Lead finding from *Pterocarpus santalinus* with hepatoprotective potentials through *in silico* methods

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Abstract

Review of the literature revealed that, many compounds have been isolated from *P.santalinus* but there is no report of screening its potency in protecting CCl_4 induced liver damage. Hence in the present study an attempt has been made to study the hepatoprotective potency of the active constituents in *P.santalinus* through *in silico methods*. Lipenski's Rule of Five was applied on all the compounds to evaluate their drug likeness and pharmacological properties. Only the compounds satisfying the Lipenski's criteria were considered for further computational operations. Compounds that cleared the Lipenski's barrier were prepared for docking studies by their energy minimization in Marvin Sketch. HBx protein of hepatitis B virus served as a target (receptor) for docking studies. Its structure was retrieved from PDB (ID:3I7H). Energy minimized compounds were subjected to receptor-ligand interaction study using Hex docking tool. Based on the docking scores of energy minimized compounds it was concluded that pterocarpol and cryptomeridiol required minimum energy to bind to HBx. ADME-TOX analysis of these compounds showed that their AMES test value was significantly lower than some of the commercially and widely used drugs to treat Hepatisis. Hence these compounds hold good prospective of being used as medicine that targets HBx for hepatitis treatment.

Keywords: In silico study, *Pterocarpus santalinus*, active constituents, HBx Protein.

Introduction

Pterocarpus santalinus L.f. (Fabaceae) also called as Red sanders (English) is an endangered and endemic to Andhra Pradesh. It grows well in hilly regions with hot and dry climate. *P. santalinus* is highly valued for its heavy, dark claret-red heartwood which yields 16% of red colouring matter to santalin[1]. Santalin is used as coloring agent in pharmaceutical preparations and food stuff. Fruit extract have found many medicinal uses in treating inflammation, headache, skin diseases, chronic dysentery, etc. The plant wood has the potential to heal cuts, wounds and inflammation. It also aids in treating headache, skin diseases, fever, boils, scorpion string and to improve sight. The stem bark extracts of the plants have been found to exhibit antibacterial, antidiabetic, anti-hyperglycaemic activity and hepatoprotective activity. Despite several medicinal uses, the compounds constituting the plant extract have not been fully explored for their medicinal values[2, 3, 4]. In the present study, we evaluate the hepatoprotective activity of the plant compounds through *in silico* analysis.

Hepatitis B viral infection leads to infectious hepatitis and is strongly associated with development of hepatocellular carcinoma[5]. Genome of Hepatitis B virus embraces four open reading frames viz. P, S, C and X[6]. ORF P is the longest of all the ORF's in the genome and codes for polymerase protein[25]. ORF S encodes for three surface proteins namely pre S1, pre S2 and S[7]. ORF C codes for core protein[8]. Our focus is on ORF X that directs the synthesis of 17 kd hepatitis B virus X protein (HBx)[9, 10]. Functions of the proteins encoded by ORF's P,S and C have been lucidly studied in the past[27,26,7,8]. Studies on HBx protein have been very limited in the past, mainly due to the notion that it may not actively involve in infection. But its role in infection has been revealed and now, more and more studies are focussing on HBx's mechanism to induce infection[11]. Studies show that HBx serves as a transcription activator of polymerase II and III promoters. It is responsible for upregulation of many cellular and viral genes[12]. It interacts with transcriptional factor CREB and enhances its DNA binding ability to regulate transcription[13]. It increases the

calcium release into the cytoplasm leading to alteration of cytosolic calcium which is an essential requirement for HBv replication1. This renders HBx a potential molecular drug target for hepatitis treatment. Most of the commercially available drugs targets Polymerase (p) protein for hepatitis treatment[15, 16, 17] . There are virtually no drugs targeting HBx which has a crucial role to play in hepatitis infection[17]. Keeping the need for HBx targeting drugs in mind and its essentiality in infection, we selected HBx as the drug target for docking analysis *in silico* with the compounds occurring in *P.santalinus*[11].

Active constituents of *P.santalinus* include alpha and beta santalol, Cedrol, pterocarpol, isopterocarpol, santalin A and B, pterocarpin, cryptomeridiol, santalin Y and many other compounds[28]. Review of the literature revealed that, so far no *In Silico* attempt has been made to study the efficacy of these compounds ability to ameliorate the ccl4 induced hepatic toxicity. In our earlier report, we reported the ability of crude stem bark extract of *P.santalinus* protecting the liver from CCL_4 toxicity[2]. The present paper describes the *In silico* analysis of lead compounds to exacerbate the hepatic toxicity.

