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FUTURE RESEARCH GAPS IN ECHINACEA PURPUREA THERAPEUTICS

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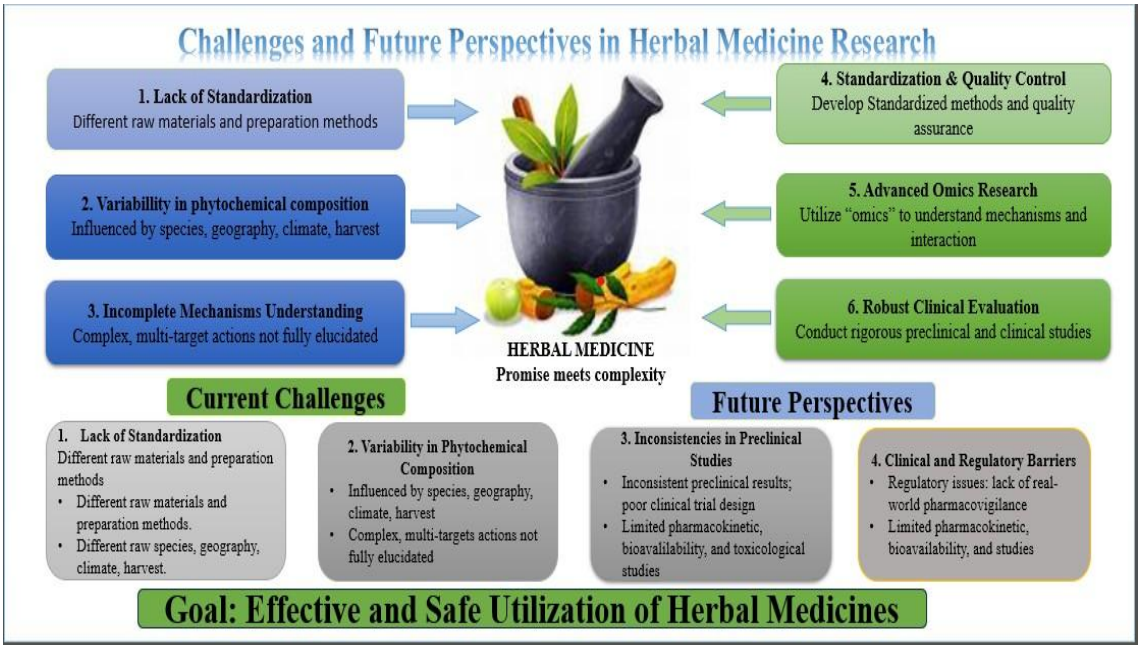
Abstract
Herbal medicine research faces various hurdles that hinder its inclusion into current healthcare system. A major concern is the non-uniformity and non-uniformity of the phytochemical constituents, affecting therapeutic uniformity and reproducibility. Further limiting scientific confirmation is a not sufficient understanding of mechanisms of action. Moreover, comprehensive studies of complex and complicated herbal compositions are constrained by the limited application of state-of-the-art omics technologies. Clinical trials are often limited to therapeutic indications and poorly designed, whereas preclinical studies often have discrepancies. Poor pharmacokinetic and bioavailability data compound the process of dosage optimization. The toxicological profile, the interactions between drugs and herbs, and long-term safety issues have not yet been sufficiently investigated. Adulteration, quality control, formulation and administration of the drug are other factors contributing to market variability. Regulatory hurdles add to the delay in clinical translation, as do real-world data limitations. To overcome these limitations and promote the development and wide-spread use of herbal remedies, standardized procedures, cutting-edge technologies, and comprehensive clinical evaluation are required.

Keywords: Herbal Medicine, Standardization, Phytochemical Variability, Pharmacokinetics, Clinical Translation.



1. Introduction:

The Echinacea purpurea (L.), one of the most popular and widely researched medicinal herbs used in modern phytotherapy. Moench. Native to North America, the species belongs to the Asteraceae family and has been ethnomedicinally used by Native American tribes for wound healing, infection, snake bite, and inflammatory diseases for long. [1]. E. purpurea is used as a herbal medicine in modern medicine, notably as an immunity stimulator, to either prevent or cure upper respiratory infection diseases like common cold.[2]. Despite its popularity and significant economic value, the effectiveness and reliability of Echinacea purpurea remains a subject of debate, and there are still areas that require further investigation. Phytochemical studies identify a wide range of physiologically active compounds in Echinacea purpurea, such as alkamides, caffeic acid derivatives (including cichoric acid and chlorogenic acid), polysaccharides, glycoproteins, flavonoids and volatile oils. These compounds are believed to have combined antibacterial, anti-inflammatory, immunomodulatory and antioxidant properties. However, the individual function of each component and their interactions at cellular and molecular levels are not clear. As most of the pharmacologic effects are not associated with any one marker compound but to the overall phytochemical composition of the plant this results in a significant research gap. [3Future research on Echinacea purpurea therapies will be emphasized for their critical deficiencies in standardized compositions, kinetics of action and extensive clinical validation. To demonstrate efficacy and therapeutic reliability, strong trials, advanced molecular studies and safety profiling are needed, given the variable effects of the extract and the variable clinical results.[4] The lack of standardized preparations is one of the biggest obstacles in the treatment of Echinacea purpurea. [1,5]. One of the greatest challenges to treating Echinacea purpurea is the non-standardized preparations. [1,5]. Differences in phytochemical composition and biological activity can be observed between different plant species, different plant parts (roots, aerial parts or whole plant), growing conditions, geographical area, harvesting period, post-harvest processes and extraction methods.[5]. The phytochemical constituents of Echinacea purpurea differ, rendering the preclinical and clinical studies hard to replicate and often inconsistent or contradictory. The lack of standardized quality control processes and known biomarkers compounds the problem of reliability when moving from experimental to clinical settings. Furthermore, the molecular processes underlying its immunomodulatory activities are still not well understood. According to available data, E. purpurea affects immunological responses via a variety of mechanisms, such as increased phagocytic activity, macrophage activation, cytokine production modification, and interaction with the Cannabinoid receptor type 2 (CB2) signalling system.[6]. Although the results are encouraging, the evidence is mostly from in vitro studies and animal models, which hinders direct clinical application. While still underutilized, new molecular approaches such as transcriptomics, proteomics, metabolomics and systems biology have enormous potential to elucidate the multi-target mechanism of action of Echinacea purpurea at a systems level, enabling a more accurate therapeutic validation and drug development.[7].

Results from clinical studies on Echinacea purpurea have been inconsistent and frequently unclear. There have been many randomized controlled trials that have evaluated its efficacy in the treatment and prevention of upper respiratory tract infections, but the results have been inconsistent, with variations in patient populations, extract formulations, dosage regimens, and study design. While some trials indicate no discernible difference from a placebo, others claim small advantages in terms of symptom duration or intensity. These discrepancies emphasize the necessity of carefully planned extensive clinical trials using uniform preparations in order to demonstrate certain therapeutic efficacy.[8] Small sample sizes, brief intervention times, inconsistent study designs, non-standardized extractions, and inconsistent dosage schedules are common problems in research. Interpreting clinical results is further complicated by variations in research endpoints and placebo effects. [8]. These restrictions show how urgently large-scale, carefully planned clinical studies using standardized preparations and precisely defined outcome criteria are needed. Echinacea purpurea's medicinal potential is still mostly unknown outside of respiratory illnesses. New research points to potential advantages in immunological dysregulation, healing wounds, dermatological issues, oxidative stress-related diseases, and chronic inflammatory disorders.[9]. There is currently little clinical evidence to support the long-term therapeutic use of Echinacea purpurea, despite encouraging pharmacological and preclinical results. The majority of studies that are now available are brief, and there is a dearth of thorough information on long-term safety. Although Echinacea is considered safe and has only mild side effects such as gastrointestinal upset and allergic reactions, its immunostimulatory properties have raised concern for its use in individuals with autoimmune disorders or compromised immune systems. Moreover, long term clinical trials and careful patient-specific assessment are required due to the lack of satisfactory proof of the safety of extended treatment.[10]. Thus, pharmacovigilance data and thorough toxicological studies are crucial. Another important research need is to elucidate the pharmacokinetics and biological availability of the constituents of Echinacea purpurea. Very limited information is available

regarding the absorption, metabolism, distribution and elimination of its bioactive compounds in human. [3,7].
The recent advancements in phytopharmaceutical technology underscore the importance of standardization and creative delivery systems to harness the therapeutic benefits of Echinacea purpurea and other herbal treatments. Standardisation reduces variability and enhances the predictability of pharmacologic responses by maintaining a fixed amount of the bioactive agents. At the same time, the low bioavailability, solubility and instability of phytochemicals have been overcome by applying some nano delivery technologies to them, like liposomes, nanoparticles and nano emulsions. These technologies can help to incorporate herbal medicines into modern evidence-based pharmacotherapy, with targeted delivery, avoiding degradation of active components and enhanced therapeutic effect.[11]. Emerging techniques such as phytopharmaceutical standardization and nano delivery systems show promise, but this approach has been limited and experimental in its application with Echinacea purpurea and has not been widely used in clinical practice. Regulatory issues including regional variations in the regulation of herbal medicines that can affect the consistency of assessment of safety and effectiveness. Problems of product adulteration, raw material variability, lack of uniform labelling and quality control further impede its use in medical systems, both evidence-based and conventional. These restrictions indicate the need for stricter regulations, tested quality standards and improved postmarket surveillance to ensure the reliability of products made from Echinacea.[12]

1.1 Lack of Standardization of Herbal Preparations

One of the most significant and timeless research needs for Echinacea purpurea medicines is the absence of standardization on herbal formulations, which can greatly influence repeatability, clinical efficacy and regulatory approval. The lack of consistency in the products available on the market, in terms of their botanical source, parts of plant used, extraction process and dosage form, also causes a great variability in the phytochemical content and the pharmacological effect demonstrated by these products, limiting considerably the conversion of experimental results to evidence-based therapeutic applications. [13,14].

1.2 Species Variation

Echinacea purpurea, Echinacea angustifolia and Echinacea pallida are the three species of Echinacea most commonly employed medicinally.
However, plants differ in their phytochemical profile, and biological properties are very different.[15] Numerous commercial formulations and even scientific studies are not appropriately labelled with regard to different species of Echinacea used alternately or together for unclear medicinal results. E. angustifolia, for instance, contains more amounts of alkamides, and E. purpurea is particularly rich in polysaccharides and cichoric acid.[15]. Species' diversity continues to be a major constraint on Echinacea product standardization. The phytochemical composition of some species like Echinacea purpurea, Echinacea angustifolia and Echinacea pallida varies considerably, particularly in their alkamide, derivatives of caffeic acid and polysaccharides. This variability may lead to unpredictable clinical efficacy and inconsistent biological activity. In addition, several and/or unknown species are often used in commercial formulations, complicating the issue of repeatability and quality assurance. Consequently, it is essential to establish the strict species authentication, standardisation of extraction methods and regulations to ensure uniformity and therapeutic reliability.[16]. These variations directly affect the immune and inflammatory functions. The absence of chemical, botanical, or genetic species confirmation adds to this problem. Echinacea species have been reported as being adulterated and/or substituted in commercial products, posing a threat to safety and effectiveness.[17]. From a research standpoint, conflicting and even contradictory results arise when the species employed in experimental and clinical investigations are not precisely defined. As a result, accurate taxonomy identification and species- specific standardization techniques must be given top priority in future study.

1.3 Plant Parts Used

The utilization of various plant parts, such as roots, aerial parts, flowers, or entire plants, is another significant source of variety. Different phytochemical compositions found in each plant section provide different pharmacological effects. [14]. Due to inadequate standardization of herbal formulations, which causes variations in chemical composition and therapeutic benefits, there is a substantial gap in Echinacea purpurea study. Variability in pharmacologically active components is further influenced by species differences within the Echinacea genus and the use of different plant parts (such as roots, leaves, or flowers). These discrepancies make it difficult to compare studies accurately and restrict the creation of trustworthy, evidence-based treatment applications.[18]

1.4 Extraction Methods

Echinacea purpurea standardization attempts frequently depend on phenolic ingredient profiling, especially for derivatives of caffeic acid as cichoric acid. Studies with well-known analytical methods have established significant differences between the various species of Echinacea, between plant sections and in the various commercial formulations. Extraction characteristics, such as solvent type and processing conditions, have a significant impact on this variability because they can change the stability and production of bioactive phenolics through chemical transformation or enzymatic degradation. We must understand the challenges of quality control and repeatability in goods production from echinacea, given these discrepancies.[19]. Significant variation in biological activity has been caused by the lack of consistent extraction procedures among research. For example, extracts rich in polysaccharides mainly activate innate immunological responses, whereas extracts rich in alkamides exhibit a higher immunomodulatory activity via CB2 receptor interaction. [20]. However, reproducibility is still limited because of the lack of exactness in reporting and standardization of extraction parameters like solvent type, solvent concentration, extraction time and temperature. Future research needs to be directed towards the development of standardized and validated extraction procedures and the correlation of extraction methods and specific pharmacological effects.

