, 4-hydroxyisoleucine, demonstrated significant anti-diabetic effects, suggesting its potential as an adjunct therapy for type 1 and type 2 diabetes. Fenugreek extracts also exhibit promise in cancer treatment, particularly against pancreatic and breast cancer cell lines, by inducing apoptosis and autophagy. Fenugreek further displays strong antimicrobial activity, especially against multi-drug-resistant bacteria, positioning as a natural antimicrobial strategy to replace synthetic antibiotics. Aqueous and ethanolic extracts of fenugreek have also shown significant gastro-protective effects, reducing gastric peptic ulcer severity persuaded by ethanol and aspirin. This study underscores the health-promoting properties of fenugreek seeds in various medical conditions and calls for further research to fully unlock their medicinal benefits.

Keywords: Fenugreek, Anti-Inflammatory, Antioxidant, and Blood Sugar Lowering Effects

Introduction:

Fenugreek (*Trigonella foenum-graecum*) is a multifaceted herb that has long served as both a common culinary ingredient and a medicinal resource. Indigenous to the Mediterranean, Southern Europe, and Asia, fenugreek has been cultivated for thousands of years for its seeds and leaves, which are extensively utilized in cooking and traditional healing practices. In recent times, scientific research has expanded the herb's applications, confirming many of its historical uses for enhancing human health. This dual role—as a flavorful spice and a powerful natural remedy—positions fenugreek at the forefront of the growing field of functional foods and integrative medicine.[1] In ancient Egypt, fenugreek was used in embalming, while traditional Chinese and Ayurvedic medicine employed it to address a variety of health concerns, such as digestive issues, wounds, and inflammation. Known for its distinct, slightly bitter taste and maple syrup-like scent, fenugreek has become a key ingredient in spice blends like Indian curry powders and Ethiopian berbere. Its therapeutic potential is primarily attributed to its rich composition of bioactive compounds, including saponins, flavonoids and alkaloids, which contribute to its growing reputation as a powerful health-promoting agent.[1]

Scientific research increasingly supports fenugreek's potential in improving human health, particularly in the management of diabetes, high cholesterol, and hormonal imbalances. Studies indicate that fenugreek seeds may aid normalise blood sugar levels and increase insulin sensitivity, offering promise in the prevention and treatment of non-insulin dependent diabetes mellitus. Moreover, the herb's ability to reduce cholesterol and promote heart health is gaining attention. In addition to its metabolic properties, fenugreek has been investigated for its broader therapeutic effects on lactation, with many breastfeeding mothers using it to increase milk production, a practice both culturally common and scientifically supported.[1-3]

Fenugreek's appeal extends to its potential to influence testosterone levels and enhance libido, particularly in men. Supplements containing fenugreek extract are increasingly marketed for their purported benefits in boosting energy, mental alertness, mood, and supporting sexual health. While the evidence is still inconclusive and further research is required, early results appear promising.[4] The rising interest in natural and holistic health solutions has further increased fenugreek's relevance. As more people seek alternatives to pharmaceutical drugs, plant-based remedies like fenugreek have become a focal point. Given its long history as a safe culinary ingredient and its growing body of

clinical evidence, fenugreek is well-positioned within this broader shift toward natural health interventions. However, it's important to note that while fenugreek is generally considered safe, excessive consumption or the use of concentrated extracts may lead to potential side effects.

In conclusion, fenugreek stands as an example of the intersection between traditional knowledge and contemporary scientific inquiry. From its role as a modest kitchen spice to a subject of modern biomedical research, its story reflects a larger trend of revisiting natural substances for their therapeutic potential. This review will delve into fenugreek's various health benefits, focusing on its bioactive compounds, mechanisms of action, clinical evidence, and future prospects as both a food and a medicine.

Inflammation-Reducing Effect

This research examined the potential health-enhancing properties of *Trigonella foenum-graecum* (fenugreek) seeds by assessing their antioxidant and anti-inflammatory effects. The findings indicated that the extracts were capable of minimizing oxidative damage and suppressing inflammation-related enzyme activity. Further investigation led to the discovery of several bioactive constituents, including triglycerides, fatty acids, saccharides, and flavonoid-C-glycosides. Except for the saccharides, these compounds exhibited significant biological effects in the assays. Notably, this study is the first to report the occurrence of certain sabstances in fenugreek seeds, underlining their potential as natural therapeutic agents for combating oxidative stress and inflammation.[5] In one study, the petroleum ether extract of fenugreek seeds was assessed for its inflammation lowering and arthritis-suppressing effects using multiple rat models. The extract significantly reduced paw swelling in both carrageenan and formaldehyde-induced edema models, inhibited granuloma development in the cotton pellet test, and improved liver function by reducing elevated enzyme levels linked to arthritic conditions. These therapeutic effects are likely due to the high content of linoleic and linolenic acids identified in the extract.[6]

This research indicated that mucilage extracted from fenugreek exhibits strong inflammation lowering and antioxidant impacts in a rat model of adjuvant-persuaded arthritis. The treatment effectively reduced joint inflammation, surpassing the performance of the standard drug indomethacin. It decreased the expression of key inflammatory markers, including cyclooxygenase-2, myeloperoxidase, and TBARS, while augmenting the action of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, along with elevating glutathione and vitamin C levels. Additionally, fenugreek

mucilage helped restore altered blood parameters, correcting elevated ESR and WBC counts and improving RBC, hemoglobin, and CRP levels. Histological evaluation further confirmed a reduction in tissue edema and inflammatory cell infiltration, highlighting the potential of fenugreek mucilage as a natural treatment for arthritis.[7] This study investigated the pain-suppressing and inflammation lowering effects of different fractions from fenugreek seeds. The methanolic extract was divided into six fractions, which were tested for their effects by means of formalin and carrageenan-persuaded paw edema models. The methanolic extract revealed notable pain-suppressing and inflammation lowering properties at a dose of 100 mg/kg. Among the segments, the alkaline chloroform fraction, which contained alkaloids, showed the strongest antinociceptive effects, similar to morphine. Additionally, both the aqueous and acidified chloroform (ACC) fractions effectively reduced paw edema, with the ACC fraction demonstrating a dose-dependent anti-inflammatory effect. Phytochemical analysis identified flavonoids in the aqueous and ACC fractions. These findings suggest that alkaloids are likely responsible for the antinociceptive effects, while flavonoids contribute to the anti-inflammatory properties of fenugreek seeds.[8]

