

INTEGRATED NETWORK TOXICOLOGY AND MOLECULAR DOCKING ANALYSIS REVEALS MOLECULAR MECHANISMS OF PRILOCAINE-INDUCED METHEMOGLOBINEMIA

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Abstract

Prilocaine is a widely used local anesthetic known to induce methemoglobinemia, a hematologic disorder characterized by oxidation of hemoglobin iron from ferrous (Fe²⁺) to ferric (Fe³⁺) state, resulting in impaired oxygen transport and tissue hypoxia. Despite clinical recognition of this adverse effect, the molecular mechanisms underlying prilocaine-induced methemoglobinemia remain incompletely understood. This study aimed to investigate the toxicological mechanisms of prilocaine-induced methemoglobinemia through an integrated network toxicology and molecular docking approach. Potential molecular targets of prilocaine were identified using PubChem and SwissTargetPrediction, while methemoglobinemia-associated genes were collected from GeneCards, DisGeNET, and Ensembl databases. Overlapping targets were determined and subjected to protein-protein interaction network construction using GeneMANIA. Functional enrichment analyses, including Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis, were performed using STRING. Molecular docking was conducted using AutoDock Vina to validate interactions between prilocaine and key protein targets. Three common targets—CYP2C19, CASR, and JAK2—were identified as potential mediators of prilocaine-induced methemoglobinemia. Protein interaction network analysis highlighted these genes as central regulators linked to oxidative stress and metabolic pathways. Functional enrichment revealed significant involvement in oxidation-reduction processes, cellular oxidative stress response, heme binding, oxidoreductase activity, and xenobiotic metabolism. KEGG pathway analysis identified cytochrome P450-mediated drug metabolism, PI3K-Akt signaling, and JAK-STAT signaling as major pathways implicated in toxicity. Molecular docking demonstrated favorable binding affinity of prilocaine toward CYP2C19 (−6.9 kcal/mol), CASR (−6.8 kcal/mol), and JAK2 (−6.4 kcal/mol), supporting their functional relevance in mediating toxic effects. This study reveals that prilocaine-induced methemoglobinemia is driven by a multi-target and multi-pathway mechanism, primarily involving cytochrome P450-mediated metabolic activation, oxidative stress generation, and downstream intracellular signaling dysregulation. The identified molecular targets provide mechanistic insight into prilocaine toxicity and may serve as potential biomarkers or therapeutic targets for prevention and management of drug-induced methemoglobinemia.

Keywords:

Prilocaine, Methemoglobinemia, Network Toxicology, Molecular Docking, CYP2C19, JAK2, CASR, Oxidative Stress, Cytochrome P450, Computational Toxicology

1. INTRODUCTION

Methemoglobinemia is a serious haematologic disorder. Under regular conditions, the iron present in hemoglobin is bivalent (Fe⁺⁺). By oxidizing the iron in hemoglobin,