1.5 Dosage Forms and Formulation Variability

The dosage forms of Echinacea purpurea include tablets, capsules, tinctures, syrups, teas and topical preparations. The active ingredients' stability, bioavailability and therapeutic effects are influenced by every dosage form. [15,21]. Different forms of Echinacea purpurea (tablets, capsules, tinctures, liquid extracts) have different pharmacokinetic and stability properties. Solid formulations often are more chemically stable and have a longer shelf life, although they may take longer to be absorbed and take effect than liquid formulations. But the conflicting results of treatment have been caused by the different types of formulation, methods of extraction and doses used in different research trials. Finding definitive clinical conclusions is more difficult, due to the lack of recommendations regarding optimal dose, treatment duration, and dosage form. The lack of specific marker molecules to guide dose adjustments is another obstacle to clinical translation. Formulation optimization, dose-response relationships, and the creation of standardized phytopharmaceutical products with distinct chemical fingerprints should be the main topics of future research.[22]

1.6 Future Directions

There is a need for a multi-disciplinary approach that incorporates pharmacognosy, analytical chemistry, pharmacology and clinical sciences to overcome the lack of standardization of Echinacea purpurea. Utilization of certified analytical methods, along with compliance to regulatory quality requirements and Good Agricultural and Collection Practices (GACP) is required to ensure consistency and reliability. Further, the development of Pharmacopeial monographs and internationally accepted reference standards would greatly improve the clinical credibility, product quality and repeatability of study results in the development of Echinacea-based therapies.[23]

2. Variability in Phytochemical Composition

Variation in phytochemical composition of Echinacea purpurea is one of the major knowledge gaps that currently constrain the therapeutic activity of this species and hinder its clinical use. Although numerous studies have identified bioactive compounds with an immunomodulatory, anti-inflammatory in nature and antioxidant activity, there are significant qualitative and quantitative differences in the nature and quantity of these compounds found in different preparations of Echinacea. These variations are primarily due to seasonal and regional influences, cultivation and harvesting practices, as well as absence of generally accepted marker compounds and quality control standards. These differences prevent the development of standardized phytopharmaceutical product and complicate the analysis of the pharmacological and clinical data. [24,25].

2.1 Seasonal and Geographical Variation

The phytochemical profile of Echinacea purpurea is significantly influenced by seasonal fluctuation. Alkamides, caffeic acid derivatives (cichoric acid, chlorogenic acid), and polysaccharides are examples of secondary metabolites whose biosynthesis and accumulation vary throughout the plant's growth phases. [26]. Seasonal and geographic factors have a major impact on the phytochemical content of Echinacea purpurea. Climate, soil composition, and harvesting time are some of the factors that affect the concentrations of active ingredients such alkamides and derivatives of caffeic acid. Standardization and reproducibility of herbal formulations are difficult because of these environmental variables, which cause variances in biological

activity and medicinal efficacy.[27]. Even when same cultivation techniques are used, comparative studies of E. purpurea cultivated in North America and Europe have shown significant variances in chemical makeup. Research and commercialization are severely hampered by this seasonal and geographic variations. Even when the same nominal species is used, clinical trials that use plant material from different places or gathered at different times may provide conflicting findings. Therefore, in order to reduce phytochemical variability, future research must focus on regulated growing conditions and rigorous assessment of seasonal and geographical variables.[28] Environmental factors including soil properties and climate have a big impact on Echinacea purpurea's phytochemical composition. Alkamide production can be impacted by changes in soil nutrients, and plants grown in warmer climates often have lower amounts of caffeic acid derivatives than those grown in temperate climates. Furthermore, the efficacy of these components as individual indicators of quality is limited because to their extreme sensitivity to harvesting and processing circumstances. Because of their interaction with the Cannabinoid receptor type 2 (CB2), alkamides are thought to have a significant role in immunomodulatory activity. However, their quick metabolism and lipophilic nature make it difficult to quantify and standardize them.[29]

2.3 Marker Compounds and Quality Control Issues

In order to address phytochemical variability and guarantee quality control of Echinacea purpurea preparations, it is essential to identify and validate appropriate marker chemicals. Nowadays, a number of substances are frequently employed as chemical markers, such as cichoric acid, Echinacoside, chlorogenic acid, and total alkamide content. [27,30]. However, the complex phytochemical profile responsible for therapeutic efficacy cannot be captured by relying on a single or small number of markers. Because cichoric acid is abundant in the aerial sections of E. purpurea and has immunomodulatory and antioxidant qualities, it is frequently employed as a quality marker.[30]. However, processing methods and environmental factors have a significant impact on its concentration, making it an unreliable single indicator of quality. Alkamides, on the other hand, interact with cannabinoid receptor type-2 (CB2) to play an important part in immunomodulatory activity; nevertheless, their lipophilic nature and quick metabolic turnover make it difficult to accurately quantify and standardize them in herbal products.[31].

The alteration and mislabelling of commercial Echinacea products worsen quality control problems. Studies using chromatographic fingerprinting and DNA barcoding have shown that many commercial products have inadequate amounts of active ingredients, unreported plant parts, or incorrect species. [32]. These discrepancies present safety and regulatory issues in addition to undermining treatment efficacy.

High-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), and metabolomic profiling are examples of advanced analytical techniques that present promising instruments for thorough quality assessment. [30]. Future quality control methods should take a multi-marker or fingerprint-based approach instead of depending solely on single marker compounds in order to better represent the comprehensive nature of Echinacea purpurea treatments.

2.4 Implications for Research and Clinical Translation

The diversity of phytochemicals in Echinacea purpurea can have profound implications for clinical trials, pharmacological studies, and regulatory approval. Inconsistent clinical results are due to varying chemical composition leading to problems with dose standardization and interfering with mechanism-of-action studies. [24,25]. More understandings of the mechanism and improved standards are needed for the clinical translation of Echinacea purpurea. Future research efforts should prioritize repeatable cultivation methods, well-known analytical techniques, and the combination of metabolomics with pharmacological data, all in order to discover reliable bioactive markers. In the clinical setting, these could help increase safety, effectiveness and uniformity. Moreover, the clinical trials designed well are crucial to ensure the therapeutic outcomes and to optimize the dose. Moving forward, the universal applicability of evidence-based medicine and quality assurance will be further facilitated by harmonization of regulatory systems and consideration of full phytochemical profiles in pharmacopeial specifications.[33]

3. Incomplete Understanding of Mechanisms of Action

Despite being one of the most popular medicinal herbs for boosting immunity, Echinacea purpurea's exact mode of action is still unclear. Several experimental and clinical studies demonstrated immunomodulatory, anti-inflammatory and antiviral properties, although the molecular mechanisms involved are complex, multifaceted and not fully understood. [34,35]. The therapeutic efficacy of E. purpurea is attributed to the synergistic effect of multiple bioactive compounds, such as alkamides, polysaccharides, and caffeic acid derivatives, on multiple immunological targets, not to any single compound or to any specific pathway. This complexity poses a big research challenge and impedes clinical translation, dose adjustment, and reasonable

standardization. Clinical translation, dose adjustment, and reasonable standardization are all hampered by this intricacy, which is a significant research gap.

3.1 Immunomodulatory Pathways

Rather than being a straightforward immune stimulant, Echinacea purpurea is better regarded as an immunomodulator. According to preclinical data, its extracts have the ability to control both innate and adaptive immune responses; the effects vary depending on the physiological or immunological setting, dose, and phytochemical makeup. This bidirectional activity demonstrates its ability to support a more balanced and adaptive immune regulation mechanism by either boosting or suppressing immune functions based on the biological environment. [36]. While lipophilic alkamides alter immune signalling pathways at the cellular level, polysaccharides and glycoproteins found in aqueous extracts are known to improve innate immunity by promoting phagocytosis and antigen presentation.[37].

Interaction with these receptors starts intracellular signalling cascades that result in the transcription of immune-related genes, such as nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. Enhanced host defensive mechanisms, such as increased production of cytokines and chemokines involved in early immunological responses, are the outcome of activating these pathways. [39].

Echinacea purpurea mainly affects immunological responses by activating macrophages and controlling inflammatory mediators. It modifies the production of cytokines like TNF-α and nitric oxide by influencing important signalling pathways like NF-κB. Its function in boosting host defence and controlling immunological homeostasis is facilitated by these pathways. Mechanistic interpretation is further complicated by differences in extract composition between experiments. Therefore, mapping the immunomodulatory networks impacted by E. purpurea requires sophisticated systems-level techniques.[40].

3.2 Cytokine Regulation and Macrophage Activation

Macrophages play a key role in controlling inflammation and innate immunity. Important cytokines including TNF-α, IL-1β, IL-6, and IL-10 are released upon activation and are involved in immunological signalling, pathogen removal, and tissue repair. Echinacea purpurea has been found to modulate inflammatory pathways and cytokine release to change macrophage activity. Its bioactive components, particularly polysaccharides and alkylamides, trigger macrophage responses more than simply stimulating the immune system, thus maintaining immunological balance. The dual effect of E. purpurea indicates its therapeutic potential as an immunomodulatory herbal medicine, which promotes host defense and also helps to regulate overactive inflammatory responses.[41]. In this pioneering study it was demonstrated that purified arabinogalactan, one of the major polysaccharides of Echinacea purpurea, directly activates macrophages to stimulate the production of key pro-inflammatory cytokines including interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α). These results provided preliminary evidence, that polysaccharide-rich extracts of Echinacea stimulated host defence against infections and other immunological challenges by activating innate immunity, including activation of macrophages and production of cytokines.[42] By inhibiting excessive cytokine production, nitric oxide synthesis, and other inflammatory mediators under activated conditions, alkamide-rich extracts, on the other hand, demonstrate anti-inflammatory benefits [43]. Echinacea is truly an immune stimulant, rather than just an immunomodulator. It enhances immunological response when immune system is weak and reduces the hyperactive inflammatory response when immune system is hyperactive. This context-dependent control guarantees a suitable reaction to pathogenic stimuli and contributes to immunological homeostasis. Echinacea helps to regulate immune responses such as macrophage function, cytokine production, and inflammatory pathways, thus supporting efficient host defence without causing detrimental inflammation. Originally it was observed that these properties make it a useful therapeutic agent to combat infections, inflammatory diseases and immunological imbalance related diseases.[44]. The two-way nature of Echinacea purpurea's immunomodulatory activities indicate that it can play more than just a role in enhancing immune responses but also supports immunological homeostasis. This impact also correlates with the activation of macrophages and regulation of inducible nitric oxide synthase (iNOS) that modifies the creation of nitric oxide.[45]. NO levels can also be too high, leading to tissue damage and chronic inflammation, but low levels can also promote antibacterial activity. Studies have shown that Echinacea promotes effective host defence and inflammatory regulation by inhibiting NO generation. Upstream signalling pathways that regulate this, however, remain largely unstudied and are a subject of great interest. [46].

3.3 CB2 Receptor and Molecular Targets

The discovery that Echinacea purpurea's alkamides are endocannabinoid system modulators, namely through interaction with the Cannabinoid receptor type 2 (CB2), is a major advancement in our knowledge of the

plant's pharmacological effect. Activation of this receptor, which is mostly expressed in immune cells, helps control inflammatory signals and immunological responses. The results provide insight into the multi-targeted effects of E. purpurea and provide a mechanistic basis for its immunomodulatory properties.[47]. Alkamides from Echinacea are acknowledged as significant bioactive components with noteworthy immunopharmacological activity. These lipophilic substances interact preferentially with the cannabinoid type-2 (CB2) receptor, a G-protein-coupled receptor primarily expressed on immune cells, such as macrophages, B lymphocytes, T lymphocytes, and dendritic cells, because of their structural similarity to endogenous cannabinoids. Echinacea alkamides that activate CB2 receptors alter intracellular signalling pathways related to immune cell motility, inflammatory control, and cytokine generation. Alkamides are important contributions to Echinacea's therapeutic actions, as this receptor-mediated mechanism explains the plant's immunomodulatory and anti-inflammatory qualities.[48].

Cannabinoid type 2 (CB2) receptor activation is essential for controlling inflammatory and immunological reactions. CB2 signalling limits excessive inflammatory reactions by reducing the release of pro-inflammatory mediators such TNF-α, IL-1β, and IL-6. Moreover, CB2 receptor engagement modulates chemotaxis and leukocyte trafficking to inflammatory areas and inhibits the growth and activation of immune cells, such as T lymphocytes and macrophages. CB2 activation is a key biological target for immunomodulatory and anti-inflammatory treatments because of these coordinated effects that support immunological homeostasis, lessen tissue damage, and encourage inflammation resolution. [49]. Echinacea alkamides function as partial agonists of cannabinoid CB2 receptors to produce immunomodulatory effects. When they bind to receptors, they activate mitogen-activated protein kinase (MAPK) cascades including ERK, JNK, and p38 and alter intracellular second-messenger systems, especially cyclic AMP. Immune cell signalling, cytokine synthesis, and macrophage activation are all impacted by these pathways, which control transcription factors involved in inflammatory gene expression. The ability of Echinacea alkamides to regulate inflammatory and immunological responses in a context-dependent way is explained by these molecular interactions.[50]. The anti-inflammatory and immune-regulatory properties of E. purpurea extracts are explained molecularly by these interactions. Even with these encouraging results, there are still a number of significant unsolved questions. Individual alkamides differ greatly in their affinity and effectiveness for CB2 receptors, and little is known about how they behave pharmacokinetically in humans.[51]. Echinacea purpurea's immunological effects are mediated by CB2-independent pathways in addition to its interaction with the Cannabinoid receptor type 2 (CB2). These include attenuating oxidative stress pathways, controlling inflammatory enzymes, and modifying transcription factors like NF-κB. These varied molecular interactions reflect E. purpurea's function as a complex immunomodulator functioning through many signalling systems rather than a single receptor-dependent pathway, highlighting its multitarget nature.[52]

3.4 Integration of Multiple Molecular Targets

Rather than one molecular target, Echinacea purpurea's therapeutic actions most likely stem from the concurrent regulation of several immunological pathways. A complicated, multi-target method of action is suggested by crosstalk between TLR signalling, cytokine networks, macrophage activation, and the endocannabinoid system. [38]. Despite increased interest, the majority of current research on Echinacea purpurea ignores its wider, system-level pharmacological effects in favor of concentrating on specific signalling pathways. This reductionist view is limited in its understanding of its immunomodulatory processes. The use of emerging technologies, such as proteomics, metabolomics, and transcriptomics, offers powerful tools to explore complex molecular interactions and global immune responses, furthering the understanding of E. purpurea's therapeutic potential.[53].

