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THALASSEMIA TREATMENT WITH *AZADIRACHTA INDICA* (NEEM): A COMPREHENSIVE REVIEW USING AN *IN SILICO* METHOD

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Abstract
Thalassemia, a pervasive genetic blood disorder, stems from impaired hemoglobin synthesis, leading to chronic anemia and substantial global health burdens, particularly in regions like Pakistan. Existing treatments, such as transfusions of blood and chelation care, are life-saving but come with significant costs and adverse effects like iron overload, alloimmunization, and Thalassemia-Induced Bone Disease (TIBD). Advanced therapies like gene therapy and hematopoietic transplanting stem cells remain largely inaccessible due to high costs and infrastructure demands, especially in developing nations. This review emphasizes the critical need for novel, affordable, and accessible therapeutic options, particularly from natural sources. Azadirachta indica (Neem), a plant with a rich history in traditional medicine, exhibits documented anti-inflammatory, antioxidant, and immunomodulatory properties, suggesting its relevance in mitigating thalassemia-related complications, including TIBD. This article explores the application of in silico drug discovery methodologies—including target prediction, ADMET profiling, molecular docking, and network pharmacology—to systematically identify and evaluate potential therapeutic compounds derived from Neem. The proposed in silico pipeline aims to bridge the current research gap by rigorously investigating Neem's phytochemicals against thalassemia-related protein targets, offering a promising avenue for developing affordable and effective treatments that address both primary hematological issues and secondary morbidities like TIBD.

Keywords: *Thalassemia, Azadirachta indica, Neem, In Silico Drug Discovery, Molecular Docking, Thalassemia-Induced Bone Disease (TIBD), Phytochemicals, Natural Products, Computational Biology, Drug Repurposing.*

1. Introduction

Thalassemia is a hereditary genetic disorder caused by mutations in the genes that govern hemoglobin chain synthesis, resulting in diminished levels or total absence of these chains. This genetic disorder causes hemolytic anemia a chronic condition marked by inadequate erythropoiesis and accelerated red blood cell loss, thereby compromising the body's capacity to produce healthy erythrocytes (Bajwa & Basit, 2023). It is prevalent in Africa, Southeast Asia, the Middle East, and South Asia (Kattamis *et al*., 2020). Among the two primary forms of thalassemia, alpha and beta, beta-thalassemia is the more severe variant, frequently necessitating lifelong blood transfusions and chelation of iron to address iron overload resulting from repeated transfusions (Langer, 2024b). While advantageous, these treatments do not cure the hereditary problem and pose risks such as iron overload, alloimmunization, and transfusion-related infections (Patterson *et al*., 2022).

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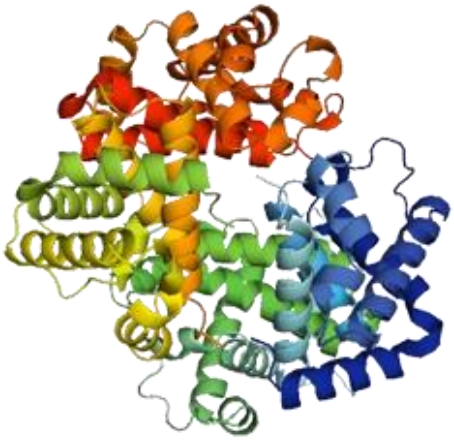

Fig1: 3-dimensional structure of human hemoglobin. Visually seen and modified in PyMOL, the crystal structure came from the Protein Data Bank (PDB ID: 1A3N) tetrameric arrangement of two alpha and two beta units.

