Original Research Article

Molecular docking and in silico ADMET studies of silibinin and glycyrrhetic acid anti-inflammatory activity

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Abstract

Purpose: To use in silico docking analysis and ADMET prediction of silibinin and glycyrrhetic acid to determine their pharmacokinetic and pharmacodynamic properties as therapeutic molecules against inflammatory disorders.
Methods: The study utilized plant-derived compounds with known anti-inflammatory activity. Three important enzymes, including COX-2, 5β-reductase and phospholipase A2, that are involved in the mediation of inflammatory processes, were chosen as protein targets for the ligands (silibinin and glycyrrhetic acid). Q-Site Finder and admetSAR were employed for active site prediction and ADMET profile, respectively. Furthermore, protein-ligand complexes were visually inspected by LigPlot and Chimera.
Results: Post-docking analysis confirmed strong interaction of silibinin and glycyrrhetic acid with their respective targets. ADMET profiles for both compounds were very promising. Both ligands (silibinin and glycyrrhetic acid) showed strong binding energy for all three target proteins (-7.5 to -10.9 kcal/mol). Moreover, Asp347, Gln350, Gly354, Gln192, His351, Ser579 and Phe580 were the common interacting residues in the target proteins for both ligands.
Conclusion: Glycyrrhetic acid possesses superior ADMET profile to silibinin. Hydrophobic interactions between the two ligands (glycyrrhetinic acid and silibinin) and the three target proteins (COX-2, phospholipase A2 and 5β-reductase) are significant.

Keywords: Silibinin, Glycyrrhetic acid, ADMET, Docking studies, Phospholipase A2, COX-2, 5β-Reductase

INTRODUCTION

Xenobiotics and toxins injure the liver and can result in substantial hepatic pathology. The liver metabolizes and excretes drugs and xenobiotics. All drugs have some side effects, and many affect hepatocytes including cisplatin, tegafur, cyclophosphamide (anti-cancer drugs), nefazodone (an antidepressant), and some diabetes medications [1]. Hepatocytes are also responsible for the excretion of drugs from animals [1]. Drug overdose, toxins, chemotherapeutic agents like acetamide, and a hepatotoxic agent, carbon tetrachloride (CCl_4) can damage hepatocytes and lead to liver inflammation (hepatitis) and cirrhosis [2]. Plants and their extracts have been usedto treat human diseases since ancient time. Among these, some plants have been reported to have additional medicinal value and beneficial characteristics including anti-inflammatory, immune-modulatory and anti-viral actions on hepatoprotective

properties [3-5]. The secondary metabolites of herbal treatments have become more prominent for the treatment of liver disease, with evidence-based outcomes being established through promising clinical trials and validation.

Silybummarianum, commonly known as milk thistle, produces seeds that have medicinal value when ripe [6,7]. Silymarin (SLN), an important secondary metabolite of *Silybummarianum*, consists of a complex mixture of four isomers (flavonolignans): silybin, isosilybin, silydianin, and silychristin. SLN has anti-inflammatory, anti-lipid, immuno-modulatory, anti-oxidative, and hepatocyte-regenerating actions. However, it is not thought to be anti-viral [1,2].*Glycyrrhizaglabra* is a leguminous plant belonging to the Leguminosae family [8]. The root extract of *Glycyrrhizaglabra* contains various chemical compounds of medicinal value including saponin, triterpines, flavonoids, and other chemicals like sugars, coumarins, amino acids, choline, and tannins [4,9,10].Moreover, in Japan, glycyrrhetic acid GLN is used for the management of chronic hepatitis C [11]. Glycyrrhizin metabolism is important because its metabolites inhibit the production of aldosterone and suppress 5-β reductase commonly called hepatic pseudoaldosterone syndrome. There is an inhibition of phospholipase A2 activity which is important in various inflammatory processes. Glycyrrhizin has the ability to interfere with cyclooxygenase and prostaglandin production involved in the progression of inflammatory mechanisms in biological system [18].