4. Limited Application of Advanced Omics Technologies

Although Echinacea purpurea has a long history of pharmacological study and its wide therapeutic use, the modern omics-based techniques have not been completely introduced in research on the plant. Most studies are still conducted with conventional phytochemical studies, in vitro immunological studies and small-scale clinical trials. Although these approaches have provided valuable information, the multitarget mechanisms of E. purpurea are too complex to be fully explained. On the other hand, contemporary methods like systems biology, proteomics, metabolomics, and genomes provide thorough approaches to understanding systemic immune responses and molecular interactions. However, their applications in Echinacea remain limited and thus mechanistic understanding is lacking, uniformity is lacking, and progress toward evidence-based therapeutic treatments is hampered.[54]

4.1 Genomics and Transcriptomics

By making it possible to identify genes involved in biosynthetic processes, stress responses, and therapeutic activity, genomic and transcriptomic techniques have transformed the study of medicinal plants. Compared with other medicinal plants, Echinacea purpurea has less genomic resources, which limits our understanding of the genetic regulation of the generation of bioactive compounds. [54].
Recent developments in transcriptomics and genomes have shed further light on Echinacea purpurea's molecular processes. Key genes and transcription factors involved in the manufacture of bioactive chemicals such alkamides and derivatives of caffeic acid have been identified by high-throughput sequencing and gene expression profiling. These methods aid in the comprehension of metabolic pathways, genetic diversity, and regulatory networks, which supports the creation of better medicinal applications and quality control techniques.[55]. Few research have examined changes in gene expression in Echinacea purpurea tissues or in immune system cells treated with its extracts, despite breakthroughs in molecular biology. There are substantial gaps in our knowledge of transcriptional regulation's molecular mechanisms because the majority of current research does not sufficiently investigate it. Deeper understanding of how E. purpurea affects immune-related gene networks and cellular signalling pathways may be obtained by extending the use of genomics and transcriptomics.[56].
Genes that exhibit differential expression and are involved in important metabolic pathways such phenylpropanoid metabolism, fatty acid-derived alkamide synthesis, and polysaccharide production can be found using genomic and transcriptomic techniques, especially RNA sequencing (RNA-seq). These high-throughput methods offer comprehensive insights into the molecular control of medicinal plants' production of secondary metabolites.[57]. The discovery of differentially expressed genes involved in important Echinacea purpurea metabolic pathways, such as phenylpropanoid metabolism, alkamide biosynthesis, and polysaccharide production, is made possible by high-throughput methods like RNA sequencing (RNA-seq). Furthermore, transcriptome profiling of immune cells treated with Echinacea purpurea extracts indicates alteration of key signalling pathways and transcription factors such NF-κB, AP-1, and STAT, which are involved in controlling inflammation and immunological responses.[58]. The lack of such molecular studies is a significant research restriction. Specifically, a greater understanding of the genetic diversity of Echinacea species is limited by the absence of comparative transcriptome investigations. In commercial marketplaces, this problem is made more difficult by the frequent misidentification or substitution of species, which compromises the authenticity and quality of products. Transcriptome-based molecular markers may be useful instruments for quality control and species authenticity in this situation. To better understand genetic variation, control of biosynthetic pathways, and consistency in therapeutic efficacy, genomics and transcriptomics must be integrated in future research.[59]

4.2 Proteomics and Metabolomics

By connecting expression of genes to functional effects, proteomics and metabolomics offer complementary viewpoints. Direct evaluation of protein abundance, post-translational changes, and protein–protein interactions all crucial components of immunological signalling pathways impacted by Echinacea purpurea is made possible by proteomic techniques. [60]. There is limited proteomic research conducted on Echinacea purpurea, but great potential exists. Proteomics offers a powerful tool in immune pharmacology to identify and characterize the proteins, signalling pathways and mediators that are influenced by the extracts of E. purpurea. These investigations can provide insights into the molecular mechanisms of action of these extracts on the activity of immune cells including lymphocytes, dendritic cells and macrophages.[61]. Different expression of cytokines, kinases and transcription regulators, for example, could explain the pro- and anti-inflammatory reactions observed in Echinacea. Most research to date, however, focuses on enzyme-linked immunosorbent tests (ELISA) which detect specific cytokines rather than on global proteome profiling. [62].
Metabolomics has been applied increasingly frequently in Echinacea research, especially for plant extract quality control and phytochemical profiling.[63]. Advanced analytical techniques like nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography–mass spectrometry (LC–MS), and gas chromatography–mass spectrometry (GC–MS) have been used to identify and characterize important bioactive compounds like alkamides, phenolic acids, and polysaccharides, enabling detailed chemical profiling of plant metabolites.[64]. Metabolomics has been comparatively underutilized for researching host-herb interactions and in vivo metabolic processes, despite its expanding use. It may be possible to understand how Echinacea purpurea affects oxidative stress reactions, immunological modulation, and endogenous inflammatory metabolic pathways in biological systems by untargeted metabolomic techniques.[65]. By connecting particular chemical profiles to observed biological activities, metabolomic fingerprinting offers a method that goes beyond straightforward compound abundance measurement. By identifying possible bioactivity-related markers, this approach facilitates the establishment of relationships between phytochemical composition and pharmacological effects.[66].

4.3 Systems Biology Approaches

One significant gap in Echinacea research is the absence of bioactivity-guided metabolomic investigations, which restricts the ability to clearly connect metabolite profiles with biological effects.

Systems biology techniques are ideal for comprehending Echinacea purpurea's intricate therapeutic mechanisms because it acts through a variety of chemicals and targets. [67]. A thorough knowledge of Echinacea's medicinal mechanisms is limited by the fact that the majority of current research on the plant still does not use integrated systems biology or multi-omics techniques. By methodically connecting active substances with biological targets and disease pathways, network pharmacology a method within systems biology—has acquired significance in the research of herbal medicines. [68]. Echinacea purpurea may function through important regulatory nodes involved in immune modulation, such as oxidative stress pathways, CB2 receptor signalling, and cytokine-mediated communication networks, according to network pharmacology research. Despite their great promise, thorough network-based investigations of Echinacea components are currently rather rare. Host-microbiome interaction analysis is another developing field of study. There is growing evidence that the therapeutic effects of herbal remedies may be significantly influenced by gut microbiota regulation.[69]. Echinacea purpurea may have an impact on microbial composition or metabolite production, which may have an impact on immune function, according to omics-based microbiome research. This aspect has not received much attention up to this point.

By anticipating synergistic interactions among Echinacea ingredients and determining the best combinations for medicinal application, computational modelling and machine learning techniques could further improve systems-level understanding. [70]. Personalized herbal medicine techniques that adhere to precision medicine principles may also be made possible by integrating clinical data with omics-derived molecular markers.[71].

4.4 Challenges and Future Directions

Due to a number of significant obstacles, the use of cutting-edge omics technologies in Echinacea purpurea research is still restricted. These include high experimental costs, a lack of standardized extract preparations, inadequate genetic resources, and little multidisciplinary cooperation across phytochemistry, bioinformatics, and molecular biology. These limitations, when considered together, are a barrier to the full realization of the potential to use omics-based approaches to understand the complex biology and therapeutic opportunities for Echinacea species.[72]. In order to tackle the current limitations to the investigation of the omics, a coordinated action is required to develop a framework for standardized data repositories, harmonized experimental techniques, and reference genetic resources for Echinacea purpurea investigations. Future research needs to be based on multi-omics techniques with standardized plant extracts and well characterized biological systems for consistency and reliability of the results. Omics data must be linked with pharmacological and clinical outcomes for the translation of biological discoveries to therapeutic applications. All of these approaches can help to further elucidate the mechanism of Echinacea-based treatments, ensure regulatory clearance, and enhance product uniformity.[73].

5. Inconsistencies in Preclinical Studies

Biomedical research and the development of new drugs rely heavily on preclinical studies as they are the initial tests that give credibility to a drug candidate for moving on to human clinical trials. These investigations involve in vitro systems, animals and computational approaches for evaluating biological activity, safety, toxicity, pharmacokinetics and mechanisms of action. Although crucial, preclinical studies are frequently affected by important discrepancies that reduce their predictive value and increase the attrition rate of clinical drug development. It is well known that many treatment candidates with encouraging preclinical results eventually fail in clinical trials because of ineffectiveness or unanticipated toxicity, underscoring basic flaws in preclinical research frameworks. [76,86].

Poor reproducibility in preclinical research is one of the main causes of inconsistency.

Several studies have shown that when trials are conducted independently, a significant portion of published preclinical findings cannot be duplicated. [84]. Variability in experimental methods, variations in cell lines or animal strains, variable reagent quality, and insufficient statistical power are some of the causes of this lack of repeatability. Small sample sizes are frequently used in preclinical research, which raises the possibility of false-positive findings and inflated effect sizes. [82]. Furthermore, bias is introduced by inadequate randomization and blinding in animal research, which results in unduly positive assessments of therapeutic success.[87]. Because research with negative or neutral results are less likely to be published, selectively reporting and publication bias further skew the scientific literature, giving the impression that it is strong and successful.[84].

The low translational applicability of in vitro models is another source of inconsistencies. Because they are affordable, scalable, and appropriate for high-throughput screening and mechanistic research, widely used are in vitro systems. Nevertheless, the intricacy of whole living creatures is not adequately captured by these systems. [77,78]. The majority of in vitro research uses immortalized cell lines, which differ from primary human tissues in terms of phenotypic diversity, aberrant metabolic activity, and changed gene expression profiles.[85]. Additionally, artificial surroundings and supraphysiological drug doses are common in in vitro experiments, which might result in an overestimation of potency and therapeutic potential.[79]. Therefore, when examined in vivo, drugs that exhibit great activity in vitro often exhibit decreased or inconsistent effects, which contributes to poor translation.

By offering a more physiologically appropriate setting for researching medication effects, animal models aim to address some of the shortcomings of in vitro methods. However, because different species have different anatomy, physiology, immune systems, and drug metabolism, animal studies add more layers of inconsistency. [76,81].

As a result, many medication candidates that seem safe and efficacious in animal models do not show clinical benefit in human studies.[86].

The absence of validated and human-relevant disease models is another significant factor causing inconsistencies in preclinical research. In addition to accurately predicting clinical responses to treatment interventions, a useful diseases model should replicate the essential pathogenic, molecular, and functional aspects of the human condition. However, a lot of widely used preclinical models are not predictive and only capture specific characteristics of the disease.[81]. However, extrapolating dosages from animals to humans is challenging due to variations in absorption, distribution, metabolism, and excretion between species.[79].

Additionally, contradictory dose-response data results from differences in experimental design, dosing schedules, and reporting standards among laboratories, which lowers confidence in preclinical predictions. [84].

The improvement of preclinical research' rigor, transparency, and a translational relevance has received more attention as a result of these difficulties. Improved experimental design techniques are acknowledged as crucial steps toward increasing reproducibility. These techniques include randomization, blinding, adequate sample size calculation, and standardized outcome measures. [87].

Preclinical study inconsistencies remain a significant barrier to the successful conversion of biological research into efficacious clinical treatments. High attrition rates in clinical development and higher costs are caused by a number of factors, including poor repeatability, limited relevance of experimental models, insufficient disease representation, and inconsistent dose-response relationships. [86]. A comprehensive approach that incorporates quantitative translational methods, human-relevant models, and rigorous experimental design is needed to address these issues. It is possible to increase the effectiveness of medication development and eventually improve patient outcomes by bolstering the predictive power and dependability of preclinical research. [86.]

5.1 In vitro vs in vivo correlation

A predicted link between a biological or physicochemical quality evaluated in vitro and the matching pharmacological or biological response found in vivo is known as in vitro, in vivo correlation, or IVIVC. Linking laboratory-based experimental data with actual biological performance in living systems is a common practice in the pharmaceutical sciences. [74,75]. Because in vitro models are frequently used to predict in vivo behavior while lowering development costs, experimental time, and ethical considerations connected with animal and human studies, establishing IVIVC is crucial in pharmaceutical sciences, toxicology, and biomedical research.[79]. The intrinsic complexity of living biological systems makes it difficult to translate results from in vitro research into trustworthy in vivo outcomes, despite tremendous technical advancements. The direct prediction of in vitro outcomes is frequently limited by the physiological variability, dynamic interactions, and systemic regulation found in real organisms.[76].

