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Screening Hepatoprotective Effective Components of *Leonotis nepetifolia* Root Based on the Molecular Docking and its Mechanism Exploring

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Abstract

Background: There is a relatively high prevalence of liver illnesses since the liver is one of the organs most prone to be harmed by interaction with xenobiotics (drugs, alcohol, drug misuse, environmental pollutants, and others). Worldwide and in India, high death rates are associated with cirrhosis, fatty liver, chronic hepatitis, and cancer. One of the most prevalent malignant diseases in humans and the second greatest cause of cancer-related death worldwide, liver cancer is a serious issue, particularly in less developed areas. Different experimental models have been developed to ascertain the mechanisms by which liver lesions arise in light of the rising frequency of liver illnesses. The plant species in the genus Leonotis and family Lamiaceae known as *Leonotis nepetifolia*, commonly called ***Klip dagga***, Christmas candlestick, or lion's ear, has a variety of pharmacological effects. ***Method:*** The primary LNR compounds were docked against the *Caspase-3* enzyme using computational methods in the current experiment. The Auto Dock software used a grid-based docking algorithm to determine the bond. Using the Merck Molecular Force Field, 2D structures of compounds were created, transformed to 3D, and then energetically decreased up to an arms gradient of 0.01. (MMFF). ***Results:*** *LNR* found to be effective hepatoprotective agent and their lead molecules effectively binds to be target protein ***caspase-3*** enzyme with binding energy -4.92 & -4.09 kcalmol^{-1} for chlorogenic acid & gallic acid respectively. ***Conclusion:*** A computationally based docking investigation revealed that both lead compound (chlorogenic acid and gallic acid) has potent ***caspase-3*** inhibitory properties. Both compounds have same covalent interaction at Phe^{128} & Met^{61}. The outcomes showed a promising docking score and a pattern of strong covalent interaction between the lead chemical and the target protein's active region. Gallic acid and chlorogenic acid work together synergistically to induce hepatoprotection in the ethanolic root extract from *L. nepetaefolia*.

Keywords: Hepatoprotective, *Leonotis nepetaefolia* root (LNR), *In-silico* molecular docking, ***caspase-3,*** Gallic acid and chlorogenic acid.

INTRODUCTION

The body's many physiological processes depend on the liver, which is the most significant organ in this regard. It plays a role in metabolism, secretion, and storage, among other essential processes. It is essential for the excretion and detoxification of numerous foreign and endogenous substances. Therefore, any damage to it or impairment of its function has serious consequences for the affected person's health. Although viral infection is one of the main causes of hepatic damage, over 18,000 people are reported to die each year as a result of liver cirrhosis brought on by hepatitis [1]. The presence of inflammatory cells in the liver's tissue distinguishes hepatitis, an inflammation of the organ. Types A, B, C, D, and E relate to the five basic types of viruses. The weight of illness and mortality is particularly concerning for these five categories. The ailment may self-limit (heal on its own) or it may worsen, leading to cirrhosis and fibrosis. Hepatitis can manifest with few or no symptoms, but it frequently results in anorexia (low appetite), jaundice, and general malaise. Acute hepatitis is defined as lasting less than six months, while chronic hepatitis lasts longer. Parasites, viruses, autoimmune illnesses, peroxidized fatty acids, fungal toxins, industrial pollutants, radioactive isotopes, alcohol, herbal remedies, and pharmaceuticals are all examples of xenobiotics that can cause hepatic problems. Particularly, types A and C are the most prevalent causes of cancer and liver cirrhosis, and they also cause chronic

disease in hundreds of millions of people [2-3]. In India, using herbal items to treat illnesses has a long history, dating back to the alternative European and Chinese medical systems of ancient times as well as Ayurvedic medicine. Hepatoprotective medications have a substantial source in medicinal plants. A number of liver ailments have reportedly been treated with more than 700 mono- and polyherbal medicines in the form of decoction, tincture, and pills. By carefully combining the benefits of the conventional medical system with the contemporary idea of evidence-based therapeutical screening, authentification, and randomised placebo-controlled clinical trials to support clinical efficacy, the 21st century has seen a paradigm shift toward the therapeutic evaluation of herbal products in liver disease models. There have been several claims that various plants and preparations offer hepatoprotective properties [4-5].