methemoglobinemia occurs and becomes triad (Fe⁺⁺⁺). Physiologically normal Methemoglobin (MetHb) level in the blood is less than 1% and when it exceeds more than 10-15%, cyanosis occurs. If the level exceeds 35%, systemic symptoms such as weakness, tachycardia, respiratory distress, nausea and vomiting develop due to tissue hypoxia; lethargy, stupor and syncope occur at more than 55% [5]. It is lethal when left non-treated with levels above 70%. Prilocaine is a local anesthetic causing methemoglobinemia [1]. The rapid onset and short duration of action make it a common medication to be used in mild invasive procedures, like cardiac pacemaker implantation. Nevertheless, regardless of advantages of prilocaine, at the doses near or above the recommended maximum 600mg, which lead to hepatic metabolite o-toluidine causing methemoglobinemia [2]. The diagnosis of methemoglobinemia is established by measuring MetHb levels in the blood. Management approaches include the use of methylene blue, ascorbic acid and N-acetylcystein. One mechanism of action of methylene blue is through an increase in NADPH-dependent MetHb reductase pathway: therefore, effectively maintaining lower MetHb levels [3]. Methemoglobinemia is a common disorder that can stem from many causes including: hemoglobinopathies, hereditary enzyme deficiencies (NADH MetHb reductase), and exposure to drugs. Notably, hemoglobinopathies and inherited enzyme defects (NADH MetHb reductase) are rare causes; drug exposure is the most common [4]. Methylene blue infusion is the first line treatment of choice in drug induced methemoglobinemia. Patients with methemoglobin > 30% should be treated, preferably with methylene blue, even if they are asymptomatic; consider treatment at a level of 20% in symptomatic patients [5]. It acts on sodium channels located on the neuronal membrane and reduces the spread of seizure activity and propagation. The therapeutic action of the prilocaine on a nerve system is due to its ability to stabilize the neuronal membrane by preferentially binds to and inactivates sodium channel activity. Justly, the plasma and its metabolism after quickly tissue uptake actions are generalized features of prilocaine that turned it into a well-suited anesthetic agent in intravenous regional anesthesia [6]. Network toxicology, an overarching method, which builds upon network pharmacology [3], integrates bioinformatics with high-throughput data obtained from genomics, proteomics and metabolomics to assess systematically the effects of environmental toxicants worldwide [7]. This study is a combined effort of network toxicology and molecular docking to ameliorate the knowledge gaps regarding Prilocaine -induced Methemoglobinemia molecular mechanisms. Network toxicology predicts potential mechanisms of Prilocaine toxicity, while molecular docking simulates the binding stability of Prilocaine to target proteins. The

integrated analysis aims to elucidate the molecular interactions of Prilocaine and Methemoglobinemia to offer novel perspectives on its underlying mechanisms, which may further contribute to a deeper understanding of its potential toxicological ramifications.

2. METHODOLOGY

2.1 BASELINE NETWORK ANALYSIS OF PRILOCAINE TOXICITY

To predict the toxicological profile of Prilocaine, a combination of network-based search algorithms and computational toxicity prediction tools was employed. Specifically, ProTox 3.0 was used as an initial screening platform. These tools enabled the evaluation of potential toxicity endpoints based on the molecular structure of prilocaine, providing preliminary insights into its pharmacokinetic behavior and toxicological properties.

2.2 COLLECTION OF PRILOCAINE-ASSOCIATED TARGETS

The chemical structure and SMILES notation of prilocaine were retrieved from the PubChem database using the keyword “prilocaine.” The three-dimensional (3D) structure is presented in Fig.1. Potential molecular targets were subsequently predicted using the SMILES representation of prilocaine and queried against SwissTargetPrediction, applying a probability threshold > 0. The results obtained from all sources were integrated to construct a comprehensive library of potential molecular targets associated with prilocaine.

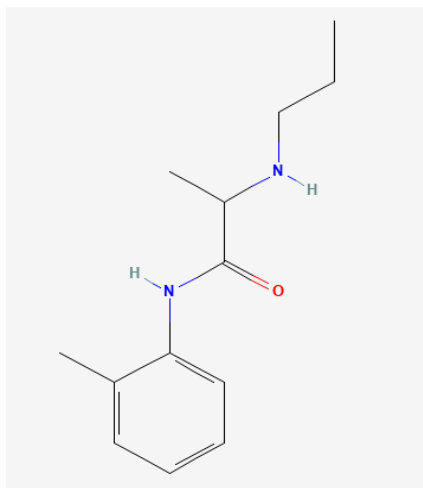


Fig.1. Chemical structure of Prilocaine

2.3 IDENTIFICATION OF METHEMOGLOBINEMIA-RELATED TARGETS

Potential molecular targets associated with Methemoglobinemia were identified using three widely recognized bioinformatics platforms: GeneCards, DisGeNET, and Ensembl. The keyword “methemoglobinemia” was used to retrieve relevant targets. Data from all three databases were integrated, and duplicate entries were removed to generate a non-redundant and unified gene set. This curated dataset represents

genes associated with prilocaine-induced methemoglobinemia through genetic, biochemical, or functional evidence and served as the foundation for subsequent network construction and pathway enrichment analyses.