The lack of reliable and clinically applicable disease models is a significant barrier to preclinical research. . Most of the animal models used today are not suitable to accurately reflect the pathogenesis of human disease, particularly complex disease such as cancer, neurological and autoimmune diseases. The failure of the translation process is due to differences in immunological response, metabolism, and genetic control between the species. This means that the therapeutic effects observed in animal models often are not reproduced in humans. Examination of the literature indicates that animal models have limited predictive power, which calls into question their reliability in drug development research. [78]. Mechanisms like drug solubility, permeability, metabolism, cytotoxicity, and receptor interactions are frequently studied using these systems.[77]. The strength and dependability of IVIVC are significantly impacted by this basic variation in biological complexity.[74]. However, a variety of physiological parameters, including gastrointestinal pH

fluctuation, motility, enzyme activity, intestinal transporters, and first-pass metabolism, affect in vivo drug absorption.[77].
These models are useful screening methods during the early stages of drug development since they have shown a respectable connection with human absorption data for numerous drugs.[78]. However, mucus layers, immunological interactions, and regional variations along the intestine all of which have a substantial impact on drug transit in vivo are absent from these models.[83]. IVIVC produced from these techniques is therefore frequently compound-specific and could not be generally applicable.[77].
In vitro tests are widely used in toxicology and safety evaluation to evaluate cytotoxicity, genotoxicity, and organ-specific toxicity. [79]. In vivo toxicity consequences are largely dependent on systemic exposure, metabolic activation, and compensatory biological responses all of which are lacking in the majority of in vitro systems even though these assays offer quick and mechanistic insights.[82]. For instance, unless metabolically capable hepatocyte models are used, liver toxicity is often overestimated in vitro.[82]. Because of this constraint, sophisticated in vitro systems with improved physiological relevance have been developed. By more accurately simulating tissue architecture, mechanical stresses, and dynamic fluid flow seen in vivo, recent developments in three-dimensional cultures, organoids, and organ-on-a-chip technologies have enhanced the predictive potential of IVIVC. [82]. The transition of medication candidates from preclinical investigations to clinical approval is still ineffective, with a high failure rate throughout clinical development, despite notable advancements in biomedical research. Preclinical models frequently employ reductionist systems, which are unable to accurately capture the intricacy of human physiology, including multi-organ interactions and dynamic biological networks. This is one of the main causes. Because human biological reactions are poorly predicted, many medication candidates with excellent preclinical efficacy fail in clinical trials.[80]. However, their regular use in drug development and safety evaluation is still constrained by issues with reproducibility, standardization, scalability, and regulatory approval.[81]. While in vivo research usually uses animal models, in vitro research generally uses human-derived cells. Animal in vivo responses and human in vitro results may not correlate well due to differences in metabolic enzymes, receptor expression, signalling cascades, and transport processes. [79]. This disparity highlights the significance of creating in vitro models that are applicable to humans and makes cross-species extrapolation more difficult.[81].
Strict mathematical modelling and statistical validation are necessary for quantitative IVIVC. [75]. Different IVIVC levels are recognized by regulatory bodies, and larger levels offer more assurance when predicting in vivo performance using only in vitro evidence.[75]. High-quality experimental data, physiologically applicable models, and a solid mechanistic understanding of drug behavior are necessary to achieve such connections.[74]. The correlations remain empirical and lack predictive validity in the absence of mechanistic understanding.[77].
IVIVC is nevertheless essential to contemporary pharmaceutical and biomedical research in spite of these drawbacks. [80]. It shortens the time it takes to develop new products, encourages logical formulation design, and minimizes needless animal testing.[79]. Predictive accuracy has been greatly increased by the ongoing improvement of experimental models in conjunction with computational techniques like physiologically based pharmacokinetic modelling, even if perfect correlation between in vitro and in vivo data is rarely achievable.[81].
To sum up, in vitro–in vivo correlation serves as a vital link between simplified experimental methods and intricate biological reality. [74]. Careful experimental design, biological significance, and quantitative analysis are necessary for its success.[75]. The gap between in vitro observations and in vivo results is anticipated to close as in vitro technologies advance toward better physiological fidelity, enhancing the translational relevance of preclinical research. [82].

5.2 Lack of validated disease models Dose response relationships

In biomedical research and drug development, the absence of validated disease models continues to be a significant obstacle, especially when trying to establish trustworthy dose-response connections between experimental interventions and biological results. [74]. Although many of the models now in use only partially capture the complexity of real disease, disease models are designed to imitate important pathological, molecular, and physiological aspects of human disorders. Because of this, dose-response data produced by such systems might not be able to reliably forecast efficacy of therapy, toxicity, or the best dosage for humans.[75].
The quantitative relationship between the amount of exposure to a drug or stimulation and the ensuing biological effect is known as a dose-response relationship. [76]. These correlations are essential for figuring out therapeutic windows, maximum tolerated doses, and minimum effective doses. Nevertheless, the observed dose-response curves may be deceptive, non-reproducible, or therapeutically meaningless when illness models lack validation. [77]. In complicated, multifactorial diseases like cancer, neurological

disorders, autoimmune illness, and metabolic syndromes, where human pathophysiology cannot be fully captured by simplified experimental systems, this problem is most noticeable. [74].
Although many animal models are unable to faithfully replicate human disease pathways, they have historically been regarded as the gold standard for researching dose-response interactions in vivo. [78]. Drug sensitivity and response patterns can be dramatically changed by species-specific variations in metabolism, immunological responses, genetics, and receptor expression. [79]. As a result, large rate of attrition during clinical development may result from doses that seem safe or effective in animal models turning out to be toxic or ineffectual in humans. [75]. The absence of validated disease models that may produce clinically significant dose-response data is highlighted by this poor translational predictability.
Immortalized cell lines and primary cell cultures are examples of in vitro disease models that provide controlled settings for mechanistic research and high-throughput screening. [80]. Although these systems are helpful in determining dose-dependent cellular responses, they frequently lack important characteristics including systemic control, tissue architecture, and cell-cell interactions.[76]. Therefore, when extrapolated to whole organisms, dose-response equations developed from in vitro models may exaggerate pharmacological potency or underestimate toxicity. [77]. The interpretation and comparison of dose-response data across various in vitro systems are made more difficult by the lack of uniform validation standards. [80].
The form and repeatability of dose-response curves are also impacted by the absence of validated disease models. Variability in illness genesis, development, and severity can result in variable responses at the same doses in poorly described animals. [74]. Because of this heterogeneity, it is challenging to discern between actual pharmaceutical effects and experimental noise, which lowers confidence in dose optimization choices. [81]. Furthermore, non-linear or paradoxical dose-response patterns seen in these models can be byproducts of inadequate models rather than real biological processes. [76].
The incapacity of many disease models to explain the disease variability seen in human populations is another significant drawback. [79]. In terms of therapy response, genetic background, and disease severity, patients frequently show significant interindividual diversity. Experimental models, on the other hand, usually depict a limited and consistent illness phenotype. This discrepancy contributes to unexpected results in clinical trials and reduces the applicability of dose-response associations produced in preclinical research. [75]. Dose selection is still mostly empirical in the absence of established models that account for this variation.
Three-dimensional cultures, organoid systems, patient-derived xenografts, and genetically modified animal models have all been developed in an attempt to enhance the validation of disease models. [82]. These methods seek to increase the predictive accuracy of dose-response relationships and more closely mimic the biology of human diseases. Despite their potential, these models still have issues with long-term stability, scalability, and uniformity. [80]. Crucially, validation necessitates a methodical comparison of clinical results with dose-response data produced from models, a procedure that is frequently inadequate or insufficient. [77].
Strong dose-response data is crucial for regulatory decision-making when evaluating the safety and effectiveness of drugs. [78]. Inadequate validation of disease models reduces regulatory confidence in preclinical dose-response data, requiring substantial clinical testing to offset uncertainty. [75]. This raises the expense of development and puts human subjects at more risk in early-phase trials.[79]. Therefore, increasing translational efficiency and patient safety requires strengthening disease model validation. In addition to improving experiments, quantitative modelling techniques like systems biology modelling and pharmacokinetic–pharmacodynamic analysis can fill in the gaps left by inaccurate illness models. [81]. These methods allow for more accurate predictions of human reactions by integrating dose-response data from many experimental systems and scales. [76]. However, the biological significance and validation state of the underlying illness models continue to determine how accurate these models are.[74].
In conclusion, the validity and translational usefulness of dose-responsive relationships in biomedical research are severely compromised by the absence of validated disease models. [75]. Dose-response data remain ambiguous and frequently deceptive in the absence of models that faithfully capture human illness mechanisms. To improve dose selection, lower clinical failure rates, and eventually improve treatment results, model validation must be advanced through increased biological relevance, standardization, and integration with quantitative modelling. [77].

6. Limitations in Clinical Trial Design

A significant obstacle to accurately assessing the safety, effectiveness, and practical applicability of treatment therapies is limitation in clinical trial design. [88]. The validity of clinical trials is largely dependent on careful study design. Internal validity and generalizability can be jeopardized by flaws such improper patient selection, insufficient randomization, poorly stated objectives, small sample size, and low statistical power. Results may be further skewed by operational issues such participant attrition, protocol

violations, and uneven execution. These restrictions may make study results less reliable, interpretable, and acceptable to regulators. Therefore, to guarantee that clinical trials produce reliable evidence for therapeutic decision-making and regulatory evaluation, meticulous preparation, sound methodology, and open reporting are crucial.[89]

The limited choice of study populations is one of the biggest constraints in clinical trial design. [90]. Narrowly defined eligibility criteria are used in many clinical trials to reduce heterogeneity and improve internal validity. Although this method enhances experimental control, it frequently restricts the registered subjects' representativeness. Patients from a variety of genetic or ethnic backgrounds, women, older persons, and people with numerous comorbidities may all be underrepresented as a result. This limited recruitment may lower external validity and make it more difficult to apply the results to standard clinical practice. Therefore, broader, more inclusive trial designs are necessary to guarantee that study results fairly represent patient groups in the actual world. [91]. Because of this, trial results might not have external validity, which would restrict their applicability to larger patient populations seen in standard clinical practice.[88].

In clinical trial design, sample size and statistical power can provide significant obstacles. [92]. The likelihood of drawing false conclusions is further increased by the fact that power calculations are sometimes predicated on assumptions about effect magnitude and variability that might not fairly represent actual circumstances. [93].

Another significant restriction influencing study results is endpoint selection. [90]. Biomarkers or intermediate outcomes are examples of surrogate endpoints that are commonly utilized to reduce trial duration and expenses. Nevertheless, there isn't always a consistent correlation between these endpoints and clinically significant outcomes like survival, quality of life, or disease progression. [94].

Although blinding and randomization are essential concepts intended to reduce bias, their application isn't always ideal. [88]. Blinding is challenging or impossible in some clinical contexts because of the nature of the intervention, such as behavioural therapies or surgical procedures, which raises the possibility of subjective result assessment. [92].

Another significant design constraint is trial duration. [93]. Long-term efficacy, delayed side effects, or illness recurrence may not be captured by short-term research. Prolonged surveillance is frequently necessary for chronic diseases in order to properly evaluate treatment benefit and risk; nevertheless, longer trial durations raise costs, complexity, and participant dropout rates.[90]. Results may be skewed by high attrition, especially if dropout is linked to adverse events or treatment response.[88].

These design decisions may have an impact on how new treatments are interpreted by regulators and used in clinical settings.[94].

Trial design and execution are further complicated by operational and logistical limitations. [92]. Data quality can be lowered and variability introduced by recruitment difficulties, procedure violations, and inconsistent data collecting across sites. Despite being essential for sufficient enrolment, multicentre studies frequently struggle to sustain protocol adherence and standardised outcome evaluation.[93]. The internal consistency of trial results may be compromised by these operational problems.

Additionally, clinical trial design is shaped by ethical issues, which also impose intrinsic constraints. [88]. Restricting dose escalation, exposure duration, or invasive examinations may be necessary to balance participant safety with scientific integrity. Although these precautions are crucial, they might make it more difficult to thoroughly describe dose-response relationships or uncommon adverse events prior to regulatory approval.[90]. Because of this, certain safety issues only surface during post-marketing surveillance.[91].

The rising complexity of current therapeutics, including biologics, gene therapies, and tailored medications, has further exposed limits in standard trial designs [94]. Small patient populations with extremely diverse diseases may not be a good fit for traditional randomized controlled trials. Although they provide additional statistical and regulatory issues that are still being developed, adaptive trial designs and the real-world evidence method offer potential solutions.[93].

In conclusion, the quality, interpretability, and translational value of clinical research are greatly impacted by restrictions in clinical trial design. [89]. Trials' capacity to accurately predict real-world efficacy and safety is diminished by a number of factors, including limited population selection, insufficient power, subpar outcomes, operational difficulties, and ethical restrictions. To increase the dependability of clinical research and improve patient outcomes, it is crucial to address these limitations through enhanced methodological rigor, creative trial designs, and integration of additional evidence sources.[88].

6.1 Small sample sizes

The validity, reliability, and interpretability of clinical research results are significantly impacted by limitations in clinical trial design, especially small sample sizes and brief study durations. [88]. Clinical trials are intended to produce data regarding the safety and effectiveness of medical therapies; yet, due to logistical,

ethical, and budgetary limitations, studies are frequently underpowered or too short to capture significant clinical effects. These restrictions increase ambiguity during regulatory approval and clinical decision-making and lower confidence in study findings.[89].
One of the most prevalent and significant restrictions in clinical trials is small sample numbers. [90]. To guarantee enough statistical power to identify real treatment effects and differentiate them from random fluctuation, an adequate sample size is crucial. False-negative outcomes, in which potentially helpful therapies appear unsuccessful due to low power, are more common in trials with a small sample number. [91]. On the other hand, when several outcomes are evaluated or subgroup analyses are carried out, small studies are equally vulnerable to inflated effect sizes and false-positive results. [92].
Small sample numbers are a major issue in early-phase trials, studies involving highly specialized or tailored medicines, and research on rare diseases. [93]. Although these studies offer valuable initial data, their small sample size limits the accuracy of efficacy estimates and the capacity to identify rare but clinically significant adverse events. [94]. Because of this, safety hazards might not be noticed until later-phase trials or post-marketing use expose larger groups.[95].
Additionally, the evaluation of treatment response variability among various patient subgroups is hampered by small sample sizes. [96]. Due to the intrinsic heterogeneity of clinical populations, therapy outcomes are influenced by variations in age, sex, genetic history, comorbidities, and disease severity. This diversity cannot be sufficiently represented in trials with small enrolment, which could result in findings that are not applicable to populations in the real world. [89]. This restriction adds to the disparities between the results of clinical trials and the efficacy shown in the real-world following licensure.[97].