Blood Sugar-Lowering Effect

This study investigates the blood sugaring lowering and cholesterol-lowering effects of fenugreek seeds, observed in both animal models and humans. The main bioactive components driving these effects are the saponins and high fibre content of fenugreek, rather than its main alkaloid, trigonelline. Fenugreek's antidiabetic effects are linked to its ability to lower blood sugar levels, primarily by delaying gastric emptying and inhibiting carbohydrate-digesting enzymes. Furthermore, fenugreek may increase plasma insulin levels, with the amino acid 4-hydroxyisoleucine promoting insulin secretion. Regarding its cholesterol-lowering effects, fenugreek enhances the conversion of liver cholesterol to bile salts and facilitates the excretion of cholesterol-saponin complexes. This leads to a decline in LDL and VLDL cholesterol levels while uplifting HDL-cholesterol in both diabetic rats and humans. Importantly, fenugreek has not been associated with any toxic effects, suggesting that its consistent ingesting may be beneficial in the regulation of blood sugar and in preventing cardiovascular complications such as atherosclerosis and coronary heart disease.[9] The study found that diabetic rats treated with fenugreek and buckthorn extracts showed improvements in biochemical and histological parameters, including reduced blood sugar, glycated hemoglobin, liver enzymes, cholesterol, and lipid

peroxidation. Additionally, there was an increase in HDL, albumin, and antioxidant activity, with reduced liver and testes damage. The seed extracts of both plants exhibited stronger antioxidant activity than the leaf extracts.[10]

The study revealed that 4-hydroxyisoleucine (4HO-Ile), an amino acid present solely in fenugreek seeds, has significant anti-diabetic properties. In a type 1 diabetes model using streptozotocin-treated rats (which have greatly reduced insulin levels), daily treatment with 50 mg/kg of 4HO-Ile for four weeks effectively reduced plasma glucose levels. Additionally, the treatment restored lipid profiles (cholesterol, HDL, LDL, triglycerides) and uric acid levels to normal, non-diabetic ranges. These results suggest that 4HO-Ile works independently of insulin to improve diabetic conditions, making it a potential adjunct therapy for both type 1 and type 2 diabetes.[11] The study demonstrated that 21 days of oral dosing of Fenugreek and Balanites extracts significantly impacted liver and kidney glycogen levels and key liver enzymes involved in carbohydrate metabolism in STZ-diabetic rats. The STZ injection led to increased blood glucose, decreased serum insulin, and reduced liver glycogen, with enzyme activity changes indicating impaired carbohydrate metabolism. Fenugreek extract reduced blood glucose by 58%, restored liver glycogen, decreased kidney glycogen, and lowered liver glucose-6-phosphatase activity. Balanites extract reduced blood glucose by 24% and similarly decreased liver glucose-6-phosphatase activity. Both extracts inhibited α-amylase activity in a dose-dependent manner, with Fenugreek showing more potent inhibition. This inhibition was competitive and was confirmed by decreased starch digestion and absorption in normal rats. These findings suggest that the hypoglycemic effects of Fenugreek and Balanites are driven by insulin-like effects and intestinal α-amylase inhibition.[12]

The study revealed that the water-soluble compound GII, derived from fenugreek seeds, exhibited significant antidiabetic effects in subdiabetic, moderately diabetic, and severely diabetic rabbits. After administering GII for 15 days in subdiabetic and moderately diabetic rabbits, and 30 days in severely diabetic rabbits, treatment resulted in a decrease in serum lipids, including total cholesterol, triglycerides, and LDL along with VLDL cholesterol, while increasing HDL cholesterol. It also lowered total lipids in the liver and heart, and improved liver and muscle glycogen levels. Furthermore, important enzymes involved in carbohydrate metabolism, such as hexokinase, glucokinase, pyruvate kinase, malic enzyme, and glucose-6-phosphate dehydrogenase, were upregulated, while enzymes linked to gluconeogenesis and the polyol pathway (including glucose-6-phosphatase,

aldose reductase, and sorbitol dehydrogenase) were downregulated. Antioxidant enzyme levels, like superoxide dismutase, glutathione peroxidase, and glutathione reductase, also increased. In addition, histopathological improvements were observed, with fatty infiltration and cellular changes in the pancreas, liver, heart, and kidneys being repaired. Lastly, GII treatment helped restore normal liver and kidney function, confirming its safety and absence of side effects.[13]

Cancer-Fighting Properties

The study demonstrated that germinated fenugreek seed extract exhibited notable anticancer activity against BXPC-3 pancreatic cancer cell lines in both in vitro and in vivo settings. In the animal study, albino mice injected with BXPC-3 cells were divided into two groups: one group was untreated, while the other received the IC50 dose of fenugreek extract. The treated mice showed enhanced survival rates and protection of pancreatic tissue from cancer-induced damage. These findings indicate that germinated fenugreek seed extract may offer a promising therapeutic approach for pancreatic cancer.[14] The study revealed that fenugreek seed extract revealed targeted cytotoxic effects on diverse oncogenic cell lines, including T-cell lymphoma, during in vitro testing. The anticancer activity was shown to be dependent on both concentration and exposure duration. Proteomic analysis further showed that the fenugreek variety associated with tumor regression in a primary CNS T-cell lymphoma patient had a distinct protein composition compared to other regional variants. These findings support the likely role of fenugreek as a valuable candidate for cancer prevention and treatment.[15] The study demonstrated that Fenugreek seed extract provided notable protection against DMBA-induced breast cancer in rats. Administered at a dose of 200 mg/kg b.wt., the extract effectively reduced mammary hyperplasia and its frequency. The findings also suggested that apoptosis could be significant in Fenugreek's chemopreventive effects on breast cancer. This is the first study to highlight the potential of Fenugreek seeds in breast cancer prevention.[16]

The study found that Fenugreek seed extract (FSE) exhibited significant anticancer effects on pancreatic cancer cells by inhibiting cell proliferation in a dose- and time-dependent fashion. FSE induced apoptosis by activating cleaved caspase-3 and Bax, and reduced cell migration by modulating the MAPK, Akt, MMP-9, and vimentin pathways. LC-MS/MS analysis identified multiple active compounds in FSE that promote apoptosis. Importantly, FSE had minimal effect on normal cells, suggesting its potential as a targeted treatment for pancreatic cancer.[17] The study revealed that fenugreek seed extract

exhibited anticancer effects by causing cell death in Jurkat cells in a dose- and time-dependent fashion. This process was marked by distinct morphological changes, including the formation of large vacuoles and increased LC3 transcript expression, suggesting the induction of autophagy. The extract contained several active compounds with anticancer properties, such as gingerol, zingerone, cedrene, vanillin, and eugenol. These results indicate that, in addition to its known ability to induce apoptosis, fenugreek may also promote autophagy-associated cell death. This study is the first to demonstrate fenugreek's ability to induce autophagy in human cells, underscoring its potential as an anticancer agent. Further studies are required to investigate the intermediates involved in the autophagic pathway.[18]