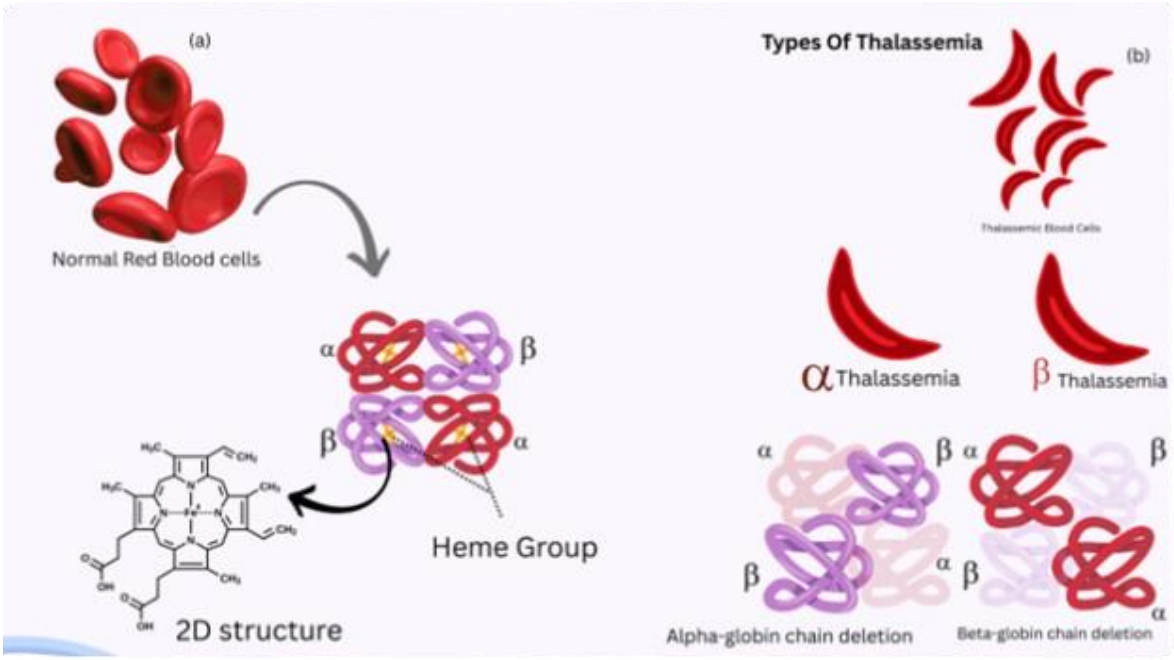

Fig 2: Overview of normal hemoglobin and types of thalassemia represented structurally. Illustration made using Canva. (a) Shows healthy red blood cells and highlights their biconcave shape. Below that, the heme group is depicted, including its 2D chemical structure with a central (Fe) atom. While (b) compares α-thalassemia and β-thalassemia by illustrating the deletion of respective globin chain of hemoglobin.

t costs, especially in less developed nations (Salzman *et al*., 2018). These constraints underscore the urgent need for novel, accessible, and cost-effective therapeutic alternatives, particularly

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from natural sources. Medicinal herbs have historically been pivotal in drug discovery. *Azadirachta indica* (NEEM) is renowned for its therapeutic benefits (Wylie & Merrell, 2022), utilized as an antimalarial, antibacterial, anti-inflammatory, antioxidant, and potential anticancer agent (Osazee & Jonathan, 2023). Neem's isolated compound, including azadirachtin, nimbin, quercetin, and imboiled, demonstrate significant biological action against various disorders (Sarkar *et al*., 2021). Computational drug discovery, leveraging *in silico* methodologies, has largely replaced traditional early-stage drug screening. These approaches efficiently predict chemical-protein interactions, including molecular docking, ADMET profiling (absorption, distribution, metabolism, excretion, and toxicity), protein-protein interaction analysis, gene enrichment studies, and virtual screening (Mellaoui *et al*., 2024). Molecular docking specifically helps identify lead drugs by assessing binding affinity and interaction patterns with disease-related proteins, facilitating experimental validation (Agu *et al*., 2023).

This study investigates novel approaches to address current thalassemia treatment limitations by exploring the therapeutic potential of NEEM-derived drugs. We utilize *in silico* methods to examine binding interactions between Neem phytochemicals and thalassemia-associated genes, aiming to identify Neem candidates for thalassemia treatment by integrating network pharmacology with pathway enrichment and visualizing protein-ligand interactions.

Thalassemia: Etiology, Classification, and Global Burden

Thalassemia is a genetic disorder encompassing diverse hereditary hemoglobinopathies, more than just a blood ailment (Bajwa & Basit, 2023b). It is characterized by quantitative deficiencies in globin chain quantity, involving the complete absence or impairment of alpha or beta globin chains of hemoglobin (Hb) (Langer, 2024). Hemoglobin, a red blood cell protein with two alpha and two beta globin chains, transports oxygen (The Editors of Encyclopedia Britannica, 2025). Disruption of this chain equilibrium leads to pathological consequences such as ineffective erythropoiesis (defective blood cells dying before maturation) and chronic hemolytic anemia (fragile mature red blood cells rapidly destroyed peripherally) (Phillips & Henderson, 2018).