Leonotis nepetifolia is also known as Klip dagga, Christmas candlestick, or lion's ear, is a species of plant in the genus Leonotis and the family Lamiaceae (mint) [6].

Phytochemistry

Alkaloids, Labdane diterpenes, Flavonoids, Iridoid glycosides. Labdane diterpenes Leonotinin,Methoxy nepetaefolin ,Nepetaefolinol ,Nepetaefuran,Nepetefolin, Leonotin, Bis-spirolabdane Diterpenoids I. Leonepetaefolin A II. Leonepetaefolin B III. Leonepetaefolin C IV. Leonepetaefolin D V. Leonepetaefolin E VI. 15-epi-leonepetaefolin A VII. 15-epi-leonepetaefolin B VIII. 15-epi-leonepetaefolin C IX. 15-epi-leonepetaefolin D X. 15-epi-leonepetaefolin E. *Leonotis nepetifolia* whole plant contains labdane diterpenoid characterized as 8β,17:9,13-diepoxylabdane-16,15:19, 6β dilactone. Coumarin characterized as 4,6,7-trimethoxy-5-methyl chromen 2-one, nepetaefolinol and leonotinine. Leaves contain labdane diterpene - nepeteafolin, methoxynepetae folin. Leonotis nepetifolia may contain morin, apigenin, 3, 6 - dihydroxy flavones, p-Coumaric acid, Caffeic acid, Kaempferol, 3, 7-Dihydroxyflavone, Galangin, Naringenin, 6-Hydroxyflavone, O-Coumaric acid and Flavone [7].

Biological Activities [7]

This plant exhibited various biological activities such as antifungal and antibacterial, antioxidant, antimalarial, antidiabetic, antitumor, antiemetic, hepatoprotective.

Medicinal uses: Every part of the plant is medicinally used. The plant is claimed to be used in pain, inflammation, microbial infection, as contraceptives and gynecological disorders [8].

Pharmacological Activity [9]

The numerous pharmacological potentials are antioxidant, antidiabetic, anticancer, antimicrobial, wound healing, antidiarrheal & anti-inflammatory.

Experimental work

***In-Silico* Molecular Docking Validation**

As per literature survey of both the plant, the root ethanolic extract contained following active phytochemicals [9]:

EELNR: Ethanolic root extract of plant contained polyphenol like gallic acid and chlorogenic acid which was selected as bioactive compound for *in-silico* molecular docking study.

Molecular docking studies

Ligand Preparation:

2D Structure of ligands like chlorogenic acid and gallic acid were drawn using ChemSketch [10], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

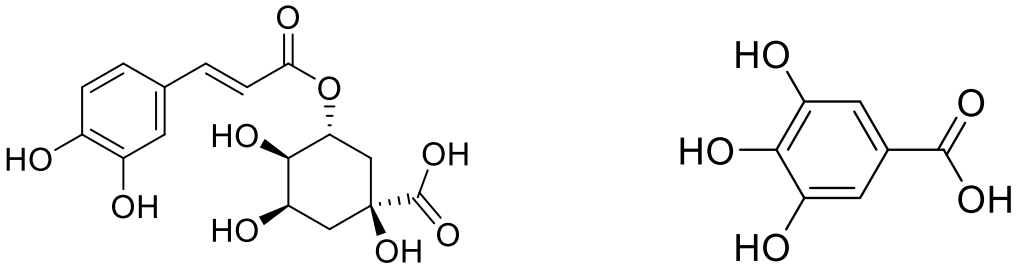


Fig. 1: 2D structure of chlorogenic acid and gallic acid

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points is given in table 1 [11].

Table 1: Grid parameters used in current docking analysis of CASPASE-3

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	CASPASE-3	40	40	40	0.375	36.357	38.829	32.088

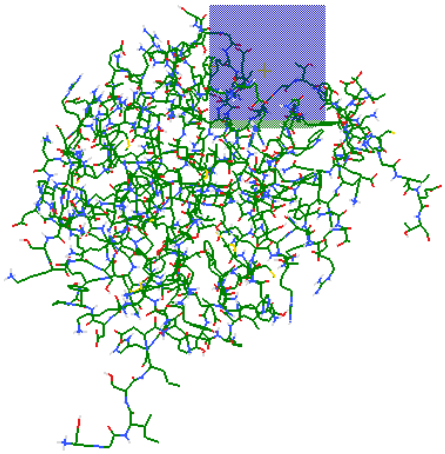


Fig. 2: Grid box covering all active sites in CASPASE-3 receptor

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [12].