Antimicrobial Activity

The study revealed that fenugreek leaf extracts displayed notable antimicrobial properties. The aqueous extract showed the most potent antibacterial effect against *Serratia marcescens*, while the methanol extract was effective against *Bacillus cereus*. Additionally, the methanol extract exhibited strong antifungal activity against *Trichoderma viridae*. The minimum inhibitory concentration (MIC) of the extracts ranged from 6.25 to 25 mg/ml. Thin-layer chromatography helped identify active compounds with antibacterial effects. The methanol and aqueous extracts were richer in phytochemicals compared to other solvents, suggesting that fenugreek leaves hold significant potential as antimicrobial agents.[19] The study found that the methanolic extract of fenugreek seeds exhibited stronger antibacterial activity than the hot aqueous extract. The methanol extract produced the highest zone of inhibition (30 mm) against *Pseudomonas aeruginosa* at a 75% concentration, while the lowest (10 mm) was seen against *Escherichia coli* at a 25% concentration. The hot water based extract also showed significant antibacterial activity, with a maximum inhibition of 24 mm against *P. aeruginosa* at 75%, but no effect at 25%. Overall, the methanol extract demonstrated superior antibacterial properties compared to the hot aqueous extract and was more effective than the commercial antibiotic amikacin.[20]

The study investigated the bacterial-inhibiting and cancer-fighting activities of fenugreek seed extract. It demonstrated significant antibacterial activity, especially against *Staphylococcus aureus* (22 mm) and *Pseudomonas aeruginosa* (17 mm). In terms of anticancer effects, the extract inhibited the proliferation of MCF-7 breast cancer cells at a 400 µg/ml concentration after 72 hours, although it did not induce notable apoptosis or necrosis. Furthermore, the extract showed no anticancer activity on liver cancer HCAM

cells or Vero non-cancerous cells. Overall, the study suggests that fenugreek seed extract holds potential as both an antibacterial and anticancer agent.[21] The study revealed that fenugreek seed extracts, particularly flavonoid extracts, exhibited notable antimicrobial activity against various uropathogenic bacterial strains. The extracts, which are rich in polyphenols, showed stronger antibacterial effects than aqueous extracts, especially against Gram-positive bacteria like *Staphylococcus aureus*. Flavonoids had an activity index (AI) ranging from 1 to 2.5 for *S. aureus*, and 1 to 1.21 for Gram-negative bacteria such as *E. coli*, *Citrobacter freundii*, and *Pseudomonas aeruginosa*. The tested uropathogenic strains were multidrug-resistant, suggesting fenugreek's potential as an alternative antimicrobial treatment.[22]

Gastro-Protective Effect

The study found that both aqueous and ethanolic extracts of fenugreek seeds effectively protected against ethanol-persuaded gastric ulcers in rats. Pre-treatment with the extracts (500 mg/kg body weight) significantly reduced ulcer development in terms of lesion number, size, and area, with results showing statistical significance. No significant difference was observed between the aqueous and ethanolic extracts in their protective effects. The findings suggest that both extracts, in addition to omeprazole, have gastro-protective properties.[23] The study revealed that both fenugreek and cabbage aqueous extracts effectively protected rats from aspirin-induced gastric ulcers. Whether used separately or together, the extracts helped reduce ulcer severity, ulcer index, gastric volume, and acidity, while also increasing gastric pH. Histological examination indicated that the combination of extracts (T5) produced results similar to the anti-ulcer drug omeprazole, with the T5 group showing a healthy stomach lining, whereas the untreated group exhibited ulceration and congestion. The combined extracts were as effective as standard anti-ulcer treatments.[24]

Conclusion

This chapter highlights the diverse therapeutic potential of fenugreek, emphasizing their anti-inflammatory, antioxidant, anti-diabetic, and cancer-fighting properties. Rich in bioactive compounds like triglycerides, fatty acids, flavonoid-C-glycosides, and saccharides, fenugreek seeds demonstrate significant health benefits across various experimental models. Their anti-inflammatory effects, particularly through mucilage and petroleum ether extracts, and their role in reducing blood sugar levels and improving lipid profiles showcase their metabolic health benefits. Additionally, fenugreek's antimicrobial, cancer-

fighting, and gastro-protective activities position it as a promising natural therapeutic agent. Further research is needed to fully explore its medicinal applications.

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HEALTH BENEFITS OF SWEET POTATO

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Abstract:

According to contemporary standards, sweet potatoes, which are also referred to as *Ipomoea batata* L. or Lam., are considered to be an extremely intriguing and nourishing dish. This is due to the fact that they contain a significant number of secondary metabolites that are advantageous to one's health, in addition to the fact that they contain a large quantity of complex carbohydrates. In order to accomplish the purpose of this review, the objective is to present a condensed synthesis of the most recent literature that has been published on this root vegetable. Specifically, the bioactive phytochemical components of this vegetable, as well as the possible effects on health and the effects of processing methods, will be the focus of the attention that will be paid. As a result of their antioxidant, anti-inflammatory, and hepatoprotective characteristics, as well as their cardiovascular protection and anti-cancer capabilities, sweet potatoes have been linked to several health advantages. These benefits include enhancements in neurological and memory ability, metabolic problems, and intestinal barrier function. Both carotenoids and polysaccharides are found in high concentrations in sweet potatoes, making them a remarkable source of both nutrients. Additionally, the purple sweet potato, which has a high anthocyanin content, not only offers consumers a novel food alternative but also has the potential to be a source of useful components for the manufacturing of healthy food items. This is because the purple sweet potato contains a high concentration of anthocyanin. The implications of commercial processing and household cooking processes on the bioactive chemicals contained in sweet potatoes require greater investigation in order to develop an understanding of how to reduce the loss of these components. When seen in this perspective, the consequences of these procedures on the bioactive chemicals should be considered important.

Keywords: Anthocyanins, Antioxidant, Hepatoprotective, Metabolic Disorders, Anti-Cancer Properties

Introduction:

A member of the Convolvulaceae family, the sweet potato (*Ipomoea batata* L.; Lam.) is a type of dicotyledonous vegetable. After wheat, rice, maize, potatoes, barley, and cassava, it ranks as the seventh most produced crop globally, and as the fifth in developing nations [1,2]. The tubers, leaves, and shoots of sweet potatoes are nutritious for both people and animals; in fact, around half of the harvest goes toward feeding pets. Digestive fiber, protein, starch, and minerals like manganese, copper, potassium, and iron are just a few of the macronutrients found in sweet potato tubers. The micronutrients include provitamin A (in the form of carotenoids), minerals, vitamins (primarily B complex, C, and E), anthocyanins (the color of sweet potatoes), flavonoids, and coumarins [3]. The sweet potato is richer in carbs, proteins, and several vitamins and minerals than other root and tuber crops [4]. It also includes more minerals, provitamin A, and vitamin C than wheat and rice [5].