Beta-thalassemia, particularly its severe form, is globally prevalent associated with over 200 chromosome 11 HBB gene variants (Needs *et al*., 2023). These mutations range from significant beta chain production decrease to complete synthesis cessation (Advani, n.d.). Clinically, beta thalassemia presents in three forms: minor (mild, often asymptomatic anemia, heterozygous carrier state), major (most severe, requiring lifelong transfusions), and intermedia (intermediate, often without regular transfusions) (Bajwa & Basit, 2023c). The disease is notably prevalent in the thalassemia belt, Mediterranean, Middle East, South Asia, and parts of Africa, correlating with historical malaria endemicity where carrier status might have conferred a survival advantage (De Sanctis, 2017). Thalassemia places a substantial global health burden on regions with high carrier frequencies. The World Health Organization (WHO, 2019) estimates tens of millions globally carry β-thalassemia mutations. This high carrier rate leads to a significant birth rate of affected individuals, straining healthcare systems in low- and middle-income nations. In Pakistan, the national beta thalassemia carrier rate is 5–7%, with 5,000 to 9,000 children born annually with beta-thalassemia major (Khaliq, 2022). This influx depletes healthcare resources, imposes emotional and financial strain on families, and contributes to increased child mortality and morbidity. Lack of advanced treatments and screening exacerbates the national situation, making management and preventive measures primary healthcare concerns (Ansari *et al*., 2011).

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Current Therapeutic Strategies and Their Limitations

Current thalassemia therapy focuses on supportive care to mitigate symptoms. Regular red blood cell transfusions ensure adequate oxygen supply and prevent anemia (Farmakis *et al*., 2022). However, chronic transfusions lead to dangerous iron overload, necessitating lifelong iron chelation therapy (Mba, n.d.) with agents like deferasirox, deferiprone, and deferoxamine. These remove excess iron from vital organs (liver, endocrine glands, and heart), preventing damage and prolonging survival (McDowell *et al*., 2024). For early-diagnosed individuals with suitable donors, hematopoietic stem cell transplantation (HSCT) is the only recognized curative option. HSCT is costly and involves considerable risks, such as graft-versus-host disease (GVHD) and transplant-related fatalities, necessitating the availability of a compatible donor. (Khaddour *et al*., 2023).

Self-renewing hematopoietic stem cells, modified genetically ex vivo, have transitioned from preclinical studies to clinical application. Recent breakthroughs, including lentiviral vector-based gene insertion and CRISPR-Cas9-mediated genome editing, have revolutionized therapy (Frangoul *et al*., 2020). FDA-sanctioned therapies like exagamglogene autotimer and betibeglogene demonstrate lasting transfusion independence in many patients by correcting genetic anomalies (Morgan & Schambach, 2025). Researchers are also investigating small molecules like luspatercept (Biswas *et al*., 2024), which can enhance hemoglobin synthesis and reduce transfusion reliance in beta-thalassemia. However, these advanced therapies are largely unavailable in underdeveloped nations due to their high costs and extensive infrastructural requirements.