In the current study, molecular docking strategy was performed to find out their respective binding energies along with the number of hydrogen bonds and other hydrophobic interactions. The study was further validated by the use of *in silico* ADMET prediction of both compounds (silibinin and glycyrrhetic acid) in order to check their pharmacokinetics and pharmacodynamics properties. These two compounds have already being tested as potent therapeutic compounds in various experimental trials. The compounds are being viewed as potent therapeutic molecules in the management of inflammatory disorders.

EXPERIMENTAL

Target proteins were docked with silibinin and glycyrrhetic acid using AutoDock 4.2 and binding energies were calculated.

Structure retrieval

The three dimensional crystal structures of the three target proteins were retrieved from the Protein Data Bank. Cyclooxygenase-2 (6COX) [12], 5-beta-reductase (3BV7) [13], and phospholipase A2 (2B03) [14] were taken as targets. The ligand molecules, glycyrrhetic acid (GLN) and silibinin (SLB) were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) and were refined using ACD ChemSketch (http://www.acdlabs.com/resources/freeware), a tool that offers functionalities such as structure refining, optimization, etc.

Active site prediction

The active sites of all the three target proteins were identified using Q-SiteFinder [15]. Q-SiteFinder works by binding hydrophobic probes to the protein. It then finds the clusters of probes with most favorable binding region based on energy values. It ranks these clusters according to the sum of total binding energies for individual clusters in the order of likelihood of being a binding site.

Various pharmacodynamic as well as pharmacokinetic parameters were considered including human intestinal absorption (HIA), Caco-2 permeability, aqueous solubility, blood-brain barrier penetration, renal organic cation transportation, cytochrome P (CYP) inhibitory promiscuity, cytochrome P_{450} inhibition, AMES toxicity, fish toxicity, rat acute toxicity, *Tetrahymenapyriformis* toxicity, human ether-a-go-go-related genes inhibition, mutagenic, tumorigenic and reproductive risks (Table 1).

Docking studies

Target proteins were docked with silibinin and glycyrrhetic acid using AutoDock 4.2. The free energy of binding between the ingredients of ligands and proteins were calculated. AutoDock 4.2 uses charge-based desolvation force fields and well defined improved models of the unbound state. Docking analysis attempts to bind the ligand into the obtained binding sites of the target protein and produces the best docked conformations with minimal energy, as the output. Semi-flexible docking protocol was applied, wherein the target proteins were kept rigid while the phytochemical ligands were kept flexible for being docked upon. A 5A° grid was built surrounding the binding pocket. Grid maps dimensions were set as 60 × 60 × 60 points with spacing of 0.375A° to yield the receptor model that included atoms within 0.5A° of the grid center. All the other parameters were kept at default and Lamarckian Genetic Algorithm (LGA) was chosen to predict the best conformers. The protein - ligand complexes were viewed by LigPlot viewer (http://www.ebi.ac.uk/thornton-

srv/software/LIGPLOT) and Chimera (http://www.cgl.ucsf.edu/chimera).

Drug-likeness analysis

Chosen compounds were subjected to further selection on the basis of Lipinski's rule of five (Ro5) [16]. Lipinski's screening was performed using Molinspiration server (http://www.molins piration.com/cgi-bin/properties) and physicoche-mical properties of ligands were calculated. Drug scores were calculated using ORISIS property explorer (http://www.organic-chemistry.org/prog/peo).

Absorption, distribution, metabolism, elimination and toxicity (ADMET) studies

The molecular structure of both ligands were submitted to ADMET-SAR server (http://lmmd. ecust.edu.cn:8000) to examine their drug likeliness and different pharmacokinetic and pharmacodynamic parameters including blood-brain barrier penetration, human intestinal absorption, Caco-2 permeability, cytochrome P450 solubility, cytochrome P (CYP) inhibitory promiscuity, renal organic cation transportation, human ether-a-go-go related genes inhibition, rat acute toxicity, fish toxicity, *Tetrahymena pyriformis* toxicity and Ames toxicity.