Sweet potatoes are becoming more popular in many circles, including the scientific community, the food industry, and individual consumers, all because of the abundance of beneficial secondary metabolites they contain [6]. Both its health benefits and its use as a component in functional meals have attracted a lot of interest in it recently [7]. Numerous physiological benefits have been demonstrated by the bioactive components found in sweet potatoes, including antioxidants, flavonoids, carotenoids, and phenolic acids. Among their many benefits, these chemicals can aid in reducing inflammation, improving metabolic health, and mitigating oxidative stress. Hence, sweet potatoes, whether eaten whole or included in functional food items, may help with general health, disease prevention, and lifespan. Combining the health advantages of these phytochemicals can improve consumers' well-being even more than when they are taken alone [8].

The health benefits of this food are associated with its hue [9]. There is evidence that cultivars with lighter flesh have more phenolic compounds, while varieties with a more intense yellow hue tend to have a larger proportion of carotenoids, specifically ß-carotene. Purple sweet potatoes have a lot of anthocyanins, while yellow and orange sweet potato cultivars are rich in phenolic acids [10,11].

Current understanding of the bioactive composition and potential health benefits of sweet potato leaves was reviewed in a recent review [12]. Protocols for plant regeneration were also discussed as an alternate technique to provide planting material that is disease-free [13]. Sweet potato roots have more than simply a long history of use as a carbohydrate

source; they also have a lot of untapped potential, as has been observed in the leaves and cultivation methods of sweet potatoes. These days, people know that they're a great source of nutrients and can help stave off chronic ailments. Supporting this claim, we have incorporated new data on their presence in bioactive chemicals and thoroughly revised the in vitro and in vivo proof of its positive effects on human health. Finally, we have investigated how processing sweet potatoes affects phenolic chemicals, which are essential to human health.

Beneficial Health Effects of Sweet Potatoes

Due to their high starch content, sweet potatoes have traditionally been an important source of carbohydrates and energy for both humans and livestock. Because of their long-lasting energy content, they have been a mainstay in many cuisines for ages. Nevertheless, sweet potatoes have recently come to light for their wider nutritional value and their part in promoting general wellness, especially in warding off chronic diseases. This change in thinking is mostly because of the wide variety of vital nutrients they contain. The high fiber content of sweet potatoes is especially noteworthy since it aids digestion and blood sugar regulation, which in turn helps prevent obesity, type 2 diabetes, constipation, and other related health problems. They also have sugars that occur naturally; the body absorbs these sugars slowly, so they provide energy steadily rather than the rapid surges that refined carbohydrates cause [14].

Sweet potatoes are packed with nutrients, including a substantial amount of protein. While it's not quite as high as in animal products, this protein helps with muscle repair and immune system support. Vitamin A, in the form of beta-carotene, is vital for healthy eyes, skin, and the immune system, and these foods are rich in it. Vitamin C, another essential ingredient in sweet potatoes, is an antioxidant that increases cell protection, collagen formation, and iron absorption.

Not only are these vitamins and minerals abundant in sweet potatoes, but they also play an essential role in maintaining heart health through regulation of blood pressure and the transfer of oxygen throughout the body. Sweet potatoes are good for your bones and teeth because they are high in calcium, which helps keep your bones healthy. Sweet potatoes also include very little salt and cholesterol and very little fat overall, particularly saturated fat. This makes them a good option for those looking to lower their risk of cardiovascular disease, which is largely due to a diet low in cholesterol and saturated fats. In this way, sweet potatoes are a potent weapon in the fight against

numerous long-term health issues, such as obesity, cancer, and cardiovascular disease. As shown in The Okinawan Diet, frequent intake of sweet potatoes or extracts rich in their bioactive phytochemicals appears to have positive effects on human health as well. While some good health outcomes have been documented in animal models, the majority of these reports have relied on in vitro investigations, which will be discussed further below. There has been just one human trial conducted thus far [15].

Antioxidant Properties

The anthocyanin and carotenoid content of purple sweet potatoes is largely responsible for their high antioxidant potential. A reduced risk of diabetes, cardiovascular disease, cancer, and cognitive function is connected with the consumption of anthocyanin-rich goods because these chemicals exhibit free radical scavenging activity [16]. In comparison to anthocyanins found in red cabbage, grape skin, elderberry, and purple maize, as well as vitamins C and E, anthocyanins from purple sweet potatoes exhibited a greater ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals [17,18].

Researchers found that white-fleshed cultivars had a higher concentration of phenolic acids and carotenoids, which gave them a higher antioxidant potential. They concluded that eating these foods might protect humans from oxidative stress [19]. In extracts of purple-fleshed cultivars "Miyanou-36" and "Bise," chlorogenic acid, not anthocyanins, was the primary DPPH radical scavenger [20]. In contrast, the primary pigment of yellow or orange sweet potatoes, β-carotene, is a source of provitamin A and has a considerable antioxidant potential because of its conjugated double bonds [21].

Hepatoprotective Effects

Only one study has been conducted on humans, while the majority of investigations on hepatoprotective effects have been conducted on animal models. Furthermore, it is to be mentioned that each of this research have concentrated on assessing the protective impact of sweet potato anthocyanins.

The anthocyanins provide hepatotoxin protection by free radical scavenging and inhibition of lipid peroxidation. The serum levels of several liver enzymes, especially gamma-glutamyl transferase (GGT), were found to be dramatically lowered in healthy males with borderline hepatitis after consuming purple sweet potato beverages [22]. Animal studies have shown that sweet potato polysaccharides and anthocyanin-rich purple sweet potato extract [23, 24] can protect against acute liver damage caused by CCl4 [25]. The delivery of anthocyanin-rich sweet potato flakes to male rats on a high-cholesterol diet

inhibited lipid peroxidation [26], and the same was seen in the acetaminophen-induced hepatotoxicity mouse model [27]. Anthocyanin, a pigment found in purple sweet potatoes, reduced oxidative stress and inflammatory response in mouse livers exposed to D-galactose [28]. In rats, an extract from the Shinzami variety of purple sweet potatoes protected the livers from damage caused by ischaemia-reperfusion by enhancing their antioxidant status [29]. Animal research have shown a protective effect, however to prove that purple sweet potato anthocyanins have a robust hepatoprotective impact, human studies are required.