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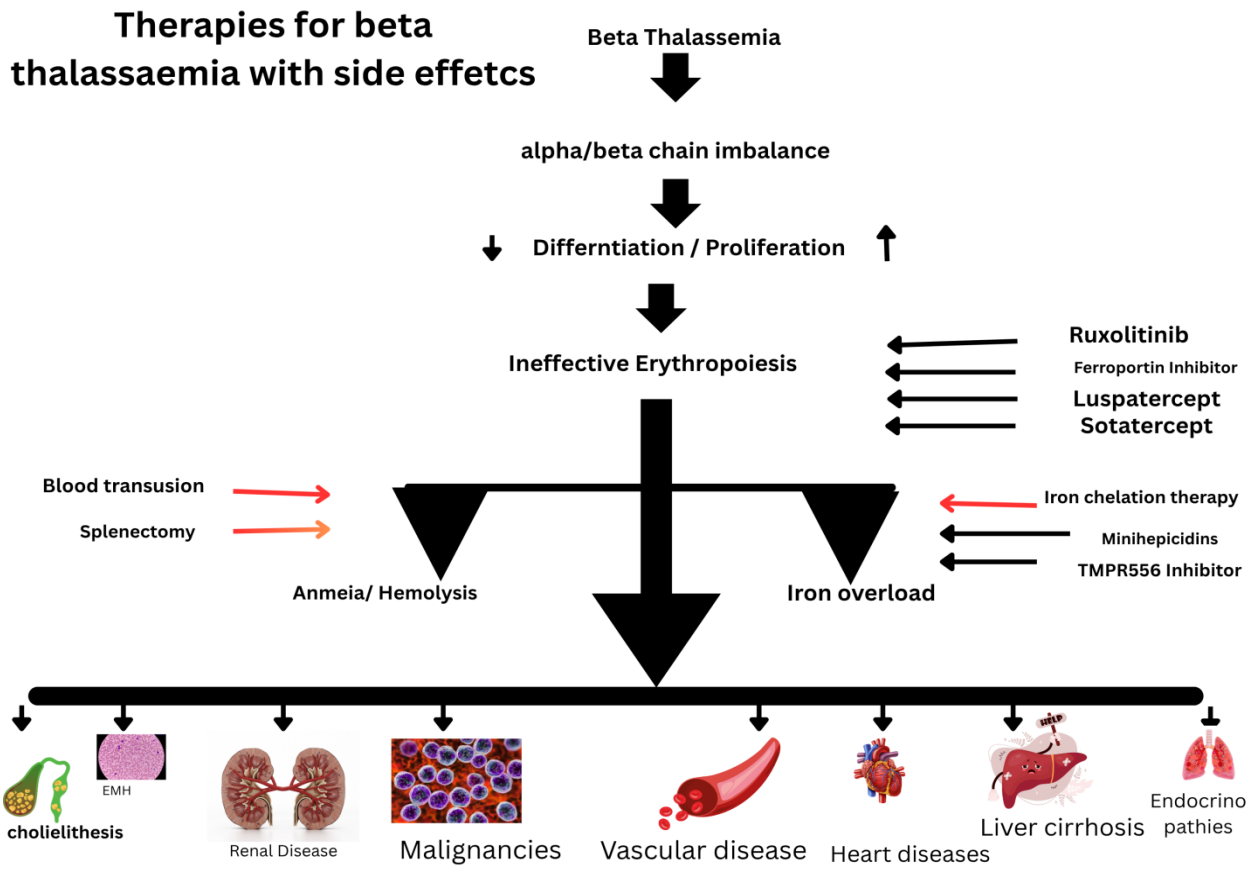

Fig 3: Approaches for managing beta thalassemia and the associated complications. This flowchart delineates contemporary treatment modalities for beta thalassemia, encompassing iron chelation therapy and TMPRSS6 inhibitors for iron overload; exploiting, luspatercept, and ferroprotein inhibitors for ineffective erythropoiesis; as well as blood transfusion and splenectomy for anemia and hemolysis. The image highlights potential adverse consequences including cholelithiasis, extramedullary hematopoiesis, renal illness, malignancies, vascular disorders, cardiovascular disease, liver cirrhosis, and endocrinopathies. This instructional graphic was created using Canva.

trabecular bone loss due to spatial competition within the marrow cavity. This also disrupts bone remodeling by inhibiting osteoblasts (bone-forming cells) and stimulating osteoclasts (bone-resorbing cells) (Gaudio *et al*., 2020). Excessive iron deposition, particularly in the pituitary gland, causes hypogonadism and reduced growth hormone levels, directly inducing osteoporosis by decreasing bone production and increasing bone resorption (Gaudio *et al*., 2019). Thalassemia also impairs bone remodeling through chronic inflammation, increasing osteoclast activity and inhibiting osteoblast development. Nutritional deficiencies in Vitamin D and calcium, crucial for bone health, are common in thalassemia patients, worsening bone deficiency (Goldberg *et al*., 2021). Furthermore, while effective for iron overload, chelation therapy can negatively impact bone metabolism, especially at high doses (J. Zhang *et al*., 2021). Comprehensive management beyond iron overload and anemia is crucial, as TIBD affects over 50% of adult thalassemia

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major patients. Problems like bone discomfort, deformities, and pathological fractures severely restrict mobility and increase morbidity, highlighting a critical unmet clinical need in thalassemia treatment (Needs *et al*., 2023b).

Medicinal Plants and Their Therapeutic Potential

Medicinal plants, rooted in ancient healing traditions, are increasingly explored for novel and accessible thalassemia treatments. Their naturally occurring chemicals offer antioxidant, anti-inflammatory, immunomodulatory, and gene-modulating effects. These properties can reduce issues like iron overload and oxidative stress, showing significant potential for thalassemia management and enhancing existing therapies (Mucha *et al*., 2021). Plant-derived compounds may be viable alternatives due to lower toxicity, cost-effectiveness, and cultural acceptance in high-prevalence regions where modern pharmaceuticals are less accessible.