Docking Study

Crystal structure

The crystal structure of the protein consisting of CASPASE-3 receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (2xyg.pdb) registered in the Protein data bank was used [13]. The complex ligand was separated by using Chimera software.

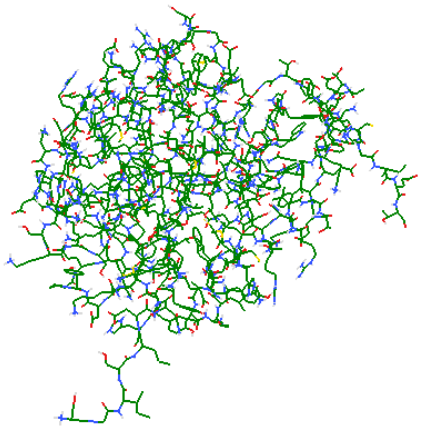


Fig. 3: Crystal structure of CASPASE-3 receptor (PDB ID-2xyg)

Processing of Protein

The downloaded receptor protein is having only one chains, i.e. chain A, which has been selected for experimental purpose and complex ligand was removed from it. The bound ligand was separated from the macromolecular complex by using software Chimera [14].

Molecular Docking Simulation Studies

Docking of ligands like chlorogenic acid and gallic acid against CASPASE-3 receptor was performed by Autodock. All the bonds of each ligand were kept flexible, while no residues in receptor were made flexible [15-16].

Toxicity & ADME-T Studies

The ligand molecules *viz.* chlorogenic acid and gallic acid were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [17-19].

RESULT & DISCUSSION

The first selection of *L. nepetaefolia* was made because of the plant's availability and potential for medicinal usage. The root was consequently taken into account for further study as a significant source of active plant components. The scientific validation of the current investigation was done by computational based molecular docking study of lead molecules of *LNR* against ***Caspase-3*** enzyme. In the family of cysteine-containing aspartate-specific proteases known as caspases, caspase-3 is one of the most significant apoptotic proteases. It can be triggered by the upstream initiator and operate on the particular substrate, causing morphological changes in the cells that eventually lead to apoptosis, as it is a downstream effector in the cascade reaction. The cytoplasmic form of caspase-3 normally exists as an inactive zymogen. A death complex is created when tumour necrosis factor (TNF) and Fas-associated death domain (FADD) join. This complex activates caspase-2, caspase-8, and other enzyme sources upstream before activating caspase-3 downstream through a process known as transactivation. Caspase-3 controls cell death by cleaving structural and regulatory proteins in the nucleus and cytoplasm. Caspase-3 inhibitors are a key focus of hepatoprotective medication research in the clinic [10].

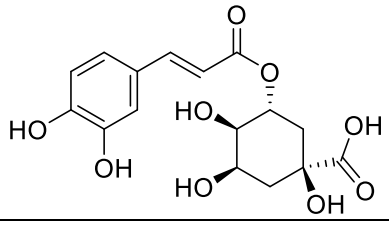
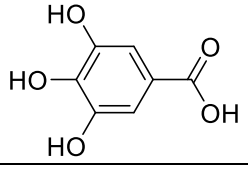
On the Caspase-3 enzyme, we conducted an *in-silico* computational aid screening of the phytoconstituents gallic acid and chlorogenic acid. To better understand the mechanism behind LF's hepatoprotective effects, the interaction between the two chemicals and ***caspase-3*** can be investigated.