RESULTS

Both ligands (silibinin and glycyrrhetic acid) have shown strong binding energy with all three target proteins (-7.5 to -10.9 Kcal/mol).The said compounds followed the Lipinski's rule in Table 2 of five without any violation with respect to molecular weight (≤ 500KDa), an octanol-water partition coefficient (log P ≤ 5), molecular

refractivity (40 - 130), number of H-bond acceptors (≤ 10) and number of H-bond donors (≤ 5). Lipinski's rule of five analyzes various physiochemical properties [16]. These includes Log P anoctanol water partition coefficient which should be greater than or equal to 5, number of H-bonds donors and acceptor ≤5 and ≤10 respectively, molecular weight of greater than 500 and a molecular refractivity in the range of 40-130. The Lipinski's screening is an essential filter that determines if a compound is suitable for drug designing. Upon docking, both silibinin and glycyrrheticacid (Figure 1) formed at least one hydrogen bonds with all three target proteins. Docking with cyclooxygenase-2 (6COX) hydrogen bonds formation were observed with Phe580 residue having bond length of 2.84Å (silibinin) and 3.09Å (glycyrrhetic acid) as shown in Figure 2. Additionally, Asp347, Gln350, Gly354, Gln192, His351, Ser579, Phe580 were found to be common interacting residues in the target proteins for both ligands.

Significant numbers of hydrophobic interactions were observed between the two ligands and all three target proteins. When docked with 5-beta-reductase (3BV7) Pro221, Lys273, Ile271, Gly24, Tyr26, Trp230, Val309, Ser225, Ser220 residues interacted with silibinin and glycyrrhetic acid in addition to a few other residues. Similarly, Phospholipase A2 (2B03) docking analysis revealed Leu41, Tyr111, Leu19, Phe106, Ile9, Met20, Asn23, Leu31, Gly30, Leu2 and Tyr69 as common amino acid residues responsible for scoring a high binding energy of -8.8 kcal/mol and -10.9 kcal/mol for silibinin and glycyrrhetic acid respectively (Figure 3). All docking results are summarized in Table 3.
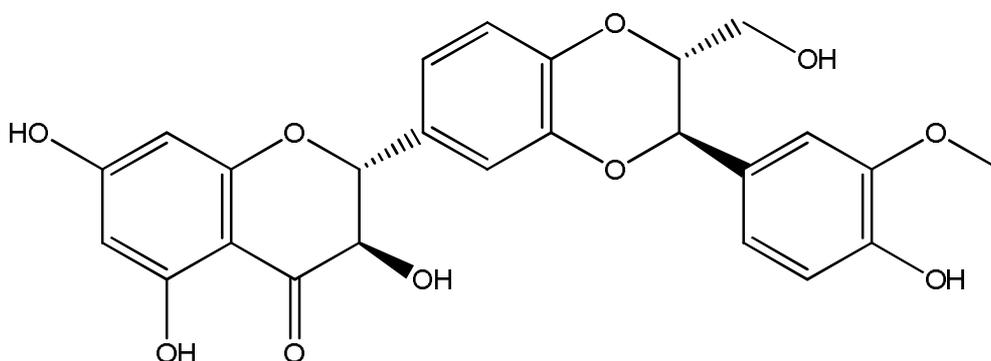
(2*R*,3*R*)-3,5,7-trihydroxy-2-((2*R*,3*R*)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)chroman-4-one

Silibinin
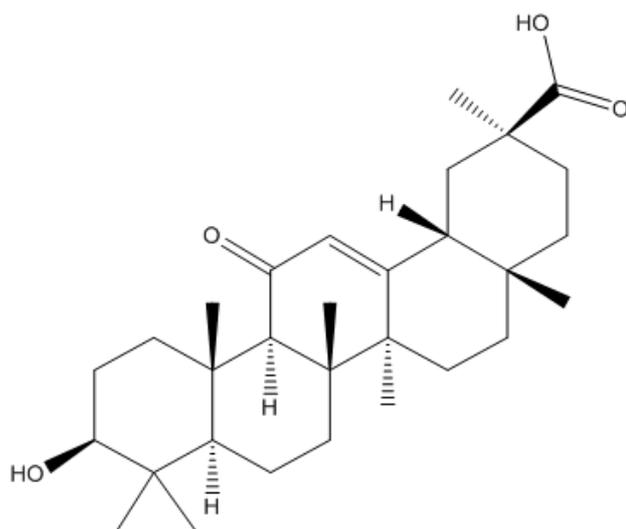
(2*S*,4a*S*,6a*S*,6b*R*,8a*R*,10*S*,12a*S*,12b*R*,14b*R*)-10-hydroxy-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid

Glycyrrhetic acid

Figure1: Molecular structures of silibinin and glycyrrhetic acid
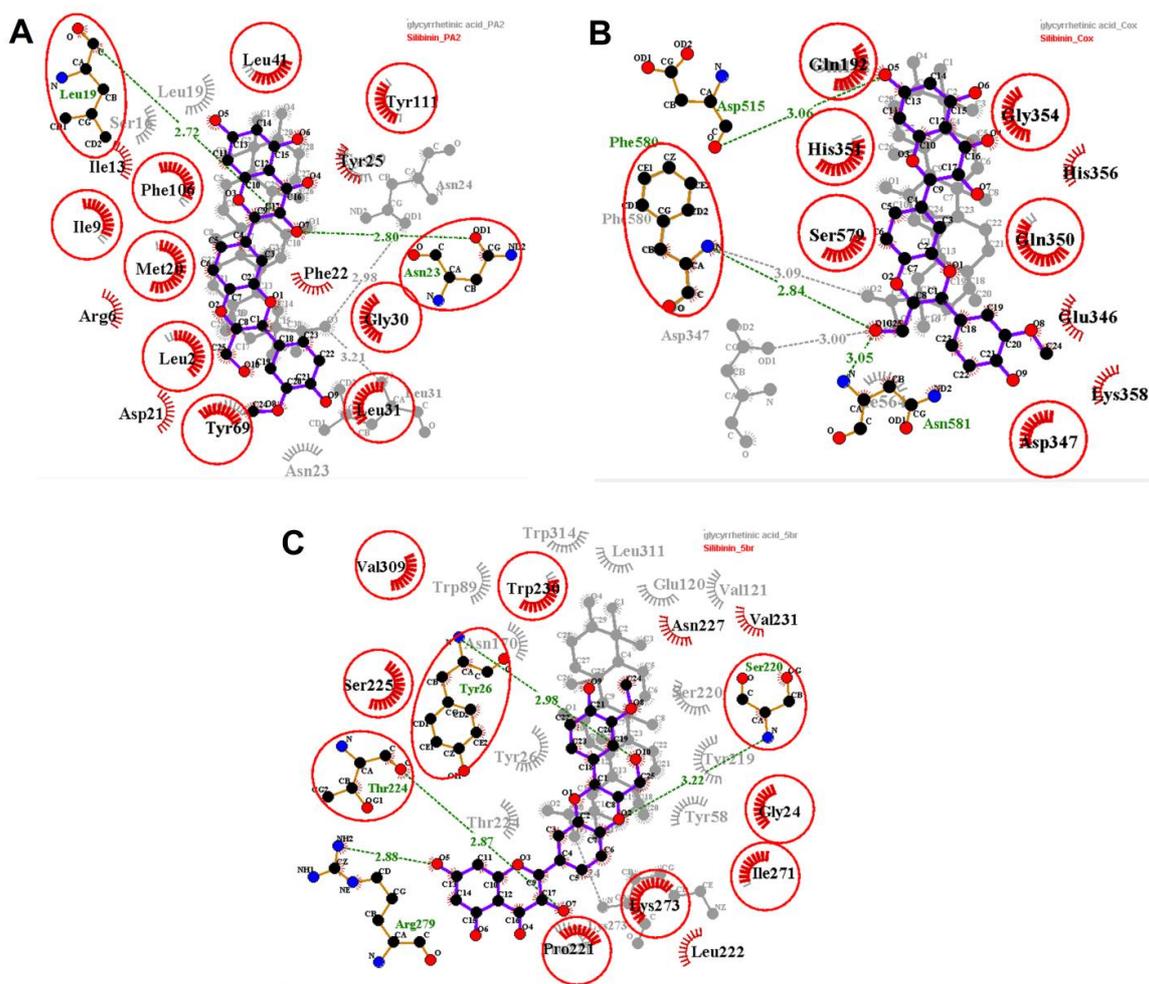