***Azadirachta indica* (Neem): A Botanical Marvel**

Azadirachta indica (Neem), revered for millennia in Ayurvedic and Unani medicine, is increasingly validated by scientific research. Its complex compounds, including polyphenols and terpenoids like naringenin, quercetin, azadirachtin, imboiled, and nimbi (Sarkar *et al*., 2021b), contribute to its wide range of pharmacological properties: antibacterial, antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory, and antineoplastic effects ("IN-DEPTH REVIEW ON TAXONOMY, PHYTOCHEMISTRY, TRADITIONAL USES AND PHARMACOLOGICAL SIGNIFICANCE OF AZADIRACHTA INDICA PLANT," 2024).

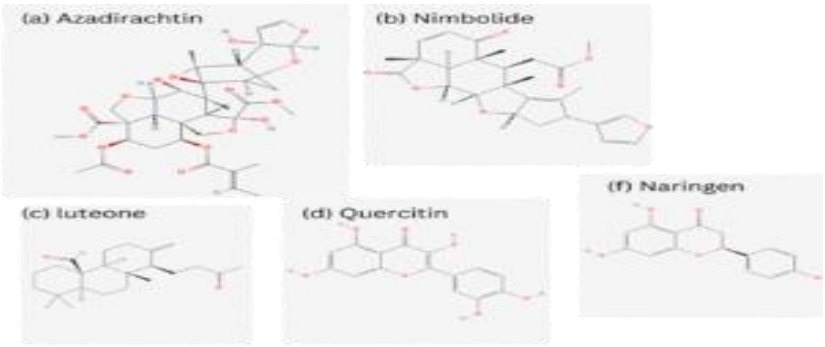

Fig 4: 2D chemical structures of NEEM compound (a) Azadirachtin (b) nimbolide (c) luteone (d) quercetin (f) naringen taken from pubchem created by writers utilising canva.

Neem's characteristics significantly contribute to managing Thalassemia Induced Bone Disease (TIBD). Its anti-inflammatory and antioxidant properties notably mitigate oxidative stress, a major contributor to bone loss in thalassemia (Alzohairy, 2016). Quercetin, a prominent flavonoid in neem, shows osteoprotective qualities in animal studies, promoting bone formation

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Compound	Class	Source	Molecular Formula	Molecular Weight (g/mol)	Key Properties
Naringenin	Polyphenol	Citrus fruits (e.g., grapefruit)	C15H12O5	272.25	Antioxidant, anti-inflammatory helps to regulate lipid metabolism.
Quercetin	Polyphenol	Onions, apples, berries	C15H10O7	302.24	potent anti-cancer, cardioprotective, antioxidant
Azadirachtin	Terpenoid	Neem (*Azadirachta*	C35H44O16	720.71	Insecticidal, anti-parasitic with low

over resorption (Sharma *et al*., 2023). It can regulate osteoblast and osteoclast activity. While direct evidence of neem's effect on TIBD in humans is limited, its predicted characteristics highlight a promising avenue for further *in silico* investigation, particularly on bone metabolism proteins and endocrine activities affected by thalassemia.

In Silico Approaches in Drug Discovery

Advances in *in silico* approaches have revolutionized early-phase drug discovery, transforming the slow and costly traditional pipeline (Marques *et al*., 2024). Utilizing computational biology and bioinformatics, these methodologies facilitate virtual screening, target protein prediction, pharmacokinetic profiling (ADMET properties), and molecular docking simulations (Wu *et al*., 2020). These sophisticated techniques accelerate discovery by providing economical and rapid assessment of compounds, prioritizing candidates, and gaining mechanistic insights into compound-target interactions. Their application is increasingly vital for rare and complex genetic disorders like beta thalassemia and associated bone disease, particularly through multi-target methodologies.

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		indica)			human toxicity
Nimbolide	Terpenoid	Neem (*Azadirachta indica*)	C27H30O7	466.52	Anti-inflammatory, anti-cancer, causes death.
Nimbin	Terpenoid	Neem (*Azadirachta indica*)	C30H36O9	540.60	Antimicrobial, antiviral, sour chemical with immunomodulation.

Target Prediction and Cross-Referencing

Target prediction is the crucial initial step in computational drug discovery, involving the identification of proteins a plant-derived bioactive chemical might interact with. Web-based platforms like Swiss Target Prediction and Pharm Mapper utilize drug-target interaction databases and advanced algorithms (e.g., ligand-based similarity, protein structural homology) to predict target genes for natural chemicals (Agamah *et al*., 2019). Following these predictions, a cross-referencing process compares compound characteristics and predicted targets with established disease-associated gene and protein databases (e.g., DisGeNET, NCBI, GeneCards) (N *et al*., 2023). This refines the focus to therapeutically relevant interactions, concentrating on promising pathways and excluding irrelevant targets, potentially including proteins involved in TIBD's dysregulated bone metabolism.