2.4 PROTEIN-PROTEIN INTERACTION (PPI) NETWORK ANALYSIS

Common targets between prilocaine and methemoglobinemia were identified by intersecting their respective datasets. These overlapping targets, representing potential mediators of prilocaine-induced methemoglobinemia, were further analyzed using the GeneMANIA database to construct protein-protein interaction (PPI) networks and identify functionally associated genes.

2.5 FUNCTIONAL CHARACTERIZATION AND PATHWAY ENRICHMENT ANALYSIS

To elucidate the biological significance of the identified targets, functional enrichment analyses were performed using the STRING database. Gene Ontology (GO) analysis was conducted to classify targets into biological processes (BP), molecular functions (MF), and cellular components (CC), providing insights into their functional roles. Additionally, pathway enrichment analysis was carried out using the Kyoto Encyclopedia of Genes and Genomes to identify key signaling pathways involved in the molecular mechanisms underlying prilocaine-induced methemoglobinemia.

2.6 MOLECULAR DOCKING ANALYSIS OF PRILOCAINE WITH CORE TARGETS

To investigate the molecular interactions between prilocaine and key protein targets implicated in methemoglobinemia, molecular docking studies were performed. The three-dimensional structures of selected target proteins were retrieved from the RCSB Protein Data Bank. Protein structures were preprocessed using PyMOL to remove water molecules and co-crystallized ligands. Further preparation steps, including the addition of hydrogen atoms and calculation of partial charges, were performed using AutoDock Tools (version 1.5.7). Docking simulations were conducted using AutoDock Vina with optimized grid box parameters and search algorithms to predict binding conformations and affinities. The resulting docking poses, binding energy scores, and interaction profiles were visualized and analyzed using Discovery Studio to gain detailed insights into ligand-protein interactions relevant to the mechanism of prilocaine-induced methemoglobinemia.

3. RESULTS

3.1 NETWORK PHARMACOLOGY AND TOXICITY ANALYSIS

The toxicological profile of Prilocaine was evaluated using ProTox 3.0, which predicted a significant association with hematotoxicity, particularly methemoglobinemia, consistent with its known clinical adverse effects. Target prediction using PubChem and SwissTargetPrediction identified a comprehensive set of drug-associated genes, while disease-related targets were

retrieved from GeneCards, DisGeNET, and Ensembl. Intersection analysis revealed three overlapping targets (CYP2C19, CASR, JAK2) showed in Fig.2, representing potential key mediators of prilocaine-induced methemoglobinemia.

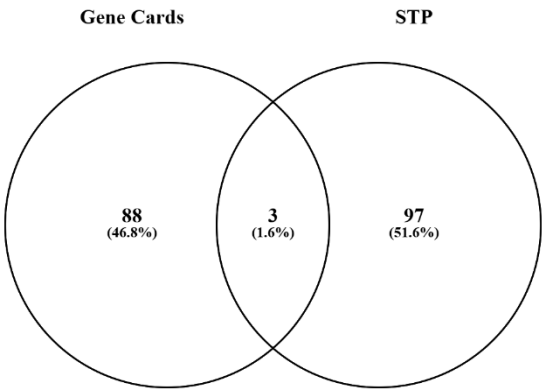


Fig.2. Venn diagram of overlapping targets

3.2 PROTEIN-PROTEIN
NETWORK ANALYSIS

INTERACTION

Protein-protein interaction (PPI) network analysis performed using GeneMANIA demonstrated a highly interconnected network among the overlapping targets and their associated genes. Hub genes such as JAK2, CASR, CYP2C19, and EPHX2 were identified and showed in Fig.3.