Figure2: Schematic diagram of binding modes of glycyrrhetic acid (in grey) and silibinin (in purple) with respective target proteins: cyclooxygenase-2 (A), 5-Beta reductase (B) and phospholipase A2 (C). The conserved residues of each target protein are shown in red circle for both ligands. This figure was produced by using LigPlot
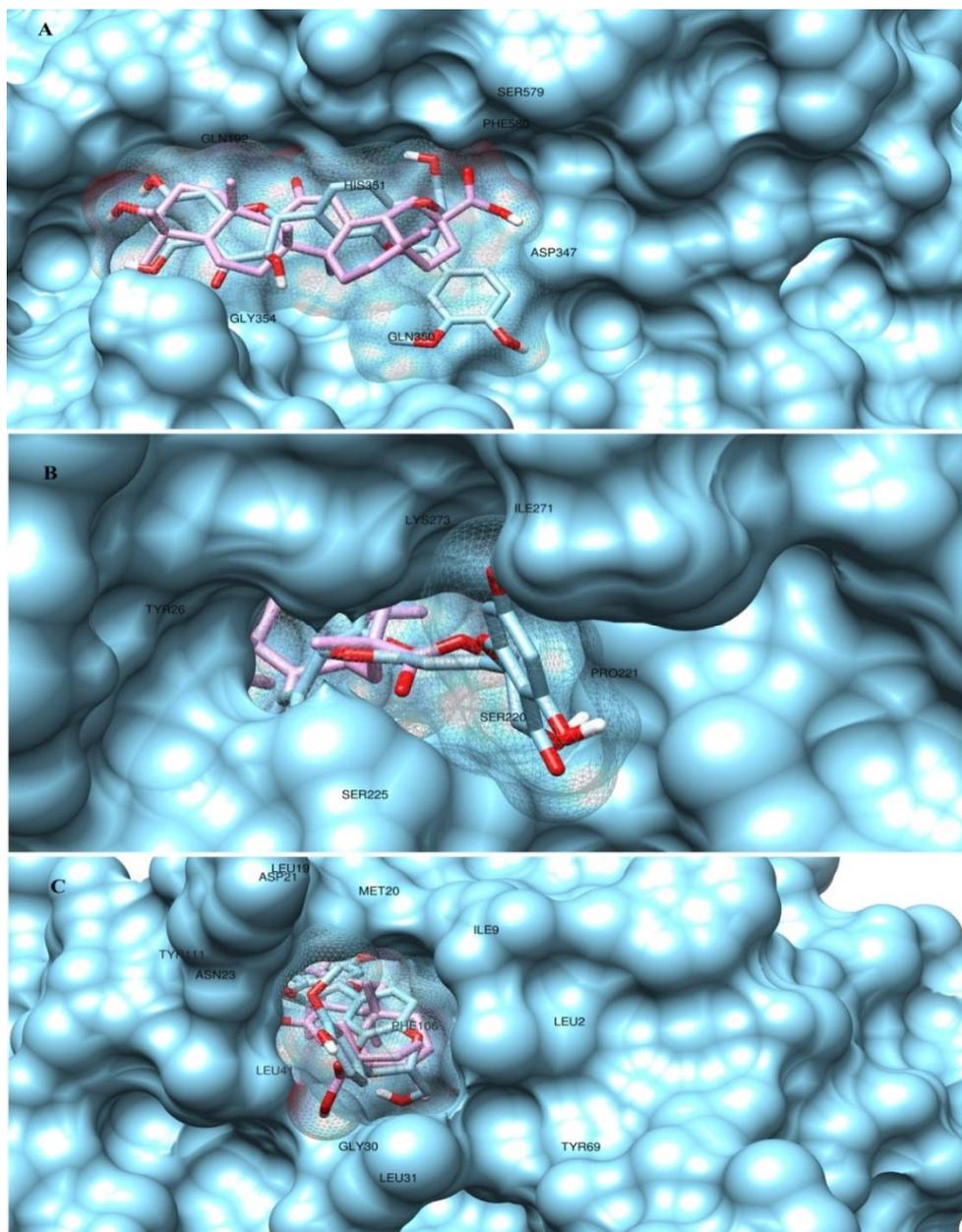
Figure 3: Molecular representation of target proteins with docked compounds. Conformation of glycyrrhetic acid (in pink) and silibinin (in blue) shown by sticks inside the binding pocket of cyclooxygenase-2 (A), 5-Beta reductase (B) and phospholipase A2 (C) shown in molecular surface representation in sky blue color