6.2 Short study duration

Another significant constraint on clinical trial design is the short research time. [98]. To cut expenses, speed up development schedules, and lessen participant burden, many trials are carried out over comparatively short durations. However, especially for chronic or slowly progressing disorders, short-term trials may not fully capture the therapeutic benefit of therapies. [99]. Short-duration trials may lead to an early conclusion that a treatment is useless if it takes longer exposure to show benefit.[88].
Short clinical trial periods may not fully capture an intervention's therapeutic potential, especially if effects take time to manifest. Additionally, they are inadequate for identifying long-term, cumulative, or delayed negative effects that might only show up after extended exposure. As a result, brief follow-up periods may result in insufficient evaluations of safety and efficacy. Because long-term results are crucial for chronic illnesses and preventive treatments, this restriction is particularly significant. For a more precise assessment of therapy benefits and hazards, longer study periods and post-marketing surveillance are crucial. [100]. Long-term exposure to a therapeutic substance may be necessary for the development of some side effects, such as organ damage, carcinogenicity, and metabolic disorders. As a result, short-term clinical trials frequently fail to detect these cumulative or delayed dangers, offering only a partial evaluation of long-term safety. This restriction is especially important for drugs meant for long-term usage. In order to identify uncommon, late-onset, or exposure-dependent adverse events that might not be visible during pre-approval testing, post-marketing surveillance and long-term observational studies are crucial.[101]. There could be serious repercussions for public health from this safety signal detection delay.[95].
Design limitations are further exacerbated by the combination of small sample sizes and brief study length. [102]. Few participants in brief trials result in few data points, which lowers statistical robustness and raises uncertainty in outcome estimations. This combination makes it more difficult to understand safety and efficacy signals, weakens dose-response evaluations, and hides temporal response patterns.[93]. Because of this, clinical and regulatory choices are frequently based on insufficient data.[90].
These restrictions also influence the choice and interpretation of endpoints.[91]. In short-term experiments, instead of clinically meaningful parameters such as survival, progression of illness, or quality of life, researchers might employ surrogate endpoints or intermediate targets. These outcomes may not always align with the therapeutic impact over a lifetime but still provide early signs of action. [103]. Using surrogate endpoints can lead to incorrect conclusions, especially in conjunction with a small number of samples.[99].
Small sample sizes and brief study durations are frequently caused by operational and ethical issues. [94]. Trial growth and prolongation are constrained by issues with recruitment, high expenses, and participant exposure to experimental therapies. When long-term effects are unclear, ethical obligations to reduce patient risk may limit follow-up time or dose escalation.[88]. Although these limitations are essential, they draw attention to the conflict between clinical research's practical viability and scientific accuracy.[96].
To lessen the effects of small sample sizes and brief durations, improvements in trial methodology have been suggested. [104]. By combining several trial phases into a single protocol, contemporary clinical trial tactics including adaptive methodology, seamless phase I/II or II/III designs, and Bayesian analytical approaches

increase efficiency. These designs allow for prospectively planned adjustments, like as dose selection, sample-size adjustment, or treatment-arm refining, based on interim results. By combining accumulating trial data with previous knowledge, Bayesian approaches further improve analysis. This can be especially helpful in cases of rare diseases or small populations. Together, these approaches reduce development timelines, conserve resources and maximize the precision and value of information from limited patient cohorts.[105]. New clinical trial techniques, such as adaptive trials, seamless phase studies and Bayesian statistics, enhance clinical trial decision-making and efficiency. These methods enable adjustments, such as dose change, sample size re-estimation, or early terminating for efficacy or futility, depending on accumulating data. When patient populations are small or data are scarce, they are very helpful. Bayesian techniques enhance estimation accuracy and facilitate more adaptable, insightful analyses by combining existing knowledge with new data. When combined, these tactics expedite the development of new drugs while optimizing the clinical and scientific benefits of each trial.[102].

Finally, the generalizability and robustness of research findings are compromised by significant limitations in clinical trial design, such as small sample sizes and short study durations. [89]. These limitations limit safety evaluation, raise the possibility of incorrect conclusions, and contribute to differences between trial and real-world results. Enhancing the credibility and translational value of clinical research requires addressing these limits through better planning, methodological innovation, and the creation of additional evidence.[97].

6.3 Non-standardized extracts and endpoints

Biomedical, pharmacological, and clinical research is severely hampered by non-standardized extracts and endpoints, especially in studies utilizing natural compounds, herbal remedies, and intricate biological treatments. [88]. Significant heterogeneity in experimental results is introduced by a lack of uniformity, which also makes it more difficult to understand the data and compromises study reproducibility. Establishing consistent evidence for efficacy, safety, and dose-response relationships is challenging when extracts change in composition and endpoints differ in definition or measurement. [89].

Non-standardized extracts contain variable amounts of active ingredients, variable amounts of impurities or degradation products and variable chemical composition. [90]. This variation can be due to variations in plant species, geographic origin, growing conditions, harvest time, processing methods and storage conditions.[91]. This means that the pharmacological activity of two extracts that are "the same intervention" can vary widely, leading to different biologic response in trials.[92]. Such discrepancies undermine the scientific basis necessary for clinical application and translational research.

The reproducibility of experiments is directly impacted by the absence of extract standardization. [93]. Reproducibility is a key requirement of scientific research, especially studies conducted with poorly defined extracts do not often succeed in replicating results in other laboratories.[94]. The composition of extracts may change and would impact metabolic pathways, target engagement and bioavailability, thus impacting the observed outcomes.[95]. This is particularly evident at the pre-clinical stage as promising findings could not be replicated at the stage of clinical trials or in later studies. [88].

Further, non-standardized extracts can add to challenges in dosage and pose problems for cross-study comparisons. [96]. Often the dosage is expressed in crude extract weight, when there is no accurate measurement of the active ingredients.[97]. This method hides actual exposure levels and makes it impossible to compare toxicity or efficacy findings between trials in a meaningful way. As a result, dose-response correlations obtained from such data are frequently inaccurate or deceptive.[89].

Simultaneously, using non-standardized endpoints makes research findings even more inconsistent. [98]. Although uneven endpoint selection, definition, and measurement techniques restrict comparability between studies, endpoints are meant to offer quantifiable markers of biological or clinical consequences.[99].

In clinical studies including complicated therapies, the issue of non-standardized outcomes is especially severe. [102]. Different scales, time periods, or significant criteria may be used in studies to evaluate equivalent constructs, such as quality of life, immunological modulation, or symptom improvement.[103]. It is challenging to compile data from many trials or reach reliable conclusions about the effectiveness of treatments because of this variability.[91]. Selective endpoint reporting may skew results in favor of positive outcomes in severe circumstances.[94].

Non-standardized extracts and endpoints also impede regulatory review. [104]. To evaluate benefit-risk profiles, regulatory bodies need evidence that is reliable and repeatable. Regulatory confidence in study results is diminished when extract composition is ill-defined and endpoints are not validated. [105]. This ambiguity frequently results in the need for more research, postpones approval procedures, or causes potentially promising therapies to be rejected.[93].

Non-standardization restricts clinical usability from a translational standpoint. [96]. To make well-informed treatment decisions, healthcare professionals need precise information on formulation, dosage, and anticipated results. Uncertainty about which formulation was successful and which results are clinically significant arises from variations in extracts and endpoints.[97]. Variable patient outcomes and inconsistent therapeutic utilization are caused by this gap between research and practice. [89].
The significance of chemical, biological, and methodological uniformity is emphasized in efforts to overcome these issues. [106]. Reproducibility can be greatly increased and variability reduced by using standardized extracts that are produced under controlled conditions and specified by established chemical markers.[90]. Adoption of validated, clinically relevant objectives also improves study comparability and fortifies the body of evidence.[98]. However, because of the variety of therapies and disease situations, reaching an agreement on suitable standards continues to be difficult.[92].
The chemical composition of complex biological preparations can vary greatly due to genetic, environmental, and processing factors, making comprehensive standardization of these preparations intrinsically challenging. Consistency, repeatability, and therapeutic predictability are all impacted by this variability, which presents serious difficulties for quality control, regulatory assessment, and the clinical application of natural product-based therapies.[107]. Finding a single marker chemical for standardization can be difficult because natural items might have effects through several elements working in concert.[95]. Thus, striking a balance between flexibility and rigor continues to be a major problem in study design.[99].
To summarize, biomedical research is severely affected by the lack of standardized endpoints and extracts, both in terms of its validity and its repeatability and translation. [88]. Uncertainty created by differences in composition of extracts and type of evidence measured complicates dose calculation, evidence synthesis, and regulatory evaluation. Standardisation of procedures is needed to enhance the quality of research and ensure study results are relevant to clinical practice and public health policy and planning decisions, while retaining scientific flexibility.[106].

7. Restricted Therapeutic Indications

Chronic inflammatory problems, especially dermatological and metabolic diseases, have received very little attention in current research due to the disproportionate emphasis on respiratory ailments. Complex immune-mediated processes are involved in conditions like type 2 diabetes, psoriasis, atopic dermatitis, and obesity. Increasing translational research in these fields may help develop tailored treatments that improve long-term clinical results by addressing underlying inflammatory mechanisms.[108]. Since respiratory and acute viral disorders have historically been the focus of therapeutic research, many bioactive treatments' wider potential has not received enough attention. Psoriasis, atopic dermatitis, obesity, type 2 diabetes, and metabolic syndrome are examples of chronic inflammatory illnesses that are increasingly understood to be immune-mediated conditions caused by continuous low-grade inflammation. However, there is still a lack of research on these indications, especially in terms of pathophysiology, biomarker creation, and long-term therapeutic approaches. Extended clinical trials verified inflammatory endpoints, and inclusive preclinical models will be necessary for future advancements. Repurposing well-known immunomodulatory medications may present affordable ways to treat unmet needs in inflammatory metabolic and dermatological conditions.[109]. This narrow focus restricts scientific knowledge, hinders innovation in the expansion of indications, and could postpone the discovery of efficacious treatments for ailments other than respiratory disorders. Due to their great frequency worldwide, seasonal burden, and substantial contribution to morbidity and mortality, respiratory infections have long been the main focus of therapeutic research. [110]. Upper and lower respiratory tract infections are appealing indications for clinical research because they are among the most frequent causes of hospital stays, antibiotic prescriptions, and medical visits.[111]. As a result, many substances, particularly those with immunostimulatory or antibacterial qualities, have been primarily investigated in relation to respiratory disorders, frequently without a thorough assessment of their effects in other clinical states.[112].

7.1 Over-focus on respiratory infections

This overemphasis on respiratory infections has resulted in limited therapeutic indications that might not adequately reflect the intervention's biological activity. [113]. For instance, despite evidence indicating broad immunological effects relevant to inflammatory, autoimmune, or metabolic illnesses, substances demonstrated to influence innate and adaptive immune responses are often assessed solely for their capacity to prevent or treat respiratory infections.[114]. Understanding a therapy's wider biological underpinnings may be hampered by limiting research to a small number of authorized uses. As a result, further research is frequently limited to the same therapeutic domain, leaving potential therapeutically significant uses unexplored. This is especially crucial for drugs that have systemic or immunomodulatory properties, as their effects might go far beyond treating acute infections. Expanding research into these broader applications

could uncover new treatment prospects and improve the overall clinical value of known interventions as the frequency of immune-mediated, metabolic, and chronic inflammatory illnesses continues to rise globally.[115].

Trials involving respiratory infections frequently evaluate outcomes like the frequency of sickness, the intensity of symptoms, and the length of recovery. A thorough assessment of therapy success may be limited by these endpoints' potential inability to adequately capture broader therapeutic effects, such as immunological modulation, quality-of-life enhancements, or long-term preventive advantages, despite their clinical utility.[116]. An evaluation of treatment efficacy may be lacking if respiratory outcomes are the only emphasis. Even though metrics like the length of symptoms and the incidence of infections are clinically significant, they might miss more extensive advantages such systemic immunomodulation, anti-inflammatory action, or protection of non-respiratory tissues. As a result, when evaluated exclusively through respiratory endpoints, therapies having significant physiologic effects may appear only marginally beneficial. This limited assessment approach may mask significant mechanism-based advantages and undervalue therapeutic value. A more thorough understanding of therapy effects might be possible if endpoint selection were expanded to include immunologic, metabolic, and organ-specific outcomes.[117]. The evaluation of numerous therapeutic medicines has been limited by the clinical research's major focus on respiratory tract infections. Benefits that go beyond preventing infections, such immunomodulatory, anti-inflammatory, or metabolic impacts, may therefore not be fully acknowledged. The evidence base required to support their implementation in other emergent health issues, such as chronic inflammatory and metabolic illnesses, is constrained by this narrow focus. Therefore, in order to properly describe therapeutic potential, increase clinical indications, and direct evidence-based integration into preventative and therapeutic strategies across multiple disease settings, it is imperative to expand clinical exploration beyond respiratory endpoints.[118]

Labelling and regulatory routes are also impacted by the predominance of respiratory indications. [119]. After a treatment is approved for respiratory infections, sponsors frequently have little financial incentive to look into other indications. Each new indication necessitates independent clinical trials, regulatory submissions, and significant financial outlay because regulatory permission is granted for specific purposes. Companies may choose to focus on other development initiatives if the expected market return is low. Because of this, potentially useful applications in other viral or inflammatory disorders may be unexplored. Although crucial for guaranteeing safety and effectiveness, this indication-specific framework may unintentionally impede therapeutic repurposing and limit the wider clinical use of proven medicines.[120]. Clinical adoption and scientific research may be unintentionally hampered by regulatory approval restricted to a particular indication. While pharmaceutical corporations frequently perceive little financial motivation to invest in expensive trials for other purposes, doctors may be reluctant to use treatments outside of approved labelling. Even in cases where greater application is supported by biological reasoning, this discourages more extensive research. As a result, research efforts continue to be focused on well-established treatment areas, perhaps ignoring possible new uses. The therapeutic usefulness of current therapies could be maximized by addressing these obstacles through targeted repurposing activities and increased clinical evaluation.[121]. In clinical practice, off-label use of drugs is prevalent, particularly when there are few approved treatments or when physicians rely on mechanical reasoning and past experience. However, the lack of solid, high-quality clinical data supporting such use frequently limits the creation of official treatment guidelines and consistent physician confidence. Decisions are often relied on observational data or expert opinion in the absence of well-designed randomized controlled trials, which might result in variation in practice patterns. For off-label prescribing to be safer, more uniform, and guidelines-driven, the evidence basis must be strengthened through rigorous clinical research.[122].