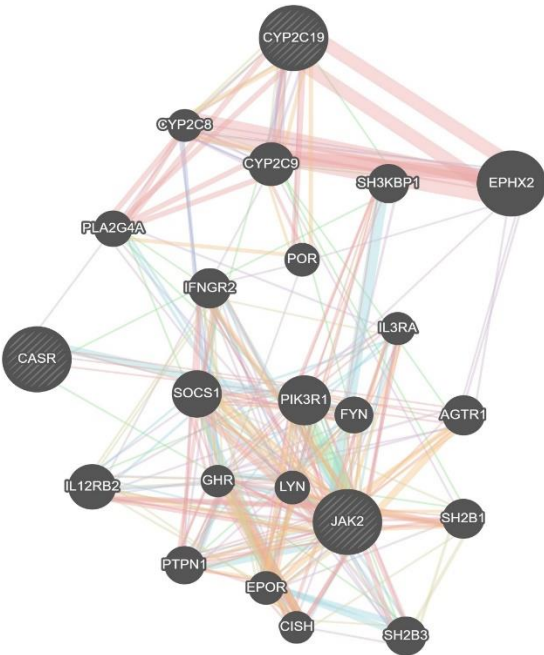


Fig.3. Protein-Protein Interaction (PPI) network analysis

3.3 FUNCTIONAL ENRICHMENT AND PATHWAY ANALYSIS

Functional enrichment analysis performed using STRING revealed that the identified targets were significantly enriched in biological processes related to oxidation-reduction, cellular response to oxidative stress, and drug metabolism (Fig.4). Molecular function analysis demonstrated enrichment in oxidoreductase activity, heme binding, and electron transfer

activity (Fig.5), while cellular component analysis indicated predominant localization in the endoplasmic reticulum and cytosol (Fig.6). Furthermore, pathway enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes identified key pathways, including drug metabolism via cytochrome P450, xenobiotic metabolism, chemical carcinogenesis, and major signaling cascades such as PI3K-Akt and JAK-STAT pathways (Fig.7). These findings collectively suggest that metabolic activation and oxidative stress play a central role in the pathogenesis of prilocaine-induced methemoglobinemia.