LNR found to be effective hepatoprotective agent and their lead molecules effectively binds to be target protein ***caspase-3*** enzyme with binding energy -4.92 & -4.09 kcalmol^{-1} for chlorogenic acid & gallic acid respectively. The result was tabulated in table 2. The grid parameter used in current docking analysis of caspase-3 was showed in table 1 & fig.3.The binding mode of selected lead molecules showed in fig.4-5. The 2D and 3D interaction of selected compound displayed in fig.6-11. The interaction of chlorogenic acid & gallic acid with active site at ***caspase-3*** enzyme showed as follows:

Compound	Conventional Hydrogen bounding	Pi-sigma bounding	Covalent bounding	Week Vander's interaction
Chlorogenic acid	ARG^{207} THR^{59}, GLY^{122}	SER^{205}	PHE^{128} MET^{61}	GLY^{60}, ARG^{64}, HIS^{121}, THR^{62}, THR^{204}, TRY^{286}
Gallic acid	GLY^{122} THR^{62}	HIS^{121}	PHE^{128} MET^{61}	CYS^{163} GLU^{123}

The pharmacokinetic profile reveals that it is having good pharmacokinetic profile but with the presence of any major toxic effects including mutagenicity, tumorogenicity and reproductive effects. The pharmacokinetic and toxicity profiling results of ligands like gallic acid & chlorogenic acid were shown in figure 12-13. Theoretically, all the ligand molecules have shown encouraging docking score.

Table 2: Results of docking of ligands like chlorogenic acid and gallic acid against CASPASE-3 receptor

Sl. No	Compound Name	Structure	Binding Energy (Kcal/mole)
1	Chlorogenic acid		-4.92
2	Gallic acid		-4.09

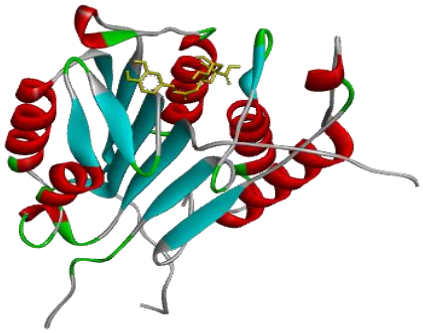


Fig. 4: Binding mode of chlorogenic acid within the active site of CASPASE-3 receptor

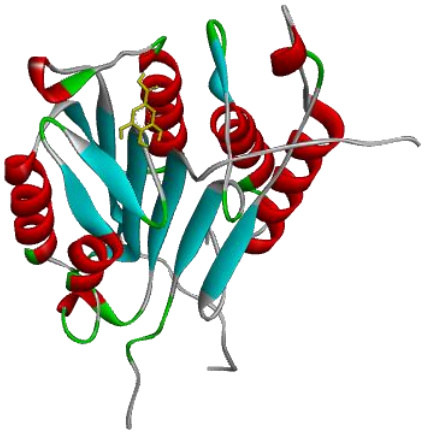


Fig. 5: Binding mode of gallic acid within the active site of CASPASE-3 receptor

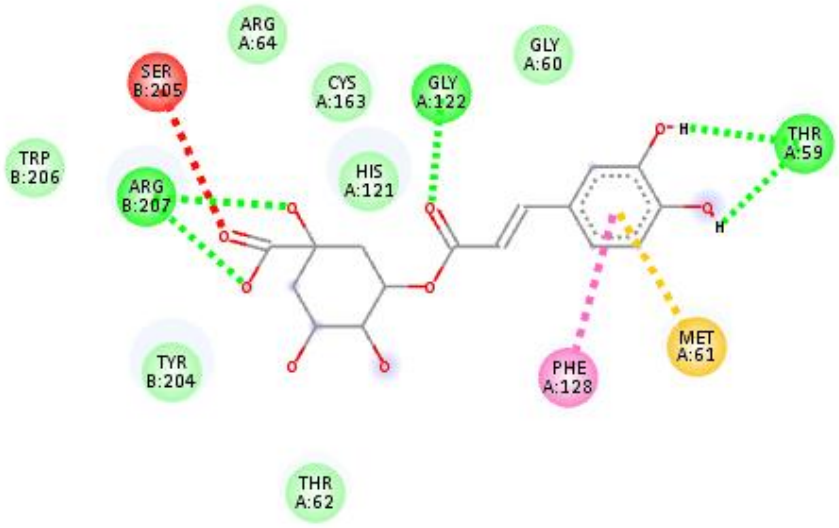


Fig. 6: Two-dimensional binding mode of chlorogenic acid within the active site of CASPASE-3 receptor