Table 2: Comparison of drug-likeness properties of silibinin and glycyrrhetic acid

Drug-likeness properties	Silibinin	Glycyrrhetic acid
Molecular weight	482.4345	470.6825
LogP	2.3627	-5.36
LogS	-3.41	-5.78
H-bond acceptors	10	4
H-bond donors	5	2
Rotatable bonds	4	1
Heavy atoms	35	34
Hydrogen atoms	22	46
PSA	155.14	74.6
RO5 violation	0	0
Refractivity	120.5501	136.8536
Drug-Likeness score	1.64	-2.36
Drug score	0.64	0.2

Table3: Comparison of binding energies (ΔG), interacting residues, H-bonds and hydrophobic interactions

Target protein (PDB code)	Docking results	Silibinin	Glycyrrhetic acid
Cyclooxygenase-2 (6COX)	Binding energy ΔG	-8.3	-8.9
	Interacting residues	Glu346, **Asp347,** Lys358, **Gln350,** His356, **Gly354,Gln192, His351,Ser579,Phe580, Asn581,** Asp525	**Asp347, Gln350, Gly354, Gln192, His351,Ser579, Phe580,** Ile564
	H-bonds	1(Asp515 - 3.06Å) 1(Phe580 - 2.84Å) 1(Asp581 - 3.05Å)	1(Asp347 - 3.00Å) 1(Phe580 - 3.09Å)
	Hydrophobic Interactions	10	8
5-Beta-reductase (3BV7)	Binding energy ΔG	-10.1	-7.5
	Interacting residues	**Pro221,** Leu222, **Lys273,Ile271,Gly24,Ser220,**Val231, Asn227, **Tyr26, Trp230, Val309,Ser225,** Arg279, Thr224	**Pro221, Lys273, Ile271,** Tyr58, Tyr219, **Gly24,** Ser220, Glu120, Val121, Trp314, Trp89, Leu311, Asn170,**Trp230, Val309, Ser225, Tyr26**
	H-Bonds	1(Tyr26 - 2.98Å) 1(Arg279 - 2.88Å) 1(Thr224 - 2.87Å) 1(Ser220 - 3.22Å)	1(Lys273 - 3.24Å)
	Hydrophobic interactions	11	16
Phospholipase A2 (2B03)	Binding energy ΔG	-8.8	-10.9
	Interacting residues	**Leu2,**Arg6,**Tyr69, Asp21, Asn23, Leu31, Gly30,**Phe22, Tyr25,**Leu41, Tyr111, Leu19,**Ile13,**Phe106, Ile9, Met20**	**Leu2,Tyr69,** Asp21,**Asn23, Leu31, Gly30,** Tyr25, Asn24, Cys29, **Leu41, Tyr111, Leu19,Phe106, Ile9, Met20**
	H-bonds	1(Leu19 - 2.72Å) 1(Asn23 - 2.80Å)	1(Leu31 - 3.21Å) 1(Asn24 - 2.98Å)
	Hydrophobic interactions	14	13

DISCUSSION

Plants secondary metabolites have medicinal values and show therapeutic potential like anti-viral, anti-inflammatory and immune-modulatory effects. The interest of investigators has been moved towards herbs because they are used as functional foods and an important source for the preparation of drugs. Hence, herbal medicines have been used worldwide for the treatment of various diseases. Inflammation is protective response involving molecular mediators, immune cells and blood vessels. Phytochemicals like silymarin and glycyrrhizin in suitable combinations are used as drugs for patients with liver disorders as they possess hepatoprotective property against inflammation [11,17]. Docking methodology facilitates structure-based virtual database screening with the ability to achieve a cost effective and quick estimation of the affinity and binding mode of a ligand for drug target. In this study, glycyrrhetic acid and silibinin have been shown to possess significant anti-inflammatory activity from ADMET prediction. Moreover, the difference between binding energies can be observed in Table 3. Both the ligands occupied the same location in the protein targets and shared common amino acid residues for the inhibition of proteins' action in inflammation.