Breast Cancer

Among female cancers, breast cancer ranks high in terms of global mortality. Using E0771 murine breast cancer cells, Kato *et al.* [30] discovered that fermented sweet potato contains lipid-soluble polyphenols, primarily caffeic acid derivatives, which have a high lipophilicity and a significant antioxidant activity. As a result, these polyphenols aggregate in the cell cytoplasm and reduce reactive oxygen species (ROS). In addition to increasing the cytotoxicity of anti-cancer drugs and inhibiting Akt activity, these metabolites halted the cell cycle at G0/G1. Sweet potato lipid-soluble polyphenols increased chemotherapy drug efficacy and decreased tumor growth, indicating that sweet potatoes may have a role as a functional food in cancer treatment.

Glycoprotein SPG-56, found in the novel sweet potato variety Zhongshu NO. 1, was found to increase apoptosis and limit proliferation of MCF-7 cells in mice in a manner that was dependent on both dose and time [31]. There was a substantial difference ($p < 0.01$) between the untreated control group and mice that were orally administered 240 mg/kg/d of SPG-56, with a 54.8% reduction in blood tumor markers CEA, 91.8% reduction in CA125, and 90.3% reduction in CA153. Scientists discovered that SPG-56 inhibited MCF-7 cell growth by influencing the expression of certain genes. As a potential new anti-tumor agent for the treatment of breast cancer, SPG-56 warrants additional investigation.

Colon Cancer

Worldwide, colon cancer accounts for a disproportionate share of cancer-related deaths. Isolated from Zhongshu-1 sweet potatoes, a novel small molecule known as glycoprotein SPG-8700 promotes apoptosis in HCT-116 colon cancer cells by modulating the expression of Bcl-2 and Bax genes [32]. It has no impact on normal cell proliferation. Another chemical derived from sweet potatoes, sporamin, shows encouraging results in both laboratory and animal studies addressing colorectal cancer [33]. By increasing

expression of genes involved in DNA damage repair, critical enzyme activity, and intracellular metal ion balance, this proteinase inhibitor altered the gene expression profile of colon cancer cells.

Lim *et al.* [34] discovered that clone P40, an anthocyanin-enriched sweet potato, protected against colorectal cancer by apoptotic mechanisms, cell cycle arrest, and inhibition of proliferation. Both cell culture in the lab and an animal model of cancer growth confirmed that this clone was active against the disease. A P40 anthocyanin extract inhibited the proliferation of human colon SW480 cancer cells in a dose-dependent manner via a cytostatic rather than a cytotoxic mechanism.

Metabolic Disorders and Intestinal Barrier Function

Sweet potatoes with purple flesh contain anthocyanin, whereas sweet potatoes with orange flesh include carotenoids; both of these extracts could be helpful as adjuncts in the fight against obesity and its complications. In vivo (in obese mice generated by a high-fat diet) and in vitro (using 3T3-L1 cells) studies investigated their anti-obesity effects [35]. As a cardio-metabolic biomarker that indicates an increased risk of heart disease and arteriosclerosis, both extracts showed promise in inhibiting fat accumulation in adipocytes, decreasing weight gain, and restoring triglyceride levels to normal through an improvement in the ratio between triglyceride and high-density lipoprotein cholesterol.

Despite the absence of comprehensive evidence from clinical trials and the paucity of research on long-term effects, a recent systematic review based on in vitro and in vivo investigations found that sweet potato is beneficial in treating hyperglycemia [36]. Atherosclerosis is the most significant cardiovascular consequence of diabetes, and cardiovascular diseases are a leading cause of death in people with type 2 diabetes mellitus [37]. Actually, it has been proven that the atherosclerotic process begins with the endothelial dysfunction seen in diabetes [38]. Researchers observed that sweet potato flavonoids reduced inflammation, inhibited platelet aggregation, and enhanced endothelial function, all of which contributed to a reduction in atherosclerosis in mice [39]. An additional study added to the existing body of evidence by stating that purple sweet potato flavonoids protected endothelium dysfunction in type 2 diabetes mellitus. This protection was achieved by lowering endothelial premature senescence and atherogenesis through the suppression of ROS levels and the NLRP3 inflammasome [40].

Other Cancers

Consistent intake of the phenolic compounds found in fruits and vegetables unquestionably confers anti-cancer advantages. Both in vitro and in vivo investigations have evaluated the potential anti-cancer effects of PSPA and polyphenol-rich sweet potato extract.

One frequent malignant condition, bladder cancer, has demonstrated in vitro antitumor effects using PSPA. In a dose-dependent manner, PSPA decreased the viability of bladder cancer cells, increased the apoptotic rate, and suppressed the cell cycle, according to Li *et al.* [41]. The anticancer benefits of PSPA are due to its method of action, which involves lowering expression of the anti-apoptotic gene Bcl-2 and upregulating genes that promote cell death. The study's findings shed light on the treatment of bladder cancer and the possible function of PSPA in cancer prevention, offering fresh information to the field. Researchers also looked at how PSPA affected the bladder cancer cell line BIU87. The groups treated with this phenolic compound showed a considerable inhibition of BIU87 cell proliferation compared to the control. It promoted cell death in a dose-dependent manner. New evidence from cell culture and in vivo prostate cancer xenograft models shows that polyphenol-rich sweet potato extract inhibits growth and induces apoptosis. And hence, the extract could be a dietary supplement that helps with prostate cancer. Despite phenolic compounds' growth and apoptosis inhibitory capabilities, human research are needed to corroborate the in vitro findings [42].

Conclusions:

One of the most important crops for human consumption, *Ipomoea batata* L. Lam., has a diverse assortment of minerals and macronutrients. Carotenoids, phenolic acids, tocopherols, anthocyanins, flavonoids, and coumarins are some of the active secondary metabolites that are abundant in this substance. Therefore, the amount of phytochemicals that are present in sweet potatoes is determined by the conditions under which they are processed and stored, in addition to variables such as fluctuation. Because of the growing body of research indicating that the bioactive components of this root vegetable have positive effects on health, it is garnering a lot of attention from the food industry, consumers, and scientists. The increased antioxidant capacity of purple sweet potatoes can be attributed, in large part, to the high levels of anthocyanin and carotenoid that they contain. These chemical substances have the ability to effectively neutralize free radicals and contribute to the limitation of lipid peroxidation, which helps to protect the liver from

hepatotoxic damage occurring. In addition, there is substantial evidence that consuming sweet potatoes that are rich in anthocyanin can enhance cognitive performance, reduce the chance of developing diabetes, cardiovascular disease, and cancer, and a variety of other health benefits. Furthermore, it has been shown that sweet potatoes contain a number of tiny sesquiterpene compounds that are beneficial to the functioning of the intestinal barrier during digestion. The majority of research on the potential of sweet potato phytochemicals has been carried either in vitro or with animal models; therefore, human intervention trials are required to demonstrate any possible health impacts from sweet potato phytochemicals. On the other hand, research has demonstrated that the anthocyanin found in purple sweet potatoes can protect healthy males who have borderline hepatitis from hepatotoxic harm. Additional research is required in this field, particularly clinical trials that are randomized and conducted on humans. In the present day, we are confronted with the challenge of ascertaining whether or not the consumption of particular sweet potatoes, particularly purple ones, over an extended period of time has an effect on oxidative stress and other disease markers. In the event that the conclusions of the investigation are confirmed, sweet potatoes have the potential to become a well-liked functional food that assists in lowering the chance of chronic diseases among customers. Because of this, new chances would become available in the market.