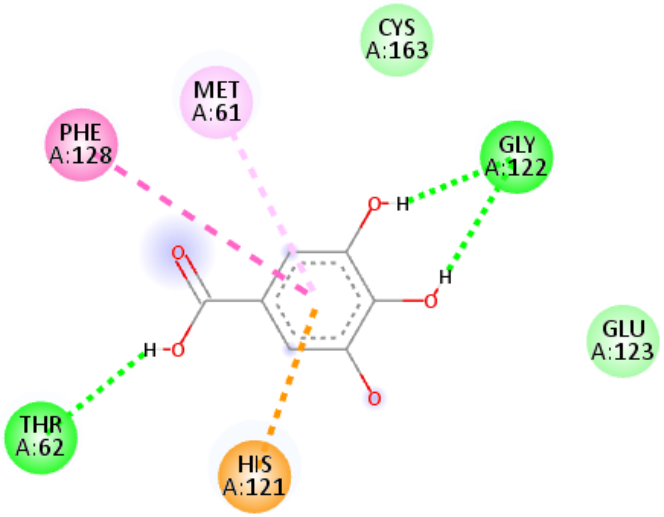


Fig. 7: Two-dimensional binding mode of gallic acid within the active site of CASPASE-3 receptor

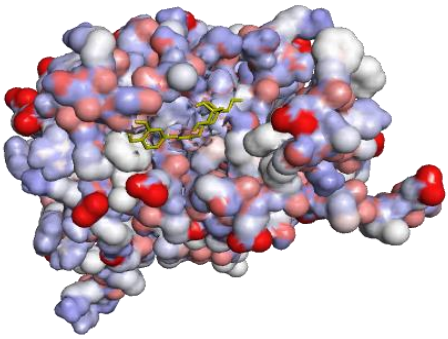


Fig. 8: Three-dimensional binding conformation of chlorogenic acid within the active site of CASPASE-3 receptor

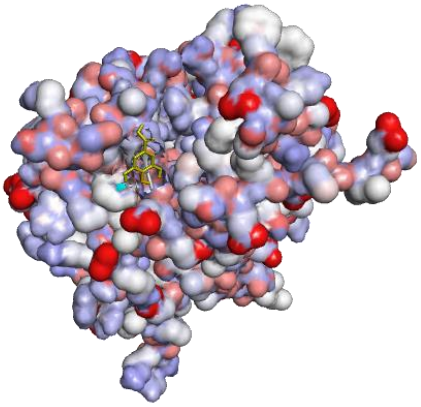


Fig. 9: Three-dimensional binding conformation of gallic acid within the active site of CASPASE-3 receptor

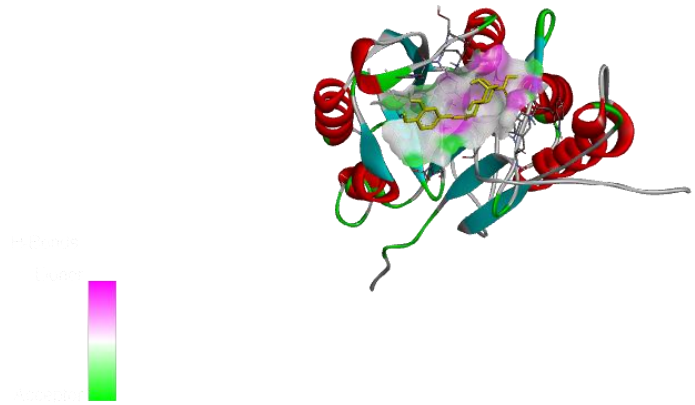


Fig. 10: Three-dimensional binding mode of chlorogenic acid within the active site of CASPASE-3 receptor

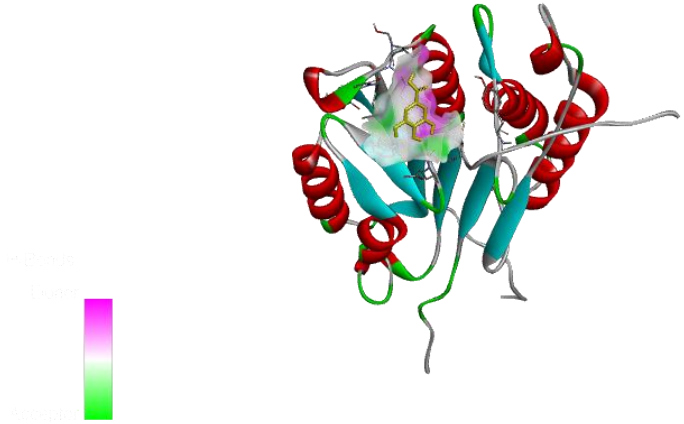


Fig. 11: Three-dimensional binding mode of gallic acid within the active site of CASPASE-3 receptor