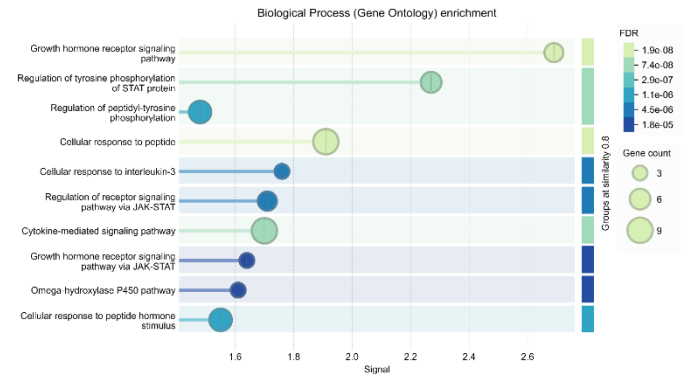


Fig.4. Gene Ontology (GO) enrichment analysis – Biological Process

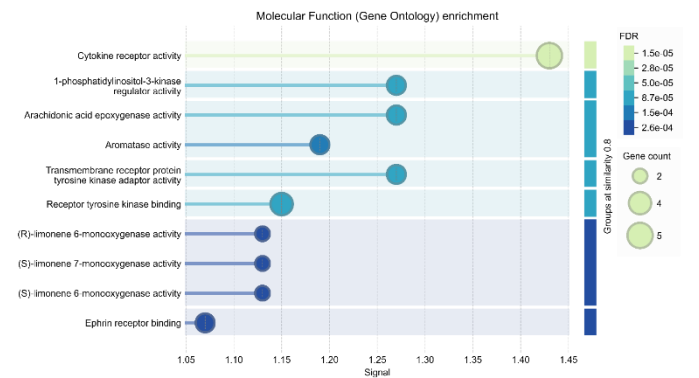


Fig.5. Gene Ontology (GO) enrichment analysis – Molecular Function

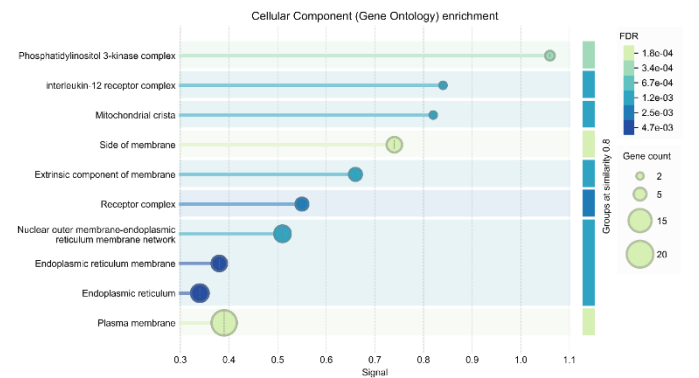


Fig.6. Gene Ontology (GO) enrichment analysis – Cellular Component

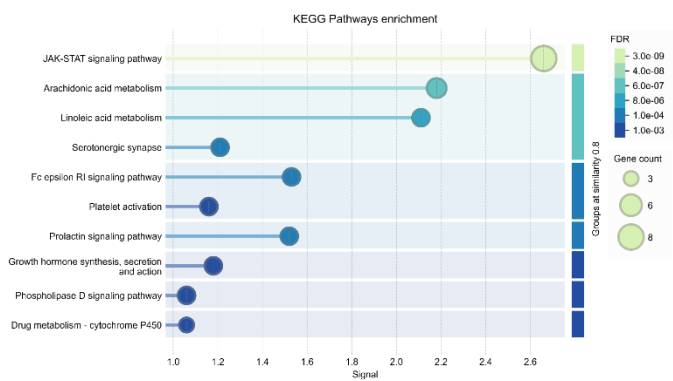


Fig.7. KEGG pathway enrichment analysis

3.4 MOLECULAR DOCKING ANALYSIS

Molecular docking analysis demonstrated stable binding interactions between Prilocaine (CID_4906) and selected core target proteins, including CYP2C19 (PDB ID: 2B7A), CASR (PDB ID: 4GQS), and JAK2 (PDB ID: 5FBH).