Depending on the patient group, disease state, and length of treatment, an intervention's safety and therapeutic efficacy can vary significantly. The results of long-term dosing in chronic inflammatory or immune-mediated disorders may not be accurately predicted by evidence from short-term use in acute respiratory infections. Efficacy and tolerance may also be impacted by differences in age, comorbidities, immunological status, and concurrent medicines. As a result, it might not be suitable to extrapolate acute-care results to chronic disease situations. To confirm sustained safety, optimize dosing regimens, and precisely describe the risk-benefit balance in these larger clinical contexts, dedicated long-term studies are crucial.[123]. Additionally, diversity of populations in clinical research is limited by the emphasis on respiratory infections.[111]. In times of severe illness, many respiratory infection trials target participants with a general health status or specific demographic, such as pediatric or geriatric adult patients. This narrow population sample makes it hard to assess how effective treatments are for people who have complex comorbidities, immunocompromised status, or chronic health conditions.[113]. Potentially beneficial

treatments run the risk of being hastily disregarded in the absence of a more thorough outcome assessment.[114]. The narrow therapeutic focus impacts public health strategies as well. [110].
In order to get over this restriction, research frameworks must be purposefully expanded. [124]. However, this expansion requires increased investment, integration among different disciplines and flexibility in regulation.[119]. Although respiratory infections are a significant and respectable field of research, an overemphasis on this area restricts translational impact, underutilizes therapeutic promise, and hinders mechanistic insight. In order to fully realize the clinical utility of therapies and to address the changing landscape of global health requirements, research must be expanded beyond respiratory indications.[124].

7.2 Underexplored chronic inflammatory diseases

7.2.1 Dermatological and metabolic applications

Among the most obvious signs of chronic inflammation are dermatological conditions, which are frequently marked by dysregulated cytokine signalling, poor barrier function, and prolonged immune activation. [110]. Additional research obstacles arise from the chronic nature of inflammatory skin disorders. Periods of remission and aggravation are influenced by environmental, genetic, and lifestyle variables, and disease development is frequently delayed and varied.[111]. These dynamics are not fully captured by short-term research or acute inflammatory models, which results in an inadequate comprehension of long-term disease behaviour and treatment response. Therefore, despite good molecular justification for their use, dermatological applications of immunomodulatory and anti-inflammatory therapies remain understudied.[110].
Despite their increasing prevalence worldwide, chronic inflammatory skin conditions and metabolic disorders are still not well studied. Psoriasis, hidradenitis suppurativa, obesity, and type 2 diabetes are all largely caused by persistent, low-grade inflammation; nevertheless, treatment approaches frequently focus primarily on downstream symptoms rather than underlying inflammatory networks. Genetic predisposition, microbiota composition, lifestyle, and concurrent conditions all influence disease expression and treatment response. However, the real-world relevance of many studies is limited due to their reliance on tightly defined populations or simple illness models. To find new therapy targets and enhance long-term results in these intricate chronic illnesses, a more comprehensive, systems-based research strategy incorporating immunology, metabolism, and host–microbiome interactions is crucial.[112].
The absence of coordinated frameworks for research that address chronic inflammation across organ systems is a prevalent problem in both metabolic and dermatological applications. [111]. Inflammatory processes that are shared by skin and metabolic tissues include immune cell infiltration, oxidative stress mechanisms, and cytokine networks. However, cross-disciplinary insights that could speed up therapy discovery are limited because research efforts are frequently compartmentalized by illness category. Increased integration of research on metabolic inflammation and dermatology may identify common goals and facilitate more effective translation of results.[110]. Metabolic and dermatological illnesses are frequently long-term, chronic conditions linked to high medical expenses, decreased productivity, and psychological distress. Reliance on symptomatic therapies rather than preventive or disease-modifying measures is sustained by insufficient funding for research. A more thorough approach to treating chronic inflammation may enhance long-term results and lessen the effects of these illnesses on society.[111]. Long-term, mechanism-driven studies that highlight chronic inflammation as a main therapeutic target are necessary to advance research in this field. [110].

8. Insufficient Pharmacokinetic and Bioavailability Data

A key element of contemporary drug discovery and development is pharmacokinetic and bioavailability characterisation, which establishes the degree and duration of systemic exposure to pharmacologically active substances. While bioavailability particularly refers to the portion of an administered dose that enters the systemic circulation in an unaltered, physiologically active form, pharmacokinetics discusses the absorption, distribution, metabolism, and elimination of a drug within a biological system. Inadequate pharmacokinetic and bioavailability data continue to be a major barrier for many promising treatment candidates, despite notable advancements in molecular pharmacology, medicinal chemistry, and high-throughput screening technology.[125], [126]. This shortcoming ultimately limits the conversion of experimental efficacy into clinical success and is one of the most frequent reasons for failure in late-stage preclinical development and early clinical trials. A considerable percentage of bioactive substances exhibit strong biological activity in vitro but are unable to get sufficient systemic exposure in vivo. Poor bioavailability, which can be caused by low water solubility, chemical instability, significant first-pass metabolism, or restricted permeability across biological membranes, is frequently the cause of this disparity. [127]

This ambiguity raises the risk related to dose toxicity or therapeutic inefficacy and makes it difficult to create therapeutic windows. Additionally, the lack of exposure data makes it difficult to compare drugs in the same therapeutic class meaningfully, which lowers the effectiveness of lead optimization initiatives.[130].
Complex molecules, such as numerous organic goods and recently created synthetic chemicals, may have significant bioavailability constraints. Unfavourable physicochemical characteristics include high molecular weight, significant polarity, low water solubility, and restricted membrane permeability may limit their therapeutic value. These elements may decrease systemic exposure following injection, limit tissue distribution, and hinder absorption. Consequently, substances with high pharmacological activity in vitro might not perform as well in vivo. Successful treatment development depends on overcoming these obstacles through sophisticated drug delivery methods, logical molecular change, or formulation optimization.[131]. Furthermore, the amount of unaltered chemical entering systemic circulation can be significantly decreased by extensive presystemic metabolism in the liver and gastrointestinal tract.[132]. Dosing tactics may be based on given dose rather than systemic exposure when bioavailability is not sufficiently evaluated, which could result in inconsistent and irreproducible results across investigations. These difficulties are made worse by the absence of standardized methods for pharmacokinetic evaluation. Cross-study comparisons are made more difficult because the generalizability of results is restricted by variations in study design, species selection, dosage routes, analytical techniques, and sampling tactics.[1].
The identification and characterisation of active metabolites are further hampered by inadequate pharmacokinetic data. Numerous substances undergo significant biotransformation, producing metabolites that may have different pharmacological or toxicological characteristics from the parent molecule.[135]. It is unknown how these metabolites contribute to overall treatment outcomes in the absence of thorough metabolic profiling. This information gap affects safety evaluation throughout clinical development and may result in incorrect attributions of harm or efficacy.[136].
The increased probability of drug-drug interactions is another significant effect of insufficient pharmacokinetic and bioavailability data. Competition for metabolic enzymes or transporters frequently results in pharmacokinetic interactions, which modify systemic exposure.[137]. It is challenging to accurately predict clinically important drug-drug interactions when drug metabolism and elimination routes are not well understood. The safe and efficient application of otherwise promising therapeutic medicines may be restricted by this uncertainty, especially in combination therapy. In order to sustain therapeutic concentrations, drugs with quick clearance frequently need greater or more frequent doses, which might raise the risk of toxicity and lower patient adherence. On the other hand, medications with poor clearance may build up in the body, resulting in extended exposure and possible toxicity, particularly in those with compromised hepatic or renal function. For safe dose optimization, it is consequently crucial to comprehend these routes.[138].
From a translational standpoint, high rates of attrition in drug development are directly caused by inadequate pharmacokinetic characterisation. Research has repeatedly demonstrated that a significant percentage of compound failures throughout preclinical and early clinical stages are caused by poor pharmacokinetics and bioavailability.[126]. These mistakes not only cause large financial losses but also postpone patients' access to innovative treatments. Therefore, incorporating pharmacokinetics evaluation early in the discovery process has been strongly recommended as a tactic to increase development efficiency and lower late-stage failure. [140].
Pharmacokinetic measurements are now much more sensitive and accurate thanks to developments in analytical technology like liquid chromatography–mass spectrometry and micro sampling methods. [141]. Simultaneously, computer methods, such as physiologically based pharmacokinetic modelling, provide strong instruments for forecasting systemic exposure and bioavailability under different physiological circumstances.[142]. Even with the availability of sophisticated modelling and analytical techniques, drug development programs continue to use them inconsistently. Pharmacokinetic factors are frequently devalued in favor of pharmacodynamic effectiveness objectives in many early-stage investigations. During the early stages of development, this imbalance may result in insufficient characterisation of the profiles of absorption, distribution, metabolism, and excretion. As a result, possible problems with bioavailability, systemic clearance, and exposure variability might only be discovered later. Therefore, it is crucial to incorporate pharmacokinetic evaluation more methodically from the start of drug discovery in order to maximize clinical development outcomes and increase translational success.[143].
A paradigm shift toward more integrated and methodical review techniques is necessary to address the ongoing problem of inadequate pharmacokinetic and bioavailability data.
Improving translational dependability requires thorough exposure profile definition, consistent reporting, and open data interpretation. A probability of therapeutic success can be increased by better aligning drug

research efforts with clinical reality by giving pharmacokinetic understanding equal weight with pharmacological efficacy.[144].

8.1 Absorption and Metabolism of Active Compounds

The pharmacokinetics behavior and therapeutic efficacy of active substances are largely determined by absorption and metabolism. While metabolism regulates a compound's chemical transformation, bioavailability, and duration of action, absorption determines how much of it enters the systemic circulation. Due to poor absorption or significant metabolic breakdown, many pharmacologically active compounds show considerable biological activity in vitro but inconsistently show efficacy in vivo.[125], [126]. One of the biggest obstacles to turning potential drug ideas into successful treatments is still inadequate pharmacokinetic and bioavailability characterization. Powerful experimental activity and real clinical success are frequently at odds as a result of this difference. Therefore, it is crucial to assess absorption, distribution, metabolism, and excretion early in the drug development process. Gastrointestinal obstacles sometimes impair the effectiveness of oral delivery, even though it is the most chosen route due to simplicity and patient adherence. Systemic drug exposure is restricted by a number of factors, including low aqueous solubility, poor epithelial permeability, enzymatic degradation, fluctuating pH levels, and active efflux systems like P-glycoprotein.[76],[128]. Physiological variations among patients have a significant impact on interindividual variability in drug absorption, which may result in uneven treatment effects. The rate and degree of medication absorption are greatly impacted by variables such changes in gastrointestinal pH, gastrointestinal motility, activity of the enzymes, and transporter expression. Changes in medication metabolism and bioavailability are also influenced by variations in the composition of the gut flora. Even when the same doses are given, these combined factors result in significant variation in systemic drug exposure. To improve the predictability of oral medication therapy in a variety of patient populations and optimize dose techniques, it is imperative to comprehend this variability.[129]. Inadequate characterisation of these parameters frequently leads to uneven pharmaceutical responses and unexpected systemic exposure.

After being ingested, active substances go through phase I and phase II enzymatic processes that convert them metabolically, mostly in the colon and liver. While phase II reactions entail conjugation activities like glucuronidation and sulfation that improve water solubility and enable elimination, phase I events, which are mostly mediated by cytochrome P450 enzymes, introduce or expose functional groups. [130]. Drug metabolism has two roles in pharmacokinetics: it is crucial for the removal and purification of xenobiotics, but it can also have a big impact on treatment results. Rapid metabolic transformation of the parent substance into inactive metabolites frequently results in decreased pharmacological activity and systemic bioavailability. On the other hand, metabolic activities can produce toxic intermediates linked to negative consequences or active metabolites that enhance therapeutic efficacy. Thus, in clinical pharmacology, metabolic pathways play a crucial role in determining both medication safety and efficacy.[132].

The pharmacological activity seen in vivo is frequently mediated by the molecule's metabolites rather than the parent compound. However, inadequate metabolic profile frequently makes it difficult to identify the actual bioactive species, making it more difficult to evaluate safety and efficacy results.[133]. Single-dose designs or constrained sampling schedules are frequently used in pharmacokinetic investigations, which only partially depict the full drug exposure profile. Important clinical characteristics such complex concentration-time behavior, time-dependent variations in drug clearance, and nonlinear pharmacokinetics may be missed by such methods. Significant interindividual variation in drug exposure also results from variations in metabolic enzyme function that are impacted by age, co-administered medications, genetic polymorphisms, and medical conditions. These variables can result in significant variations in safety and efficacy outcomes, highlighting the necessity of population-based methods and thorough pharmacokinetic modelling. These causes of heterogeneity remain poorly understood in the absence of thorough metabolic characterization, raising the possibility of unanticipated reactions in clinical settings.[134]

8.2 Tissue Distribution and Elimination

Whether active drugs reach their target site of action at therapeutically relevant concentrations depends on tissue distribution, a crucial but often overlooked aspect of pharmacokinetics. Tissue perfusion, membrane permeability, transporter expression, and physicochemical characteristics including lipophilicity, molecular size, ionization state, and plasma protein binding all effect distribution. [135]. Although pharmacokinetics are frequently described by plasma concentration–time profiles, these profiles may not accurately represent drug exposure at target tissues.