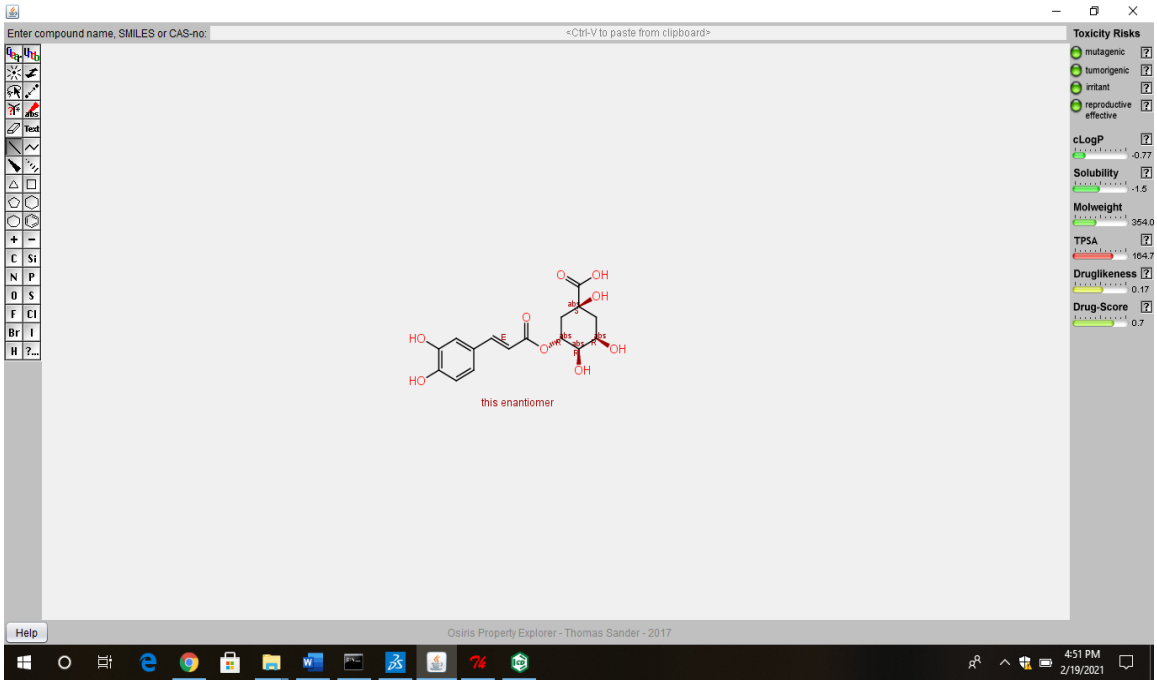


Fig. 12: Pharmacokinetic and toxicity profiling of chlorogenic acid.

