Toxicity Profiling and In Silico Evaluation of Embelia ribes Phytochemicals as Human Lysyl Oxidase-Like 2 (hLOXL2) Inhibitors

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Abstract

Human lysyl oxidase-like 2 (hLOXL2) is a copper-dependent enzyme that catalyzes oxidative deamination of lysine residues in extracellular matrix (ECM) proteins. Dysregulated LOXL2 activity is linked with pathological fibrosis, tumor progression and metastasis, and abnormal tissue remodeling. Targeting LOXL2 with selective inhibitors has therapeutic potential, yet clinically approved agents are lacking. Natural products offer rich chemical diversity for drug discovery. Embelia ribes (Vidanga), a medicinal plant with numerous bioactive phytochemicals such as embelin, has documented anti-inflammatory, antioxidant, and anticancer properties. This study performed in silico molecular docking and toxicity profiling of Embelia ribes phytochemicals against hLOXL2 to assess binding potential and pharmacokinetic safety. Embelin demonstrated favorable docking with hLOXL2 (binding energy –6.08 kcal/mol) and acceptable ADME/toxicity profiles, suggesting therapeutic potential. However, experimental validation is essential.

Introduction

The extracellular matrix (ECM) plays a fundamental role in maintaining tissue architecture, mechanical strength, and cellular signaling. Key structural proteins such as collagen and elastin undergo enzymatic cross-linking to preserve tissue integrity and elasticity. This process is primarily catalyzed by the lysyl oxidase (LOX) family of enzymes, which includes LOX and its homologs LOXL1–LOXL4. (1)These copper-dependent amine oxidases facilitate the oxidative deamination of lysine residues, generating reactive aldehydes that form covalent cross-links within

ECM proteins. While this mechanism is essential for normal physiological function, dysregulation of LOX enzymes can lead to pathological consequences.(2)

Among the LOX family members, human lysyl oxidase-like 2 (hLOXL2) has attracted significant attention due to its involvement in various disease processes. Overexpression of hLOXL2 has been strongly associated with tissue fibrosis, including liver, lung, and cardiac fibrosis, where excessive ECM deposition and stiffening impair organ function. In addition, hLOXL2 plays a critical role in cancer progression by promoting epithelial–mesenchymal transition (EMT), a process that enhances tumor cell invasion, migration, and metastasis.(3) Elevated levels of LOXL2 have been correlated with poor prognosis in multiple malignancies, including breast, pancreatic, and hepatocellular cancers. These findings highlight hLOXL2 as a promising therapeutic target for both fibrotic diseases and cancer.(4)

Despite its clinical significance, there are currently no approved selective inhibitors targeting LOXL2, emphasizing the need for novel drug discovery approaches. In recent years, several synthetic inhibitors and monoclonal antibodies have been investigated; however, limitations such as poor specificity, adverse effects, and limited clinical efficacy have hindered their successful translation into approved therapies. Consequently, identifying safer and more effective alternatives remains a priority in pharmaceutical research.(5)

In this context, natural products have emerged as valuable sources of bioactive compounds due to their chemical diversity and pharmacological potential. Medicinal plants, in particular, have historically contributed to the development of therapeutic agents with improved safety profiles and reduced toxicity. Their structural complexity often enables better interaction with biological targets, making them attractive candidates in drug discovery programs.(6)

Embelia ribes, commonly known as Vidanga, is a medicinal plant widely used in traditional systems such as Ayurveda. It contains a variety of phytochemicals, among which embelin is the most prominent bioactive compound. Embelin and related constituents have demonstrated a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Furthermore, previous studies suggest that embelin may modulate signaling pathways involved in apoptosis and cellular proliferation, reinforcing its potential as a therapeutic agent.(7)

Advances in computational biology have enabled the use of *in silico* techniques such as molecular docking, virtual screening, and toxicity prediction to accelerate drug discovery. These approaches allow for the rapid screening of compounds based on their binding affinity, interaction patterns, and pharmacokinetic properties, significantly reducing time and cost compared to conventional experimental methods. Therefore, the present study aims to evaluate the binding potential and

toxicity profile of *Embelia ribes* phytochemicals against hLOXL2 using computational methods, with the goal of identifying promising candidates for further experimental validation.(8)

Materials and Methods

Target Protein Selection

The human lysyl oxidase-like 2 (hLOXL2) enzyme was selected as the target protein due to its involvement in extracellular matrix cross-linking and its association with pathological conditions such as fibrosis and cancer metastasis.