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CUMIN AS CURE: TRADITIONAL USES AND HEALING POWERS OF JEERA

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Abstract:

This study summarizes various research findings on the therapeutic effects of *Nigella sativa* (black cumin) and *Cuminum cyminum* (cumin) in treating several health conditions. One study demonstrated that *Nigella sativa* oil offers significant protection against aspirin-induced gastric ulcers in rats, reducing ulcer size, edema, and leukocyte infiltration. Biochemical analyses further confirmed the protective benefits of *Nigella sativa* for the gastric mucosa. Another study investigated cumin essential oil's fractions, particularly the E3 fraction, which showed strong anti-ulcer properties by reducing oxidative stress, inflammation, and apoptosis, while inhibiting the NF-κB pathway. Additionally, research on cumin decoction indicated its potential as a complementary treatment to enhance Helicobacter pylori eradication when used alongside conventional therapies. Further studies highlighted the powerful anti-inflammatory and analgesic effects of *Nigella sativa* and cumin extracts, showing promise in reducing inflammation markers and alleviating pain in models of rheumatoid arthritis and colitis. Other research focused on *Nigella sativa*'s bronchodilatory effects in asthma patients and cumin's tracheal relaxant properties in guinea pigs, suggesting their therapeutic value for respiratory conditions. Cancer treatment research emphasized cumin's anticancer effects, particularly in osteosarcoma, where cumin seed extracts showed notable cytotoxicity. Black cumin oil also demonstrated antimicrobial properties, offering protection against food spoilage, and showed cardioprotective effects by improving recovery from myocardial infarction and promoting heart health in various models. Lastly, the cardioprotective benefits of *Nigella sativa* oil and its extract were demonstrated in studies involving type 1 diabetes and doxorubicin-induced heart damage, indicating their potential in managing subclinical left ventricular dysfunction and mitigating chemotherapy-induced cardiac damage. Overall, the findings suggest that both *Nigella sativa* and cumin have wide-ranging therapeutic benefits across multiple health conditions, including gastrointestinal, respiratory, cancer, inflammatory, and cardiovascular diseases.

Keywords: Cumin, Gastric Ulcers, Cardioprotective, Bronchodilatory Effects

Introduction:

Cumin seeds are derived from the herb *Cuminum cyminum*, a plant native to the region from the Eastern Mediterranean to South Asia and a member of the Apiaceae (parsley) family.[1] Recognizable by their elongated shape and yellowish-grey color, cumin seeds emit a warm, earthy aroma with a hint of citrus. For centuries, cumin has been a key spice in global cuisines, especially in Indian, Middle Eastern, North African, and Latin American cooking. In Indian cuisine, cumin is widely used in curries, stews, dals, and rice dishes like *korma*, and is a key ingredient in spice blends such as *garam masala* and *panch phoron*. Beyond its culinary role, cumin has long been esteemed in traditional medicine. In Ayurveda, it is known for stimulating digestion and metabolic activity (*agni*). It is categorized as a digestive enhancer (*deepana*) and gas reliever (*pachana*), used to treat indigestion, bloating, abdominal pain, and chronic diarrhea.[2] Often consumed as tea, powder, or decoction, cumin's carminative and mild antibacterial properties contribute to gastrointestinal health. In Unani and Siddha medicine, it is valued for its warming, tonic-like effects and has been used to manage respiratory issues, fever, and women's health conditions. Modern studies support many traditional uses of cumin. It contains bioactive compounds such as cuminaldehyde, flavonoids, and terpenes, which exhibit antioxidant, anti-inflammatory, and digestive-supportive properties. As both a culinary and medicinal agent, cumin continues to bridge traditional wisdom and scientific understanding, affirming its role as a valuable natural remedy.[2]

Black seed, also known as black cumin (*Nigella sativa*), is an annual herb from the Ranunculaceae family. Originating in Southern Europe, North Africa, and Southwest Asia, it has been cultivated for centuries in regions such as India, Pakistan, Iran, Syria, Turkey, and throughout the Mediterranean. Adapted to hot, dry climates, *Nigella sativa* is grown not only for its culinary applications but also for its recognized medicinal properties. The small, black seeds of the plant have a sharp, bitter flavor with a subtle peppery aroma. These seeds have been integral to a variety of cuisines, used both in food and traditional medicine.[3] In Indian, Middle Eastern, and North African kitchens, black cumin seeds are often dry-roasted or ground and added to curries, pulses, vegetable dishes, and pickles. They are also commonly included in bread, cheese, and other baked goods, as well as in the Bengali spice blend *panch phoron*.

In ancient times, cumin and its relatives were not only prized for their flavor but also for their preservative and medicinal properties. *Nigella sativa* has long been a staple in

medicinal traditions such as Ayurveda and Unani, where its diverse health benefits are widely acknowledged.[4] These systems have utilized black cumin seeds to treat ailments like asthma, bronchitis, rheumatism, fever, skin issues, and digestive disturbances. The seeds are consumed in various forms, including whole, powdered, or as oil, both for preventive care and therapeutic purposes.

The medicinal effectiveness of *Nigella sativa* can largely be attributed to its wealth of bioactive compounds, with thymoquinone being the primary agent responsible for many of its healing effects. Studies have shown that black cumin offers numerous health benefits, such as radical-neutralizing, inflammation-modulating, germ-fighting, hyperglycemia-lowering, hypertension-reducing, pain-alleviating, and cancer-fighting activities. Additionally, it has demonstrated potential as an immunomodulator, supporting the body's natural defences.[5] Traditionally, it has been used to manage a variety of conditions, including diabetes, hypertension, fever, digestive disorders, and liver issues. It is also known for aiding digestion, regulating menstruation (*emmenagogue*), and treating parasitic infections (*anthelmintic*). Another spice related to black cumin is *Bunium persicum*, sometimes called Shahi jeera or *Cuminum nigrum*. This variety, which also belongs to the Apiaceae family like regular cumin (*Cuminum cyminum*), is distinguished by its mild aroma and refined flavor. Popular in the cuisines of North India, Pakistan, and Iran, it remains under-studied in terms of its medicinal properties, with scientific research on its health benefits still being minimal.