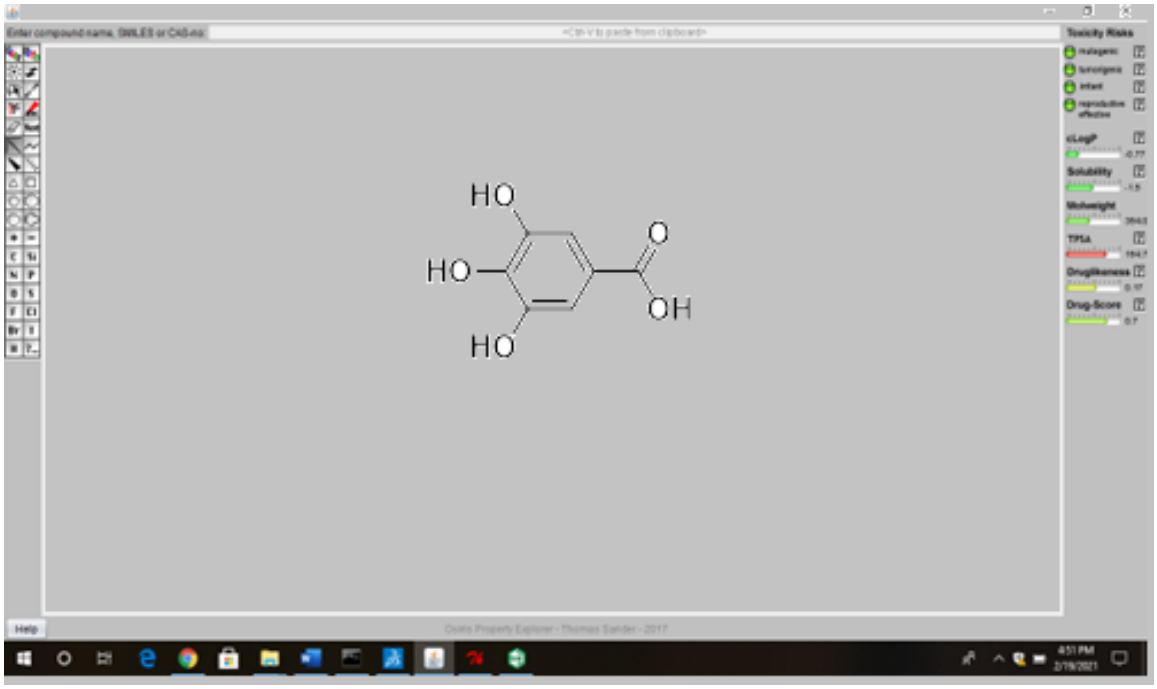


Fig. 13: Pharmacokinetic and toxicity profiling of gallic acid

CONCLUSION

Botanicals have long been an important part of the treatment of hepatotoxicity, even if modern science still has a limited knowledge of the molecular basis of these medications. To fully comprehend the molecular basis of herbal treatments for wound healing, more study is required. The most important finding is that research into the growing conditions, postharvest handling, and pharmacological preparation of these botanicals has supported their significance in terms of the chemical composition and pharmacological activity of the finished product used in pre-clinical and clinical studies.

Because of its antibacterial, anti-inflammatory, antioxidant, and analgesic properties, *Leonotis nepetifolia* (L.) R. Br, also known as dagga, klip dagga, or lion's ear, has been used to successfully cure a number of diseases and other health issues for a very long time. According to numerous studies, the secondary metabolites that make up *L. nepetifolia*, including its alkaloids, phenolics, flavonoids, tannins, steroids, glycosides, coumarins, anthocyanins, and saponins, are responsible for these biological functions. Evidence-based ethnopharmacological applications of *L. nepetifolia* include the treatment of cancer, diabetes mellitus, rheumatoid arthritis, diarrhoea, skin conditions, malaria, burns, and bronchial asthma. Although *L. nepetifolia* has a great deal of potential to treat many ailments, more isolation and identification of its medicinal phytochemical components is still needed. Additionally, the effectiveness and safety of its extracts and phytochemicals should be thoroughly investigated in preclinical and clinical trials in order to make novel treatments.

One of the most crucial organs in the body, the liver regulates many different bodily functions, with a focus on the metabolism, secretion, storage, and detoxification of both endogenous and foreign chemicals. Hepatic illnesses continue to be one of the biggest risks to public health because of these functions, and they are still a concern everywhere in the globe. Despite significant advancements in modern medicine, there are no fully effective medications that boost hepatic function, provide whole organ protection, or aid in the regeneration of hepatic cells. To find more effective and less harmful pharmaceutical alternatives for the treatment of liver illnesses, it is therefore vital to identify these alternatives. Numerous scientific studies have revealed that the use of specific plants and the consumption of particular fruits have played fundamental roles in the care of human health, and that the presence of specific chemical compounds in those plants and fruits is what is responsible for those plants' and fruits' beneficial effects.

In this work, an ethanolic extract from an *L. nepetaefolia* root bioactive are used to treat hepatotoxicity is evaluated for possible efficacy, safety, and molecular modelling against the ***caspase-3*** enzyme. A computationally based docking investigation revealed that both lead compound (chlorogenic acid and gallic acid) has potent ***caspase-3*** inhibitory properties. Both compounds have same covalent interaction at Phe^{128} & Met^{61}. The outcomes showed a promising docking score and a pattern of strong covalent interaction between the lead chemical and the target protein's active region. Gallic acid and chlorogenic acid work together synergistically to induce hepatoprotection in the ethanolic root extract from *L. nepetaefolia*.

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