Materials and Methods

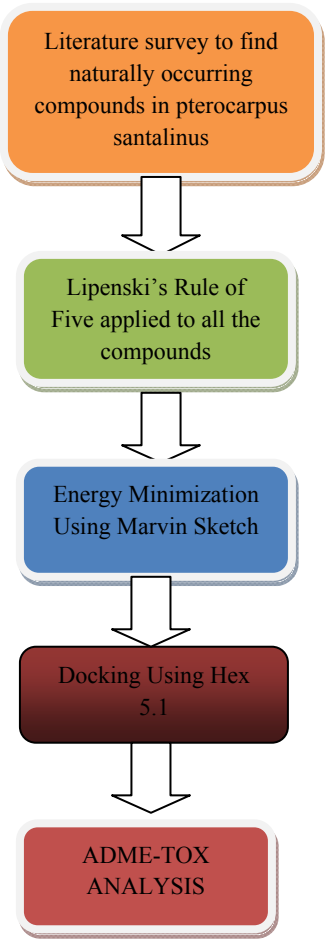

Fig 1: Overall work flow in drawing a network

All the compounds were subjected to Lipenski's Rule of Five to evaluate their absorption and permeability. For any compound to act as a drug, it has to meet a certain criteria. The criteria is given by Lipenski's Rule of Five. Compounds satisfying the criteria have better prospects of acting as drugs due to their high druglikeness. Therefore, only the compounds meeting the above criteria were considered for further computational analysis[19]

Filtered compounds were then subjected to energy minimization using MarvinSketch. MarvinSketch is a bioinformatics software used for drawing and editing chemical structures. Apart from drawing and editing it can also perform several tasks such as energy minimization, structure visualization, changing the file types, etc[20]. Energy Minimization is an essential step in computational approach towards drug discovery. This will lower the overall energy of the molecule making it flexible enough to fit in the active sites of the protein molecule with greater compatibility during the protein-ligand docking[21]. Each Lipenski cleared compound was opened in MarwinSketch and different energy conformers were obtained for the same. The least energy conformer of each compound was saved for performing docking studies[22].

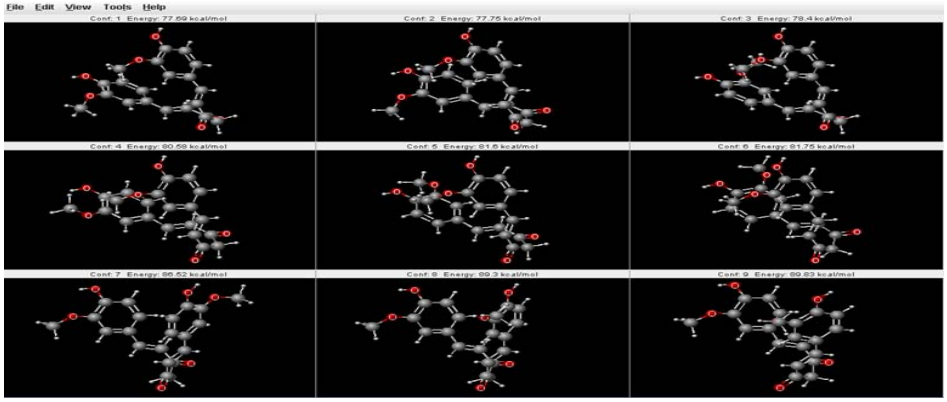

Fig 2: Different energy conformers for a compound in MarvinSketch

Drugs generally exhibit their action by binding to certain target receptor[22]. The energy required for the drug-receptor binding directly correlates to the effective binding of the drug to the target receptor. This energy at the expense of which protein-Ligand interaction occurs is denoted as G-bind value. The energy minimized compounds were ready to get docked with the target protein molecule that is hepatitis B virus X protein (HBx). Hex5.1 was used as a docking tool to carry out the docking operations. It is a freely available docking tool that can be easily downloaded using internet. Energy value (g-bind) for each ligand docked with HBx was noted down. Compounds requiring minimum energy to bind to HBx were considered as lead and were further subjected to ADME-TOX analysis[23, 24].