4.2 Molecular Docking

Molecular docking is fundamental to structure-based drug design (Joshi *et al*., 2024). It computationally predicts the optimal orientation of a small molecule (ligand, e.g., neem phytochemical) interacting with a large molecule (receptor, e.g., thalassemia-related protein) to form a stable complex. Identifying ligand binding sites on the target protein allows evaluation of these poses based on anticipated binding affinity. AutoDock Vina, with its various tools, enables precise and efficient high-throughput docking, rapidly screening numerous molecules. Its output provides a binding energy score (kcal/mol), indicating interaction strength, which helps researchers identify compounds with optimal binding characteristics for further investigation (Trott & Olson, 2009). Recent *in silico* methyltransferase investigations, primarily focused on docking to find novel enzyme inhibitors, highlight its advancement, with research now extending to bone remodeling proteins (Kaniskan *et al*., 2017).

4.3 Functional Enrichment Analysis and Network Visualization

After compound screening, the predominant targets are contextualized within broader biological systems. Functional enrichment analysis, typically performed using tools like DAVID and STRING, identifies statistically over-represented Gene Ontology (GO) terms (e.g., molecular function, biological process, cellular component) and pathway enrichments (e.g., KEGG, Reactome pathways) among the identified target proteins (S. Zhang *et al*., 2025). This analysis can reveal processes related to oxidative stress, iron metabolism, inflammation, and critically, dysregulated bone remodeling pathways in TIBD. Cytoscape further facilitates the visualization of complex target and protein-protein interaction networks (Mousavian *et al*., 2021). These networks clarify relationships between proteins in relevant biological pathways and Neem chemicals with their anticipated targets. Identifying central "hub" proteins through this method

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reveals systematic compound effects, inferring potential synergistic or pleiotropic actions, thus highlighting significant regulatory nodes and prospective therapeutic pathways.

5. Research Gap and Proposed Approach

Despite advancements in *in silico* drug discovery and documented pharmacological effects of neem, a significant research gap persists: a lack of comprehensive *in silico* analysis specifically targeting thalassemia-related proteins—including those involved in complications like bone disease—using neem-derived compounds. While numerous studies confirm neem's antioxidant, anti-inflammatory, and antibacterial properties, few have rigorously investigated its direct therapeutic significance in complex, multifactorial inherited blood illnesses such as thalassemia. Research on broader biological activities has not yet linked them to specific protein targeting in thalassemia.

This initiative directly addresses this gap. We propose a robust, multi-faceted *in silico* pipeline incorporating advanced computational methodologies: virtual screening of neem phytochemical libraries, ADMET profiling for bioavailability and safety, precise target prediction, comprehensive protein-protein interaction mapping within thalassemia pathways (including bone health), and intricate molecular docking simulations. This process will systematically identify and rank potential neem-derived lead compounds based on specific binding affinities for key thalassemia-related protein targets, enabling subsequent experimental validation.

This *in silico* pipeline offers multiple benefits. It efficiently ranks drugs by pharmacokinetics, target specificity, and binding efficacy, significantly reducing traditional drug development time and costs. Focusing on neem provides a unique advantage: it is an accessible, eco-friendly, and culturally accepted source of phytotherapy, especially crucial for regions like Pakistan with high thalassemia prevalence and limited access to expensive conventional therapies. Identifying potent compounds from a local source will facilitate the development of more cost-effective and widely used treatment strategies that can address both primary hematological issues and debilitating consequences like TIBD.

6. Conclusion

Thalassemia presents significant global challenges. While conventional medicines are invaluable, their high costs, logistical complexities, and long-term side effects like TIBD necessitate urgent research into new therapeutic techniques. Medicinal plants, particularly *Azadirachta indica*, represent a rich and historically validated source of bioactive chemicals with considerable potential to mitigate various aspects of thalassemia pathology, especially concerning bone health. *In silico* techniques offer a robust, cost-effective, and efficient framework for systematically screening promising natural drug candidates. Utilizing computational approaches to identify novel neem-derived molecules with potent anti-thalassemic activity will enhance patient outcomes.

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Author's Contribution: Hassan Saeed: Conducted literature review and wrote the manuscript. Dr. Nageen Hussain: Supervised the research and review the manuscript. Co-authors: Contributed to data interpretation and proofreading.

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