Gastro-Protective Effect

The study found that *Nigella sativa* oil effectively protected against aspirin-induced gastric ulcers in albino rats. Treatment with the oil resulted in smaller ulcerated areas, reduced edema, and leukocyte infiltration compared to the control group, which showed severe gastric mucosal damage. Biochemical analysis of gastric contents also indicated improved protection. These outcomes suggest that *Nigella sativa* oil offers significant gastro-protective benefits in aspirin-induced ulcers.[6] The study explored the impact of various fractions (E1, E2, and E3) of cumin essential oil on gastric ulcers in an ethanol-induced rat model. The results showed that all three fractions reduced the ulcer index, gastric fluid pH, and pepsin activity, although to different degrees. They also decreased the levels of prostaglandin E2, gastrin, and epidermal growth factor in the serum. Among the fractions, the E3 fraction demonstrated the most potent anti-ulcer effect. Furthermore, all fractions alleviated oxidative stress and reduced the release of inflammatory factors, with

the E3 fraction having the greatest effect. Further investigations revealed that E3 prevented apoptosis by lowering cleaved caspase-3 and Bax protein levels and inhibited the activation of the NF-κB pathway by reducing the phosphorylation of p65, IKKβ, and IκBα. The study concluded that the E3 fraction of cumin essential oil is the most effective in treating gastric ulcers by inhibiting NF-κB activation and apoptosis, thereby reducing inflammation.[7]

The study investigated the combined effect of cumin decoction and three- and four-drug protocols in treating *Helicobacter pylori* (*H. pylori*) infection in peptic ulcer patients. The results showed that all treatment groups, including those receiving cumin decoction alone and those on the three- and four-drug protocols, successfully eradicated *H. pylori*. The highest eradication rate (85.72%) was seen in the group that received cumin decoction with the three-drug protocol, though this difference was not statistically significant. The eradication rates were 61.5% for the cumin + omeprazole group, 77.8% for the three-drug group, and 58.33% for the four-drug group. The study concluded that cumin decoction may serve as a useful complementary treatment to enhance *H. pylori* eradication when used alongside conventional therapies.[8] The study explored the antidiarrheal effects of *Nigella sativa* infusion in mice. The findings showed that the infusion, containing flavonoids, alkaloids, tannins, and saponins, exhibited significant antidiarrheal activity across all tested concentrations (25%, 50%, and 100%). The 50% concentration proved to be the most effective, yielding results similar to Loperamide by reducing defecation frequency, enhancing stool consistency, delaying the onset of diarrhea, and reducing its duration. The study concluded that *Nigella sativa* infusion at a 50% concentration shows considerable antidiarrheal potential and should be further studied as a natural remedy.[9]

Inflammation Lowering Effect

The study examined the anti-inflammatory effects of cumin essential oil (CuEO) in LPS-stimulated RAW 264.7 cells. The results showed that CuEO, composed of 26 volatile compounds with cuminaldehyde as the primary component, did not exhibit cytotoxicity at the tested concentrations. CuEO significantly reduced the mRNA expression of inflammatory markers, including iNOS, COX-2, and IL-1/IL-6. Additionally, it inhibited the activation of NF-κB and decreased the phosphorylation of ERK and JNK, key proteins in inflammation signaling. These findings suggest that CuEO may act as an anti-inflammatory agent by blocking NF-κB and mitogen-activated protein kinases, indicating its potential as a beneficial dietary supplement.[10] The study investigated the anti-inflammatory and

analgesic effects of Black cumin (BC) extracts through both in vivo and in silico approaches. The findings demonstrated that BC extracts reduced writhing in the acetic acid-induced writhing test and decreased paw swelling in the carrageenan-induced edema test in a dose-dependent manner. Ingenuity Pathway Analysis revealed that compounds like ferulic acid, p-coumaric acid, kaempferol, and quercetin in BC extracts could help inhibit inflammation. The study concluded that the bioactive compounds in BC extract serve as potent anti-inflammatory and analgesic agents by modulating various inflammation pathways.[11]

This study explored the anti-inflammatory effects of thymoquinone, an active compound in *Nigella sativa* seeds, on arthritis in rats. The rats were split into five groups: a control group (0.9% NaCl), two groups treated with thymoquinone (2.5 mg/kg and 5 mg/kg), a Bacillus Calmette-Guérin (BCG) group, and a methotrexate group. Arthritis was induced using Freund's incomplete adjuvant, and various parameters, including inflammation signs, radiological changes, and levels of TNF-alpha and IL-1beta, were assessed. The findings revealed that thymoquinone effectively reduced arthritis symptoms, both clinically and radiologically, in the rats.[12] This research investigated the impact of black cumin oil on rats with experimentally induced colitis. The condition was initiated using TNBS, and the oil was given orally for three consecutive days. The treatment significantly lowered levels of inflammatory markers such as TNF-α, IL-1β, IL-6, as well as lactate dehydrogenase, triglycerides, and cholesterol, all of which were elevated due to colitis. These findings indicate that black cumin oil may offer protective effects on colonic tissue by alleviating systemic inflammation linked to ulcerative colitis.[13]

Effect on Respiratory Health

This study assessed the bronchodilatory (antiasthmatic) properties of a boiled extract of *Nigella sativa* in individuals with asthma. Administered at doses of 50 and 100 mg/kg, the extract led to significant improvements in multiple pulmonary function tests (PFTs), such as FEV1 and PEF. Its bronchodilatory effect began within 30 minutes and persisted for up to 150 minutes, comparable to the duration seen with theophylline. However, the degree of improvement in most PFTs was generally lower than that observed with theophylline and considerably less than the rapid effect produced by salbutamol at the 30-minute mark. In conclusion, *Nigella sativa* demonstrated a measurable but less potent bronchodilatory effect in asthma patients.[14] This study explored the tracheal relaxant effects of macerated and aqueous extracts of *Cuminum cyminum* (cumin) in guinea pigs. The extracts were tested under different conditions of tracheal contraction induced by

either KCl or methacholine. They produced a concentration-dependent relaxation, with more pronounced effects observed in methacholine-induced contractions, although the response was weaker compared to theophylline. Conversely, the extracts showed little to no relaxation in tissues contracted by KCl or in those that were not pre-incubated. These results indicate that *Cuminum cyminum* may have notable tracheal relaxing effects, likely through stimulation of β-adrenoceptors and/or inhibition of histamine H1 receptors.[15]