Protein Preparation

The three-dimensional structure of the hLOXL2 protein was obtained from the Protein Data Bank (PDB) and prepared using UCSF Chimera. Water molecules and other unwanted components were removed, and hydrogen atoms were added to stabilize the protein structure for docking analysis.

Ligand Selection

Phytochemicals reported in Embelia ribes were selected for the study. The chemical structures of the compounds were retrieved from the PubChem database using their respective compound identification numbers.

Ligand Preparation

The ligand structures were prepared using ChemDraw and Chem3D software. The two-dimensional chemical structures were converted into three-dimensional conformations and optimized through energy minimization to obtain stable molecular structures for docking studies.

Molecular Docking

Docking simulations between the prepared ligands and the hLOXL2 protein were carried out using the SwissDock web server. The docking process predicted the binding affinity and possible interaction sites between the phytochemicals and the target enzyme.

Toxicity Prediction

The toxicity profile of the selected phytochemicals was evaluated using computational toxicity prediction tools. The parameters analyzed included AMES mutagenicity, hERG I inhibition, hERG II inhibition, oral rat acute toxicity (ORAT), hepatotoxicity (HT), skin sensitization (SS), and minnow toxicity (MT). These parameters were used to assess the potential toxicological risks and safety profile of the compounds.

Data Analysis

The predicted toxicity values were analyzed to identify compounds with lower toxicity risks and better safety profiles. Compounds showing minimal mutagenicity, cardiotoxicity, and hepatotoxicity were considered safer candidates for further pharmacological investigations.

Results

TOXICITY TABLE

	LIGAND	AMES◊	hERG I□	hERG II●	ORAT♦	HTº	SS**	MT***
1	aloeemodine	yes	no	no	2.329	no	no	2.337
2	amentoflavone	no	0.438	no	2.527	no	no	2.685
3	astragalin	no	no	no	2.546	no	no	6.735
4	azelaic acid	no	no	no	1.355	no	no	1.601
5	boldine	no	no	yes	2.444	no	no	0.73
6	caffeic acid	no	no	no	2.383	no	no	2.246
7	Catechol	no	no	no	2.14	no	no	2.194
8	Chlorogenic acid	no	no	no	1.973	no	no	5.741
9	daidzin	no	no	yes	2.738	no	no	3.902
10	dihydrokaemoferol	no	no	yes	2.38	yes	no	0.987

11	domesticine	no	no	yes	2.38	yes	no	0.987
12	ellagic acid	no	no	no	2.399	no	no	2.11
13	embline	no	no	no	1.72	no	yes	0.251

The toxicity profile of the selected phytochemicals was evaluated using computational toxicity prediction models to determine their safety and potential toxicological risks. The parameters analyzed included AMES mutagenicity, hERG I and hERG II inhibition, oral rat acute toxicity (ORAT), hepatotoxicity (HT), skin sensitization (SS), and minnow toxicity (MT). The predicted toxicity values for all compounds are summarized in Table X.

The AMES toxicity test, which predicts mutagenic potential, indicated that most of the phytochemicals were non-mutagenic, suggesting a lower probability of causing genetic mutations. However, aloeemodin showed a positive AMES toxicity prediction, indicating a possible mutagenic risk compared with other compounds.

Cardiotoxicity was assessed through hERG I and hERG II inhibition, which are important indicators of potential cardiac arrhythmia risk. The majority of the compounds did not show inhibition of the hERG I channel, suggesting minimal cardiotoxic risk. Nevertheless, a few compounds including boldine, daidzin, dihydrokaempferol, and domesticine showed hERG II inhibition, indicating a moderate possibility of cardiac-related effects that would require further experimental validation.

The oral rat acute toxicity (ORAT) values represent the predicted lethal dose levels and provide an estimate of acute toxicity. Most compounds exhibited moderate toxicity values, indicating acceptable safety ranges for potential pharmacological use. Compounds such as azelaic acid and embelin demonstrated comparatively lower toxicity values, suggesting better safety profiles.

Evaluation of hepatotoxicity (HT) revealed that most of the screened phytochemicals were predicted to be non-hepatotoxic, indicating a low risk of liver damage. However, dihydrokaempferol and domesticine showed positive hepatotoxicity predictions, suggesting that these compounds may require careful evaluation in further studies.