Particularly for disorders affecting particular anatomical areas like tumors, inflammatory tissues, or the central nervous system, inadequate tissue penetration can significantly limit therapeutic efficiency. Despite sufficient plasma concentrations, obstacles like the blood-brain barriers or dense tumor microenvironments

may limit medication availability.[136]. On the other hand, significant accumulation in non-target tissues may raise the possibility of long-term negative consequences including off-target toxicity. Despite these consequences, there are frequently little or no tissue distribution investigations, which leads to inadequate exposure-response evaluations.
Pharmacokinetic behavior and systemic exposure are further affected by elimination processes. Renal excretion and biliary clearance are the main ways that drugs and their metabolites are removed; the rate of elimination determines the systemic half-life, frequency of dose, and potential for build-up.[137].
Reliable long-term safety prediction is hampered by a lack of knowledge about drug elimination mechanisms, particularly in prolonged therapy. Risks include drug buildup, delayed toxicity, or erratic steady-state levels increase when clearance pathways are not well understood. Drug interactions that change metabolic or excretory pathways, as well as illness conditions that affect renal or hepatic function, can also cause variations in systemic exposure. These characteristics increase the risk of side effects during long-term treatment by contributing to significant interpatient variability and making dose adjustment challenging. It is challenging to develop sensible dosage schedules or predict variations in pharmacokinetic behaviour among patient populations in the absence of thorough information on clearance mechanisms and removal kinetics.[139]

8.3 Dose Optimization Challenges
One of the trickiest problems in medication research and clinical translation is dose optimization, especially when there isn't enough pharmacokinetic information. In order to create obvious exposure-response connections, rational dose selection necessitates the combination of pharmacokinetic factors with pharmacodynamic responses. However, insufficient knowledge of tissue distribution, excretion, metabolism, and absorption frequently leads to empirical dose plans that could not accurately reflect ideal therapeutic circumstances.[140].
Dosage optimization is made more difficult by individual differences in pharmacokinetic behavior. Significant interindividual variation in systemic exposure at the same dose can result from variations in tissue distribution, clearance, metabolic capability, and absorption efficiency.[141]. For substances with limited therapeutic windows, where little variations in exposure might lead to decreased efficacy or increased toxicity, this variability is particularly problematic. Determining minimum effective dosages, maximum tolerable doses, and suitable dosing intervals is still difficult in the absence of strong pharmacokinetic characterization.
Inadequate dose optimization can lead to either excessive exposure, which raises the risk of side effects, or sub therapeutic exposure, which results in treatment failure. Reduced trust in treatment prospects and late-stage clinical trial failure are largely caused by these problems.[142]. Inadequate exposure-response data also makes it more difficult to customize treatment or modify dosage in certain groups, such as children, the elderly, or critically ill patients.
In general, limitations in pharmacokinetic and bioavailability data are directly associated with difficulties in dose optimization. In order to overcome these obstacles, pharmacokinetic evaluation must be methodically included into drug development, guaranteeing that dosage plans are informed by accurate exposure data and in line with therapeutic goals. [144].

9. Lack of Long-Term Safety and Toxicological Data
A crucial but often neglected component of the development of medicinal agents is long-term safety and toxicity assessment. Early preclinical and clinical research frequently prioritizes acute toxicity and short-term efficacy over long-term effects of prolonged exposure. This disparity is especially problematic for drugs meant for long-term use, since extended usage may result in cumulative toxicity, delayed side effects, or changes to physiological balance.[145].

9.1 Chronic use studies
Since many safety problems only become apparent after extended treatment, chronic exposure studies are crucial to the development of new drugs. Delayed toxicities including cumulative organ damage, immunity dysfunction, reproductive toxicity, or carcinogenic consequences could not be visible in short-term research. Because of this, early safety evaluations may not give a clear picture of long-term risk. When medications are used for long periods of time in therapeutic settings, this restriction generates ambiguity. Therefore, to provide a full safety profile and enable dependable, long-term therapeutic usage of novel drugs, thorough chronic use and long-duration toxicity studies are required.[146]. Because chronic safety studies are costly, time-consuming, and operationally complex, comprehensive toxic effects over time evaluation is frequently delayed. As a result, several treatments go into clinical testing or regular use with little knowledge of their

long-term safety profile. Cumulative tissue deposition, metabolic adaption, altered drug clearance, or delayed deleterious effects that are not seen in short-term tests can result from repeated exposure. Over time, these modifications may worsen toxicity or reduce effectiveness. Therefore, insufficient long-term safety data may lead to unanticipated post-marketing adverse occurrences, regulatory limitations, and the requirement for updated risk-benefit analyses.[147].

Long-term drug use might cause physiological adaptive reactions that drastically alter treatment results. The immune system modulation, receptor desensitization or decreased regulation, and inhibition or stimulation of metabolic enzymes involved in drug clearance are a few examples of these adaptations. These modifications have the potential to modify pharmacokinetics and pharmacodynamics over time, which could result in decreased therapeutic efficacy or an elevated risk of toxicity. These dynamic biological reactions demonstrate that short-term research alone are insufficient to accurately predict drug behavior over chronic exposure. In order to maintain therapeutic efficacy, fully comprehend safety, and optimize dosing techniques in clinical practice, long-term evaluation is crucial.[148]. These dynamic processes are still poorly understood in the absence of comprehensive chronic exposure research, which makes it difficult to forecast long-term results. When it comes to autoimmune illnesses, which usually demand for long-term or sustained therapeutic intervention, the dearth of long-term safety evidence is especially significant. In order to maintain disease management, patients with autoimmune disorders including multiple sclerosis, rheumatoid arthritis, or inflammatory bowel disease frequently undergo ongoing medication.[149]. Long-term treatment may cause even minor or low-grade toxicity to build up, which could eventually result in adverse effects that are clinically severe. Such effects may worsen with prolonged exposure and show up as systemic imbalance or organ dysfunction. Long-term use of immunomodulatory drugs can upset immunological homeostasis, which may make people more vulnerable to infections or compromise immune surveillance systems. Prolonged immunological change may occasionally increase the risk of cancer. These possible side effects emphasize how crucial it is to carefully monitor long-term safety and evaluate risks in treatments that affect immune function.[150].

Many drugs used to treat autoimmune illnesses are mostly assessed for their short-term anti-inflammatory or immunosuppressive effects; their long-term immunological effects have received less research attention. Immune homeostasis can be upset by persistent immune modulation, which may have an impact on immunological tolerance, decrease host defensive mechanisms, and occasionally cause paradoxical immune reactions such rebound inflammation or secondary immune dysregulation. Unintentional systemic effects that are not seen in early clinical studies may result from ongoing changes to immune pathways over time. Therefore, in order to completely comprehend safety consequences in chronic autoimmune therapy, long-term microbiological monitoring is crucial.[151]. Furthermore, long-term immune pathway activation or inhibition may have systemic consequences outside of the targeted treatment target, underscoring the necessity of prolonged toxicological surveillance in autoimmune populations. Special populations, such as children, the elderly, pregnant women, and people with impaired immune systems, raise additional safety issues. These populations are more susceptible to side effects after long-term treatment because they frequently display altered pharmacokinetic and pharmacodynamic responses.[152]. For instance, altered medication clearance and higher systemic exposure may result from immature metabolic systems in young patients or decreased hepatic and renal function in older people.[153]. For particular populations including youngsters, pregnant women, and nursing moms, relying solely on elder safety and dosage data may be insufficient. These groups' physiological characteristics can significantly change the toxicity, effectiveness, and disposition of drugs. Extended maternal exposure during pregnancy may impact fetal development, raising the possibility of teratogenicity or long-term developmental issues. Similarly, breastfeeding infants may be at danger from drug transmission through breast milk. Long-term safety data is still scarce since these populations are often underrepresented in clinical research. Therefore, focused research is necessary to determine the right dosage and guarantee the safety of mothers, fetuses, and infants.[154]. However, these populations are frequently excluded from safety studies due to ethical and methodological issues, leaving large gaps in the data. The requirement for focused safety assessments is further highlighted by the possibility that immunocompromised patients may have increased toxicity or altered immune responses over extended treatment.[155].

Such hazards could be reduced and overall therapeutic confidence raised by incorporating thorough long-term toxicological evaluation earlier in the development process. Recent developments in toxicological techniques, including as systems toxicology, biomarker-based toxicity assessment, and long-term in vivo modelling, have improved the capacity to assess chronic safety profiles. Long-term safety is still underrepresented in many development projects, and these strategies are not yet consistently applied. In order to close this gap, it is necessary to strategically prioritize both efficacy and chronic safety evaluation, especially for treatments that target autoimmune and other chronic disorders. In conclusion, a major obstacle

to the effective clinical translation of medicinal medicines is the absence of long-term safety and toxicological data. Uncertainty about prolonged usage is caused by a lack of chronic use research, a lack of evaluation in autoimmune illnesses, and insufficient safety evidence in particular populations. [149].

10. Herb–Drug Interaction Gaps

Globally, there has been a significant rise in the concurrent use of herbal remedies with traditional medication, especially among patients with long-term treatment, immunological dysfunction, and chronic illnesses. Despite the widespread belief that herbal remedies are safe, they contain a number of physiologically active ingredients that may interact with prescription medications. These interactions may change how drugs are absorbed, distributed, metabolized, or excreted, which could result in decreased therapeutic effectiveness or increased toxicity. Modulation of medication-metabolizing enzymes and transportation infrastructure is a key mechanism driving herb-drug interactions. Herbal components have the ability to either stimulate or inhibit membrane transporters including P-glycoprotein and cytochrome P450 (CYP) enzymes, which has a substantial impact on the pharmacokinetic profiles of co-administered medications. Predicting these interactions is still difficult because herbal preparations differ in terms of composition, concentration, and standardization.[156]. Nevertheless, there is still little systematic assessment of these interactions, and the information that is available frequently comes from case-based or in vitro findings rather than carefully monitored clinical trials.[157]. Interaction assessment is made more difficult by the absence of standardized formulations, variations in herbal content, and inadequate regulatory supervision. [158]. These gaps highlight the necessity for thorough research and the incorporation of herb-drug interaction data into clinical practice since they provide safety hazards, particularly for individuals using medications with limited therapeutic windows. [159,160].

10.1 Interaction with immunosuppressants

These issues are especially important for individuals using antiviral, antibiotic, or immunosuppressive medications. Because immunosuppressive medications like tacrolimus and cyclosporine have narrow therapeutic indices, even little variations in systemic contact can have detrimental clinical effects. While higher levels can raise the risk of nephrotoxicity, neurotoxicity, or other negative effects, lower medication concentrations may result in therapeutic failure, graft rejection, or disease recurrence. Anti-infective treatments carry similar concerns, as changed medication exposure may increase toxicity or reduce treatment efficacy. .[156].

Many drug-drug and herb-drug interactions have not been thoroughly investigated in well-designed clinical trials, despite their extensive clinical use. The majority of the evidence that is currently available comes from case reports, in vitro experiments, or tiny observational studies, which makes it difficult to reach firm clinical judgments. This is especially crucial for immunosuppressive medications like sirolimus, tacrolimus, cyclosporine, and mycophenolate mofetil, which are frequently used in autoimmune diseases and transplantation. Due to the limited therapeutic windows of these medications, even small variations in systemic exposure might result in toxicity or therapeutic failure, including graft rejection. Therefore, safe therapy requires systematic clinical examination of such interactions.[157]. Therefore, interactions that change how immunosuppressants are absorbed, metabolized, or eliminated are particularly concerning from a therapeutic standpoint.

Co-administered medications, such as herbal remedies and some conventional drugs, have been shown in numerous trials to have a considerable impact on the pharmacokinetics of immunosuppressants. Tacrolimus and cyclosporine are transported by P-glycoprotein (P-gp) and extensively metabolized by CYP3A4. Elevated or decreased plasma concentrations may result from inhibition or induction of these mechanisms, respectively.[158]. For example, CYP3A4-inhibiting substances may raise immunosuppressive exposure, which could lead to neurotoxicity, nephrotoxicity, and a higher risk of infection. On the other hand, enzyme induction can lower medication levels and diminish the effectiveness of immunosuppressive therapy.[159]. Long-term immunosuppressive therapy is frequently used in patients with autoimmune illnesses, which raises the possibility of interactions during long treatment durations. Despite this concern, there is still little systematic assessment of the possibility of interactions over long-term use. A gap in proactive safety evaluation is highlighted by the fact that clinical monitoring frequently depends on therapeutic medication monitoring rather than predicted interaction studies.[160].

10.2 Interaction with Antibiotics and Antivirals

It has been demonstrated that erythromycin and clarithromycin considerably increase the concentrations of tacrolimus and cyclosporine, requiring dose modification and close observation. Similarly, because they

strongly inhibit CYP3A4, azole antifungals like voriconazole and ketoconazole might result in noticeable increases in immunosuppressive exposure.[161].

10.3 Cytochrome P450 Involvement

Enzymes called cytochrome P450 are essential to these interactions. Most therapeutically used drugs are metabolized by CYP3A4, CYP2D6, CYP2C9, and CYP2C19. Pharmacological reactions and plasma drug concentrations can be dramatically changed by the inhibition or stimulation of these isoenzymes by dietary, pharmaceutical, or herbal components. This is especially important for medications like immunosuppressants, anticoagulants, and some antibacterial medicines that have limited therapeutic windows. A thorough assessment of herb-drug interactions is crucial given the growing popularity of complementary medicines. The safe integration of conventional and herbal medicines in clinical practice can be supported and unfavorable outcomes can be reduced with improved mechanistic understanding, thorough clinical inquiry, and regular medication review.[156]. Modulation of these enzymes either through inhibition or induction represents a fundamental factor of altered drug exposure. Immunosuppressants and many antibiotics and antivirals share dependence on CYP3A4 for metabolism, making this enzyme a significant node for interaction risk. While CYP enzyme suppression can lead to drug accumulation and toxicity, stimulation can result in subtherapeutic exposure and therapy failure.[158]. Furthermore, interindividual variability is exacerbated by genetic variations in CYP enzymes, making it more difficult to anticipate the results of interactions.[162].

11. Formulation and Drug-Delivery Challenges

Strong formulation and drug-delivery techniques are essential for the successful clinical translation of medicinal medicines. Many candidates fail to advance because of formulation-related issues, even though promising pharmacological activity was seen during early discovery and preclinical review. Among the most frequent obstacles to successful medication development are poor water solubility, physical and chemical instability, and uneven bioavailability.[163].

11.1 Poor Solubility and Stability

These difficulties are especially noticeable for contemporary medication candidates, such as lipophilic small compounds, natural products, and biologics, which frequently have intricated physicochemical characteristics. As a result, sophisticated formulation techniques are becoming more widely acknowledged as crucial for improving patient compliance and therapeutic efficacy. One of the main factors limiting oral bioavailability and therapeutic efficacy is poor solubility. The Biopharmaceutics Classification System (BCS) class II or IV, which is distinguished by low water solubility and variable absorption, is thought to comprise a sizable fraction of recently created medications.[164]. Reduced absorption, unpredictable dissolving in gastrointestinal fluids, and significant interpatient variability can result from insufficient solubility. Furthermore, limited solubility frequently calls for greater dosages, raising the possibility of negative side effects and systemic toxicity. Formulation development is made more difficult by stability concerns. Numerous active pharmaceutical ingredients (APIs) can be broken down by oxidation, hydrolysis, photolysis, or heat stress. Instability can lead to decreased shelf life, hazardous degradation products, and loss of potency.[165].