Table.1. Favorable binding affinities

S. No.	Protein PDB ID	Compound ID	Docking score
1	2B7A	CID_4906	6.9
2	4GQS	CID_4906	6.8
3	5FBH	CID_4906	6.4

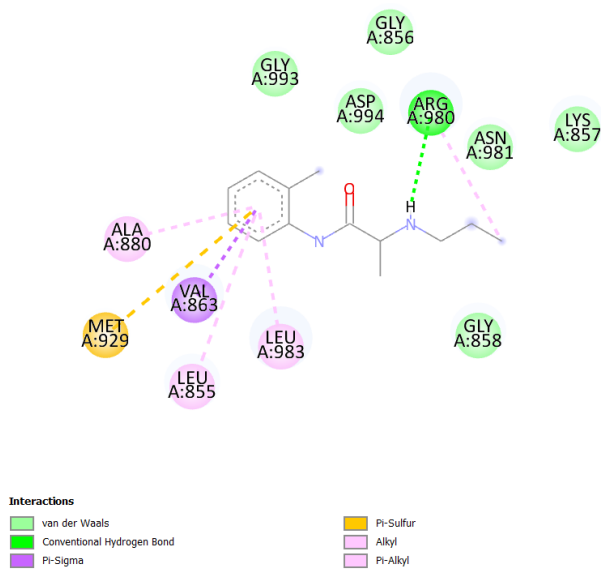


Fig.8. 2D interaction diagram of Prilocaine with CYP2C19

The docking scores obtained were 6.9 kcal/mol (2B7A), 6.8 kcal/mol (4GQS), and 6.4 kcal/mol (5FBH), indicating favorable binding affinities (Table.1). Detailed interaction analysis revealed that prilocaine formed conventional hydrogen bonds, notably with residue ARG980 in CYP2C19, along with multiple van der Waals interactions involving surrounding residues (Fig.8). In addition, significant hydrophobic interactions, including alkyl, pi-alkyl, pi-sigma, and pi-sulfur interactions, were observed with residues such as LEU, VAL, MET, and ALA, contributing to ligand stabilization within the binding pocket. In the CASR and JAK2

complexes, interactions were predominantly stabilized through van der Waals forces and carbon hydrogen bonds, along with pi-pi T-shaped interactions, further supporting stable ligand accommodation (Fig.9 and 10). These findings reinforce the potential mechanistic involvement of CYP2C19, CASR, and JAK2 in mediating prilocaine-induced methemoglobinemia.

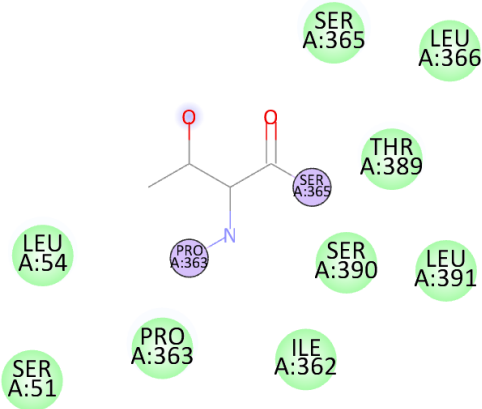


Fig.9. 2D interaction diagram of Prilocaine with CASR

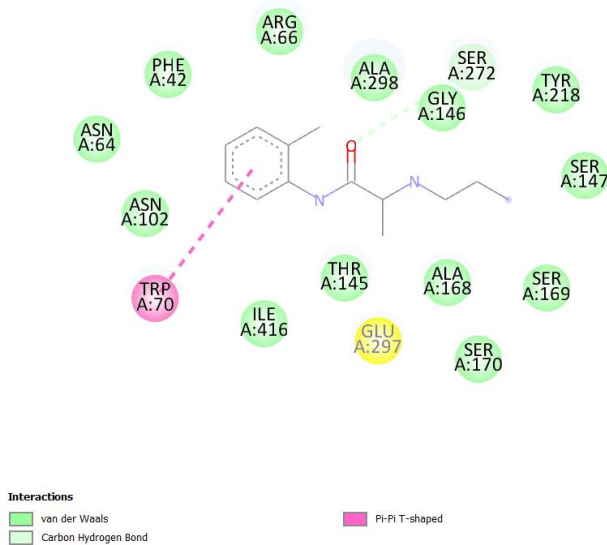


Fig.10. 2D interaction diagram of Prilocaine with JAK2

4. DISCUSSION

The present study employed an integrated network pharmacology and molecular docking approach to elucidate the molecular mechanisms underlying Prilocaine-induced Methemoglobinemia. The toxicity prediction analysis using ProTox 3.0 indicated a strong association between prilocaine and hematological toxicity, particularly methemoglobinemia, which is consistent with its clinically reported adverse effects [8]. This condition is primarily characterized by the oxidation of ferrous iron (Fe²⁺) in hemoglobin to ferric iron (Fe³⁺), leading to impaired oxygen transport and tissue hypoxia [9]. The present

computational findings provide mechanistic insights into how prilocaine may contribute to this pathological process.