This study investigated the impact of Black Seed Oil (BSO) supplementation on patients with mild to moderate COPD. After three months, the BSO group, who received it alongside their standard treatment, showed significant improvements over the control group. These included reductions in inflammatory and oxidative stress markers (TBARS, PC, IL-6, TNF-α) and increases in antioxidant levels (SOD, CAT, GSH, GPx, vitamins C and E). Moreover, the BSO group experienced notable improvements in pulmonary function. These results suggest that BSO could be an effective additional therapy for improving lung function and reducing inflammation and oxidative stress in COPD patients.[16]

Anticancer effects

This study explored the use of cumin seeds as a potential new treatment for osteosarcoma, a cancer that affects both children and adults. The cumin seed extracts were obtained using solvents such as hexane, chloroform, methanol, ethanol, and acetone, and tested for their ability to inhibit the growth of MG63 osteosarcoma cells. The hexane extract demonstrated significant cytotoxicity, with an IC50 of 86 µg/mL, inducing apoptosis, halting the cell cycle in the S phase, and inhibiting cell migration and colony formation. Additionally, the cumin extract showed strong bactericidal effects, especially against drug-resistant infections. The findings suggest that cumin seed extracts, particularly the hexane extract, have great potential as a novel cancer therapy, supported by both intrinsic and extrinsic research evidence.[17] This study investigated the cytotoxic and anti-inflammatory effects of *Nigella sativa* oil, which was extracted using petroleum ether. The cytotoxicity was evaluated with the MTT assay on AGS and PANC-1 cell lines, while the anti-inflammatory activity was assessed in RAW264.7 cells by measuring iNOS release following LPS stimulation. The findings revealed that *Nigella sativa* oil exhibited notable effects, indicating its potential for further exploration as an anticancer and anti-inflammatory treatment.[18]

Antimicrobial Effects

This study evaluated the antibacterial effects of five different oils from Turkish black cumin at concentrations of 0.5%, 1.0%, and 2.0% using the agar diffusion method. The oils demonstrated antibacterial activity against 24 pathogenic, spoilage, and lactic acid bacteria (LAB). The highest effectiveness was observed at the 2.0% concentration. *Aeromonas hydrophila* was the most sensitive, while *Yersinia enterocolitica* exhibited the greatest resistance. LAB generally showed more resistance than pathogenic and spoilage bacteria. These findings suggest that black cumin oil could be a useful antimicrobial agent for preventing food spoilage.[19] This study examined the antimicrobial, antioxidant, and cytotoxic properties of cumin essential oil. The oil showed antimicrobial effectiveness against *E. coli*, *S. aureus*, and *S. faecalis*. It exhibited significant antioxidant activity, outperforming BHT and BHA, and effectively neutralized DPPH radicals. At a concentration of 0.1 µL/mL, the oil caused 79% cell destruction in Hela cells, with its antioxidant effects likely contributing to its cytotoxicity. In a 30-day oral toxicity test on Wistar rats, cumin oil led to a decrease in white blood cell count and notable changes in blood parameters, including increased hemoglobin and platelet levels. The oil also improved the LDL/HDL ratio, suggesting beneficial nutritional effects. Overall, the high phenolic content and antioxidant properties of cumin oil demonstrate its potential for both nutritional benefits and food preservation.[20]

Cardio-Protective Effect

This study explored the protective effects of black cumin seeds on isoproterenol-induced myocardial infarction (MI) in rats. The rats were divided into four groups: a control group, a group treated with isoproterenol, a group given black cumin, and a group receiving both black cumin and isoproterenol. The results showed that pre-treatment with black cumin significantly reduced serum and tissue markers associated with MI, such as VLDL, TG, cholesterol, free fatty acids, and cardiac enzymes (AST, ALT, LDH, CK), when compared to the isoproterenol-only group. Additionally, levels of beneficial markers like HDL, LDL, and phospholipids in heart tissue were significantly higher in the black cumin-treated group. These findings suggest that black cumin offers protective benefits against MI, as demonstrated by improved cardiac markers and lipid profiles.[21] This study investigated the cardioprotective effects of Nigella sativa in children with type 1 diabetes mellitus. Sixty participants were divided into two groups: one received *Nigella sativa* seed oil alongside insulin, while the other group received only insulin. After three months, the

Nigella sativa-treated group showed significant improvements, including lower levels of cholesterol, LDL, malondialdehyde, nitric oxide, tumor necrosis factor-α, transforming growth factor-β, and troponin I, compared to both their initial levels and the control group. Additionally, this group showed better left ventricular function, as indicated by improved echocardiographic measurements. The findings suggest that *Nigella sativa* may help improve subclinical left ventricular dysfunction in children with type 1 diabetes.[22]

This study examined the cardioprotective potential of *Nigella sativa* (NS) extract against doxorubicin (DOX)-induced heart damage. The rats were assigned to four groups: control, NS extract, DOX, and DOX+NS. Results indicated that the DOX group showed elevated levels of NT-proBNP, total oxidative stress (TOS), and malondialdehyde (MDA), as well as significant heart tissue damage. In contrast, the DOX+NS group demonstrated significantly lower levels of troponin, TOS, and MDA, alongside a notable increase in total antioxidant capacity (TAC). Histological examination confirmed that the heart damage in the DOX+NS group was significantly reduced. The study concludes that NS extract has the potential to protect the heart from DOX-induced damage.[23] This study explored the effects of cumin supplementation on hypoglycemia, lipid profiles, antioxidant activity, and liver protection in rats fed a high-fat diet. The results showed that 1% cumin powder supplementation significantly reduced glucose intolerance, fat accumulation, and lipid levels (including triglycerides, total cholesterol, and LDL). It also decreased oxidative stress markers like TBARS, nitric oxide, and advanced oxidation protein products. Additionally, cumin supplementation lowered elevated liver enzymes (ALT and ALP), restored glutathione levels, and enhanced antioxidant enzyme activity (SOD and catalase). Histological analysis revealed improvements in liver tissue, including reduced fat deposition and inflammation. In conclusion, the study suggests that cumin supplementation can help mitigate dyslipidemia, oxidative stress, and liver damage caused by a high-fat diet.[24]

Conclusion:

In conclusion, this study highlights the diverse therapeutic potential of *Nigella sativa* (black cumin) and *Cuminum cyminum* (cumin) in treating a wide range of health conditions. Both plants have demonstrated protective effects in gastrointestinal disorders, respiratory diseases, inflammation, and even cancer. Their cardioprotective properties further underscore their value in cardiovascular health, particularly in mitigating damage from conditions like myocardial infarction and chemotherapy-induced heart damage. Overall,

the findings suggest that *Nigella sativa* and cumin offer promising natural remedies for managing various chronic diseases and can be explored further for their therapeutic applications in modern medicine.

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