The skin sensitization (SS) parameter assesses the potential of compounds to cause allergic skin reactions. Most compounds did not exhibit sensitization effects, while embelin showed a positive prediction, indicating a possible mild skin sensitivity response.

Environmental toxicity was evaluated using minnow toxicity (MT), which predicts the potential harmful effects of compounds on aquatic organisms. The predicted values varied among the compounds, with astragalin and chlorogenic acid showing relatively higher toxicity values, whereas embelin demonstrated comparatively lower environmental toxicity.

Discussion

Molecular Interactions Between Embelin and hLOXL2

The docking results demonstrate that embelin engages with hLOXL2 through a combination of hydrogen bonding and hydrophobic interactions. Hydrogen bonds are crucial for ligand specificity and stability, especially when formed with catalytically relevant residues. The observed distance

of 3.42 Å falls within acceptable interaction length for moderate hydrogen bonding, suggesting relevant contact.(9)

Hydrophobic interactions with nonpolar residues in the active site contribute to the overall binding stability. The benzoquinone core of embelin appears to fit well within the LOXL2 active pocket, potentially interfering with substrate access or catalytic function.

Pharmacokinetic Implications

ADME analysis suggests that embelin has drug-like properties, including acceptable predicted absorption and minimal issues with solubility. Embelin's predicted compliance with Lipinski's criteria supports its potential for oral administration. Its moderate lipophilicity could enhance membrane permeation without excessive plasma protein binding.(10)

However, in silico ADME predictions are preliminary and require empirical verification, such as Caco-2 permeability assays and metabolic stability tests.

Safety Profile Considerations

The absence of predicted AMES toxicity and hepatotoxicity increases embelin's attractiveness for further development. Hepatotoxicity is a common cause of drug failure in later stages, so early evidence of a lack of liver toxicity is promising. Similarly, the absence of significant hERG inhibition suggests a lower risk of cardiac arrhythmias, a critical safety parameter in drug development.

Mild skin sensitization may affect topical formulations but does not preclude systemic use. Further testing in dermal and systemic toxicity models is needed.

Therapeutic Relevance of LOXL2 Inhibition

LOXL2 inhibitors may exert therapeutic effects in conditions characterized by excessive ECM stiffness and remodeling. In fibrosis, LOXL2 contributes to pathological collagen cross-linking, which leads to tissue stiffening and dysfunction. Suppressing LOXL2 activity could slow fibrosis progression, reduce ECM rigidity, and improve organ function.(11)

In cancer, LOXL2 facilitates EMT, tumor invasion, and metastasis by remodeling the tumor microenvironment. Inhibiting LOXL2 could reduce metastatic potential and improve responses to chemotherapy and immunotherapy.

Comparison with Other Natural LOX Inhibitors

Natural polyphenols like quercetin, curcumin, and resveratrol have demonstrated inhibitory effects on LOX enzymes, albeit with varying degrees of potency. These compounds share common structural features that support interaction with enzyme active sites and exert antioxidant effects. Embelin, while structurally distinct as a benzoquinone derivative, displays similar bioactive properties and potential for enzyme inhibition.(12)

Although embelin's binding energy is moderate compared to some synthetic inhibitors, its favorable safety profile and natural origin may provide advantages in initial drug development phases.(13)

Conclusion

The present study evaluated the toxicity profile of selected phytochemicals from Embelia ribes using in silico toxicity prediction models. The analysis included parameters such as AMES mutagenicity, hERG channel inhibition, oral rat acute toxicity, hepatotoxicity, skin sensitization, and minnow toxicity in order to assess the safety and toxicological risks of the compounds.

The results indicated that the majority of the phytochemicals exhibited low toxicity and acceptable safety profiles. Most compounds were predicted to be non-mutagenic and non-hepatotoxic, suggesting a reduced risk of genetic damage and liver toxicity. Although a few compounds showed potential cardiotoxicity signals through hERG II inhibition and mild hepatotoxicity predictions, these findings require further confirmation through experimental studies. In comparison, compounds such as embelin, azelaic acid, and caffeic acid demonstrated relatively safer toxicity profiles with minimal predicted adverse effects.

Overall, the computational toxicity assessment suggests that several phytochemicals from Embelia ribes possess favorable safety characteristics, supporting their potential for further pharmacological development. However, additional in vitro and in vivo toxicological investigations are necessary to validate these predictions and ensure their safety for therapeutic applications.

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