Enzymatic degradation and sensitivity to pH fluctuations can make natural chemicals and biologically derived agents more unstable. These restrictions emphasize the necessity of protective formulation techniques that maintain medication integrity during production, storage, and administration. Solubility and stability issues have been somewhat resolved by conventional formulation techniques such salt production, cosolvent usage, and solid dispersions. These techniques, however, are not always appropriate and could result in other problems such precipitation, incompatibility, or low patient acceptability. [166].

11.2 Need for Nano-Formulations

Drug delivery systems based on nanotechnology have become a viable way to address stability and solubility issues. Because of their small size and large surface area, nano-formulations such as nanoparticles, liposomes, polymeric micelles, and nan emulsions offer special benefits.[167]. These systems can protect labile medicines from degradation, increase apparent solubility, and improve dissolution rates. Additionally, pharmacokinetics and biodistribution can be modified by nano-formulations. Nanoparticles can be designed to improve absorption across biological barriers and enable targeted delivery to particular tissues or cells by changing their composition and surface characteristics.[168]. This is especially advantageous for medications meant for long-term use or those with limited therapeutic windows. Additionally, by reducing off-target

exposure, nano-carriers may lessen systemic toxicity. Despite its benefits, scalability, reproducibility, and regulatory approval are issues with nano-formulations. Before widespread clinical adoption, issues with manufacturing complexity, batch-to-batch variability, and long-term stability need to be resolved.[169].

11.3 Controlled-Release Systems

By maintaining therapeutic medication concentrations over prolonged periods of time, controlled-release drug delivery systems lower dosage frequency and increase patient adherence. Additionally, by minimizing peak-trough variations, these systems can lessen the negative consequences of high systemic exposure.[170]. For chronic conditions needing long-term treatment, when steady medication levels are necessary for long-term effectiveness, controlled-release formulations are especially beneficial. Matrix systems, reservoir systems, biodegradable polymers, and implantable devices are just a few of the controlled-release techniques that have been created. Because of their biocompatibility and adjustable degradation patterns, polymers like poly (lactic-co-glycolic acid) (PLGA) are utilized extensively. [171]. By carefully modifying the polymer type, molecular mass, and matrix design, controlled-release formulations allow for the precise regulation of drug release profiles. This kind of personalization lowers the frequency of doses while maintaining therapeutic medication levels. A significant development in pharmaceutical science is the incorporation of nanotechnology into various delivery methods. In addition to offering delayed and targeted release, nano-based controlled-release platforms improve the solubility and dissolving of medications that are poorly soluble in water. This combination is a very promising approach for improved drug administration since it increases pharmacokinetic stability, boosts bioavailability, lowers systemic toxicity, and promotes more consistent therapeutic effects.[172].

12. Quality Control and Adulteration Issues

Over the past few decades, the usage of pharmaceuticals, especially those made from natural sources, has grown significantly on a global scale. This increase has revealed serious issues with quality control and product integrity, even though it also reflects the growing acceptability of complementary and alternative medicines. Product safety, effectiveness, and reproducibility are jeopardized by variations in raw materials, uneven manufacturing procedures, and deliberate or inadvertent adulteration. [173]. For herbal medicines to be safe, consistent, and therapeutically reliable, strong Quality control and regulatory control are crucial. The substitution of the wrong plant species, contamination with heavy metals, pesticides, or microorganisms, and the unreported inclusion of synthetic medications are all examples of adulteration, which is still a serious problem. Such methods can undermine public trust in herbal medicines, reduce efficacy, and have major negative impacts. Subpar or fake goods might also enter the market due to inadequate regulatory frameworks and uneven enforcement in some areas. For safe incorporation into evidence-based healthcare, it is consequently essential to strengthen standardization, authentication, and post-market surveillance.[174]

12.1 Authentication of Raw Material

A key element of herbal quality assurance is the precise verification of botanical raw materials. Ensuring consistency, safety, and therapeutic efficacy requires accurate identification of the plant species, particular plant portion, and chemotype. Variable phytochemical content decreased therapeutic efficacy, or even toxicity might result from misidentification or substitution. The accuracy of botanical verification is significantly increased by contemporary authentication methods, including as DNA-based procedures, chromatographic profiling, and macroscopic and microscopic assessment. Therefore, it is essential to establish strict authentication procedures early in the product development process in order to preserve product integrity and guarantee repeatable pharmacological results.[175]. The chemical makeup, safety, and medicinal performance of herbal preparations can all be significantly impacted by incorrect plant identification or substitution. Traditional authentication techniques, such as microscopic and macroscopic analysis, are still helpful, but they might not be sufficient for powdered or processed materials when diagnostic characteristics are lost. The precision of authentication has significantly increased thanks to contemporary analytical technology. Precise species verification and efficient adulterant or contaminant identification are made possible by chromatographic fingerprinting, spectroscopic methods, and molecular tools like DNA barcoding. Utilizing these complementing techniques is crucial for maintaining product consistency, guaranteeing the validity of raw materials, and bolstering the clinical dependability of herbal remedies.[176]. In instance, DNA barcoding has proven to be highly accurate in identifying pollutants and differentiating closely related species, even in complicated formulations.[177]. To increase everyday applicability, however, issues such DNA deterioration during processing and the absence of thorough reference databases must be resolved. To determine the presence and concentration of marker molecules, chemical profiling techniques such as mass spectrometry and high-performance liquid chromatography

(HPLC) are frequently employed. Although these techniques are useful for evaluating quality, they might not be able to identify substitution with species that are chemically identical. For strong authentication, a mix of chemical, genetic, and botanical methods is advised [178].

12.2 Regulatory Compliance

Maintaining quality standards and avoiding adulteration depend heavily on regulatory compliance. Countries have very different regulatory systems for pharmaceutical-grade items, while traditional or herbal products are subject to less regulation.[179]. Manufacturers, regulators, and consumers face difficulties as a result of this discrepancy, especially in the setting of worldwide supply chains. A basis for quality assurance is provided by international recommendations including World Health Organization (WHO) standards and Good Manufacturing Practices (GMP). [180].

Herbal and pharmaceutical products must adhere to Good Manufacturing Practice (GMP) in order to guarantee their quality, consistency, and safety. Through verified procedures, regulated production environments, and thorough documentation, GMP reduces the risks associated with contamination, batch variability, and manufacturing errors. However, implementation is still patchy, especially among smaller businesses and in environments with limited resources. Regulatory organizations are depending more and more on post-marketing surveillance, which includes product recalls, analytical quality testing, and adverse event tracking, to close these gaps. These steps are essential for spotting inferior items, enforcing adherence to regulations, and preserving public trust in medicinal products.[181]. Effective pharmacovigilance and quality control systems are frequently weakened by inadequate financing and low technical capability. Stronger international cooperation, standardized legal requirements, and integrated monitoring systems are necessary to improve medication safety worldwide. Patient safety and therapeutic efficacy are still seriously threatened by product quality variability, adulteration, and contamination. Therefore, stringent regulatory enforcement and reliable authentication techniques backed by cutting-edge analytical technology are essential to guaranteeing product integrity. Strengthening healthcare systems and advancing safe, dependable, and sustainable pharmaceutical development globally need addressing these issues through better surveillance techniques, regulatory alignment, and multi-stakeholder cooperation.[182].

13. Regulatory and Clinical Translation Barriers

The process of converting therapeutic candidates from preclinical research or conventional use into licensed clinical medications is difficult, time-consuming, and resource-intensive. The absence of standardized international regulatory procedures is a significant obstacle to the clinical acceptance of phytopharmaceuticals and other innovative treatments. The development of multi-component botanical products is greatly slowed by regulatory variability among nations, unclear approval processes, and strict evidence criteria. Additionally, distrust in the clinical and regulatory realms is exacerbated by worries about standardization, safety, and reproducibility. The successful integration of novel natural products into established evidence-based healthcare systems is hampered by these coupled issues.[183].

14. Future Perspectives and Research Priorities

Herbal medicine's future depends on its evolution from a conventional empirical practice to a precision-based, scientifically verified therapeutic approach. New developments in computer sciences, analytical chemistry, and molecular biology are opening up previously unheard-of possibilities to improve the safety, effectiveness, and repeatability of herbal remedies. Integrating herbal therapy with the concepts of personalized medicine where treatment plans are customized based on an individual's genetic profile, metabolic condition, microbiome composition, and disease phenotype is a top objective. This strategy could maximize therapeutic results while reducing side effects and response variability across individuals. [184]

The use of multi-omics technologies, such as transcriptomics, proteomics, metagenomics, metabolomics, and genomes, is one of the most promising study avenues. These technologies make it possible to thoroughly characterize both host reactions and herbal formulations, which makes it easier to find biomarkers for patient stratification, toxicity, and efficacy. These methods, when combined with systems biology and network pharmacology, help clarify the intricate multi-target mechanisms of herbal remedies, which frequently function through the synergistic interactions of multiple bioactive components. [185]

Herbal research is anticipated to be revolutionized by machine learning and artificial intelligence. Large and complicated datasets can be analyzed, herb-drug interactions can be predicted, new therapeutic targets can be found, and customized treatment algorithms may be developed with the help of these technologies. Herbal formulations, dose schedules, and quality control procedures might all be optimized with the help of AI-driven platforms, hastening the shift from conventional wisdom to evidence-based clinical application. [186]

Standardization is still a major research focus and a significant difficulty. Inconsistent therapeutic results are frequently caused by variations in phytochemical composition brought about by variations in plant species, growth circumstances, harvesting timings, and extraction techniques. Establishing widely recognized quality standards, reliable pharmacopeial monographs, and verified analytical techniques should be the main goals of future initiatives. Cutting-edge technologies like blockchain-based traceability systems, metabolomic fingerprinting, and DNA barcoding may enhance supply chain transparency, authenticity, and quality control. [19]

Large-scale, well planned randomized controlled trials with pharmacogenomic outcomes and biomarker-based stratification will be necessary for clinical translation. To demonstrate long-term safety, determine the best dosage, and validate efficacy, such studies are crucial. Simultaneously, thorough pharmacovigilance mechanisms need to be reinforced in order to track adverse events and herb-drug interactions in practical situations. [187]

Lastly, it will be crucial for pharmacologists, doctors, herbal scientists, bioinformaticians, regulatory bodies, and legislators to collaborate across disciplinary boundaries. To enable responsible implementation, it is also necessary to address equal access, ethical issues, and regulatory harmonization. Herbal medicine is positioned to play a significant role in next-generation healthcare by fusing traditional knowledge with state-of-the-art research, providing safe, efficient, and customized therapeutic options for a range of patient populations. [188]

15. Personalized Herbal Therapeutics

A new paradigm called "personalized herbal therapeutics" blends the scientific foundation of precision medicine with the holistic ideas of traditional herbal treatment. Personalized herbal therapy aims to customize treatment based on a person's genetic profile, metabolic traits, gut microbiota, environmental exposures, and illness phenotype, in contrast to traditional herbal treatments that frequently rely on broad formulations. One of the main drawbacks of conventional phytotherapy—inter-individual heterogeneity in treatment response may be addressed by this customized approach, which could increase therapeutic efficacy, decrease side effects, and improve patient adherence.[184] Pharmacogenomics, which studies how genetic differences affect reactions to bioactive substances, is a crucial scientific basis for customized herbal treatments. The absorption, metabolism, and pharmacological effects of herbal components can be greatly impacted by variations in the genes that encode cytochrome P450 enzymes, transport proteins, and cellular receptors. Clinicians may be able to anticipate which patients will benefit most from particular herbal preparations while reducing the risk of toxicity or herb–drug interactions by detecting these genetic variations.[189] The effectiveness of herbal remedies is also significantly influenced by the gut microbiota. Intestinal microbe’s bio-transform many phytochemicals into active or inert metabolites, which affects their biological activity and bioavailability. Therefore, very varied therapeutic effects may result from individual differences in microbial composition. Optimizing herbal selection and dosage, particularly in chronic inflammatory, metabolic, and gastrointestinal illnesses, may be possible by incorporating microbiome analysis into personalized herbal treatment.[190] Personalized herbal therapies are being developed more quickly thanks to developments in multi-omics technologies, such as transcriptomics, proteomics, metagenomics, metabolomics, and genomes. The identification of biomarkers linked to efficacy, safety, and treatment response is made easier by these methods, which allow for thorough analysis of both patient biology and herbal composition. When paired with network pharmacology and systems biology, multi-omics techniques help clarify the intricate, multi-target processes via which herbal mixtures work. [69] It is anticipated that machine learning and artificial intelligence (AI) would further revolutionize this industry. Large, multifaceted datasets can be analyzed by these technologies, which combine genetic, clinical, lifestyle, and phytochemical data to produce customized therapy suggestions. Additionally, AI-based predictive models could help choose patient subgroups most likely to benefit from particular herbal interventions, optimize dosing schedules, and find new herb–gene interactions.[186] Personalized herbal therapies have a number of obstacles to overcome despite their potential. The phytochemical makeup of herbal products can be greatly impacted by variations in plant species, growth circumstances, harvesting, and extraction techniques, so standardization is still crucial. Additionally, regulatory frameworks need to change to meet concerns about safety, ethical genomic data usage, quality control, and fair access to customized treatments.[187] Large-scale clinical trials utilizing pharmacogenomic, metabolomic, and microbiome-based stratification should be given top priority in future research. It will be essential for herbal scientists, doctors, pharmacologists, bioinformaticians, and regulatory bodies to work together. Personalized herbal therapies present a possible route toward safer, more efficient, and patient-centred healthcare solutions by fusing ancient herbal knowledge with contemporary precision medicine.[191]

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