Network-based target identification revealed three key overlapping genes—CYP2C19, CASR, and JAK2—suggesting that prilocaine-induced toxicity is mediated through a focused yet biologically significant set of molecular targets. Among these, CYP2C19, a member of the cytochrome P450 enzyme family, plays a critical role in drug metabolism and bioactivation [10]. The enrichment of cytochrome P450-related pathways strongly supports the hypothesis that prilocaine undergoes metabolic activation to form reactive intermediates capable of inducing oxidative stress. Such metabolites are known to oxidize hemoglobin, thereby contributing to methemoglobin formation [11]. This aligns with previous reports indicating that local anesthetics can produce oxidizing metabolites that disrupt normal redox homeostasis [12].

The protein–protein interaction network further highlighted JAK2 and PIK3R1 as central hub genes, indicating their involvement in intracellular signaling pathways associated with oxidative stress and inflammatory responses. Activation of the JAK-STAT pathway has been widely linked to cellular stress signaling and immune modulation [13], suggesting that prilocaine-induced oxidative damage may also trigger downstream inflammatory cascades. Similarly, the involvement of PI3K-Akt signaling implies a role in cell survival and stress adaptation mechanisms, potentially reflecting compensatory cellular responses to toxic insult [14].

Functional enrichment analysis provided additional evidence supporting the role of oxidative stress and metabolic dysregulation in the pathogenesis of methemoglobinemia. The significant enrichment in biological processes such as oxidation–reduction and cellular response to oxidative stress, along with molecular functions including oxidoreductase activity and heme binding, underscores the central role of redox imbalance. Furthermore, the localization of these targets in the endoplasmic reticulum suggests that metabolic enzymes involved in drug biotransformation are key contributors to toxicity [15]. The identification of xenobiotic metabolism and chemical carcinogenesis pathways further reinforces the importance of metabolic activation in generating reactive oxygen species (ROS), which can directly oxidize hemoglobin [16].

Molecular docking analysis provided structural validation of these findings by demonstrating stable interactions between prilocaine and the selected target proteins. The observed binding affinities, particularly with CYP2C19 (6.9 kcal/mol), indicate a strong likelihood of enzymatic interaction and metabolic processing. The presence of hydrogen bonds with residues such as ARG980 and ASP994, along with multiple hydrophobic interactions involving LEU, VAL, and MET, suggests that prilocaine is favorably accommodated within the active site of CYP2C19, facilitating its bioactivation. Similarly, stable interactions with CASR and JAK2 indicate that prilocaine may influence calcium signaling and intracellular kinase pathways, further contributing to its toxicological effects.

Taken together, the findings of this study support a multi-target, multi-pathway mechanism underlying prilocaine-induced methemoglobinemia. The primary mechanism appears to involve cytochrome P450-mediated metabolic activation leading to oxidative stress and hemoglobin oxidation, while secondary

mechanisms may include dysregulation of signaling pathways such as JAK-STAT and PI3K-Akt. These results not only enhance the current understanding of prilocaine toxicity but also highlight potential molecular targets for therapeutic intervention and risk assessment.

However, it is important to acknowledge certain limitations of this study. The findings are based on in silico predictions and computational models, which, although robust, require experimental validation. Future studies involving in vitro and in vivo models are necessary to confirm the identified targets and pathways. Additionally, patient-specific factors such as genetic polymorphisms in CYP enzymes may further influence susceptibility to prilocaine-induced methemoglobinemia and should be explored in future investigations [17].

5. CONCLUSION

This study elucidates the molecular mechanisms underlying Prilocaine-induced Methemoglobinemia using an integrated computational approach. The results indicate that prilocaine toxicity is primarily mediated through cytochrome P450-dependent metabolic activation and oxidative stress generation. Key targets, including CYP2C19, CASR, and JAK2, were identified as critical regulators of this process. Enrichment analysis highlighted the involvement of drug metabolism, xenobiotic pathways, and signaling cascades such as PI3K-Akt and JAK-STAT. Molecular docking confirmed stable binding interactions, supporting the functional relevance of these targets. Collectively, these findings reveal a multi-target and multi-pathway mechanism driving methemoglobinemia. Further experimental validation is required to confirm these computational insights and their clinical significance.

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