Poor pharmacokinetics and toxicity in the biological system lead to failure in drug development. With the help of ADMET profile during the process of drug discovery one can removeincompatible compounds as well as exhibit significant role in reducing cost and efforts [19]. For a drug to be approved for use, tedious toxicological analysis are performed to ensure its safety upon ingestion. *In silico* ADMET analysis is a quick approach to find if a compound has acceptable pharmacokinetics and pharmacodynamics property. The toxicity risks and bioavailability of silibinin and glycyrrhetic acid were predicted based on their ADMET profile [20]. Further analysis exhibit CYP inhibitory promiscuity as silibinin inhibit two cytochromes including CYP450-2C9 and CYP450-3A4.

Table1: ADMET-predicted features of glycyrrhetic acid and silibinin

Property	Glycyrrhiticacid	Silibinin
Blood-brain barrier	BBB+	BBB-
Human intestinal absorption	HIA+	HIA+
Caco-2 permeability	Caco2+	Caco+
P-glycoprotein substrate	Substrate	Substrate
Renal organic cation transporter	Non-inhibitor	Non-inhibitor
CYP450 2C9 substrate	Non-substrate	Non-substrate
CYP450 2D6 substrate	Non-substrate	Non-substrate
CYP450 3A4 substrate	Substrate	Non-substrate
CYP450 1A2 inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C9 inhibitor	Non-inhibitor	Inhibitor
CYP450 2D6 inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4 inhibitor	Non-inhibitor	Inhibitor
CYP inhibitory promiscuity	Low CYP inhibitory promiscuity	High CYP inhibitory promiscuity
AMES toxicity	Non-AMES toxic	Non-AMES toxic
Carcinogens	Non-carcinogens	Non-carcinogens
Fish ioxicity	High FHMT	High FHMT
Tetrahymenapyriformis toxicity	High TPT	High TPT
Honey bee ioxicity	High HBT	High HBT
Biodegradation	Not readily biodegradable	Not readily biodegradable
Acute oral toxicity	III	III
Carcinogenicity (three-class)	Not-required	Not-required

Depending upon the acute oral toxicity (ADMET prediction profile), compounds are categorized into four groups. Category I contains compounds with LD_{50} values less than or equal to 50 mg/kg. Category II contains compounds with LD_{50} values greater than 50 mg/kg but less than 500 mg/kg. Category III includes compounds with LD_{50} values greater than 500 mg/kg but less than 5000 mg/kg. Category IV consisted of compounds with LD_{50} values greater than 5000 mg/kg.

The role of cytochrome P_{450} enzymes is very important as they are responsible for the metabolism of drug in biological system and its clearance from the body. Inhibition of any isoform of CYP leads to the malfunctioning of drug metabolism and elevation of toxicity [21].

After ADMET analysis of compounds under study it was observed that glycyrrhetic acid possesses better ADMET profile when compared to silibinin, because it can cross blood-brain barrier but not the silibinin, it is non-inhibitor for all CYP-inhibitors while silibinin is inhibitor for two CYPs like CYP-2C9 and CYP-3A4. Moreover, glycyrrhetic acid presented low CYP inhibitory promiscuity as compared to silibinin (Table 1). Both compounds were non carcinogenic and showed no AMES toxicity. Both compounds were found to be fit for drug development.

CONCLUSION

Glycyrrhetic acid possesses better ADMET profile than silibinin. Furthermore, hydrophobic interactions were significantly high between two ligands (glycyrrhetic acid and silibinin) and three target proteins (COX-2, phospholipase A2 and 5β-reductase). Ligand-receptor complex showed that strong hydrogen bonding and van der Waals interactions were formed indicating that these two phytochemicals are suitable options as anti-inflammatory agents as well as can be considered safe for development into a commercial drug when compared to other drugs in terms of their relatively fewer side-effects.

DECLARATIONS

Acknowledgement

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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