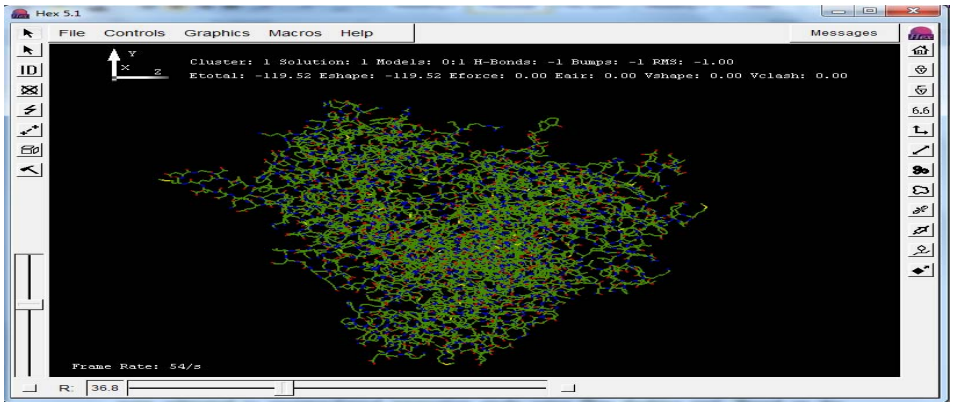

Fig 3: Docking Result in Hex 5.1

ADME-Tox drug properties viz. absorption, distribution, metabolism, elimination and toxicity, are properties that decide overall efficacy of the drug molecule. It is possible to study these properties in silico using ADME-TOX web server. It is an online server giving information about twelve major pharmacokinetics and pharmacodynamics features of the molecule. Toxicity of the molecule can also be investigated using this server which includes acute toxicity, genotoxicity and organ specific health effects. Genotoxicity result is in the form of ames test value. It correlates to the ability of the compound to act as a mutagen. Features like bioavailability, solubility, drug plasma binding protein, volume of distribution and ames test were considered for the comparison studies. A comparison was made with commercially used drugs for hepatitis treatment[24].

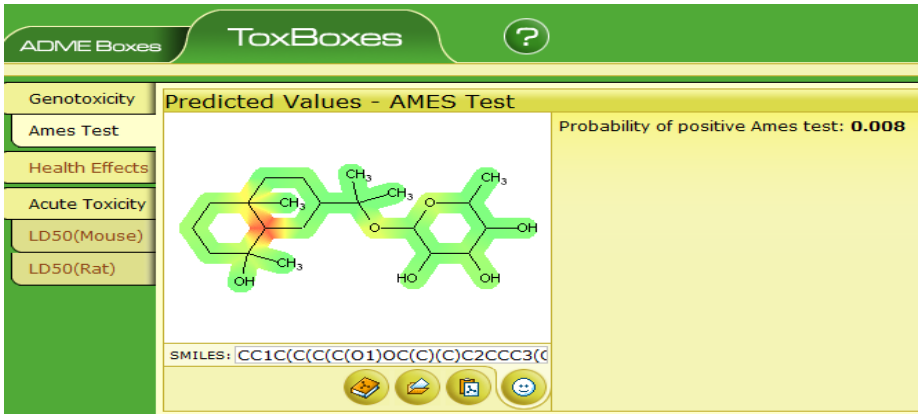

Fig 4: Ames test result for cryptomeridiol in ADME-TOX web

Results and Discussion

An intensive literature survey to find compounds occurring in *P.santalinus* resulted in 50 such compounds which includes triterpenes, flavones, coumarins, tannins, phenolic acids, polysterols, essential oils, etc. Out of these 50 compounds, 28 followed the Lipenski's criteria for druglikeness. These 28 compounds were taken as targeting agents which are suspected (based on evidence) to be responsible for inhibiting the biological processes important in causing hepatitis. Compounds were prepared for docking analysis through their energy minimization in Marvin Sketch. HBx's vital role played in hepatitis infection and a need for HBx targeting drugs rendered it as a target for our study. The docking studies were carried out in Hex5.1. It was found that two compounds namely Pterocarpol and Cryptomeridiol showed relatively better interactions requiring minimum energy to bind to the receptor[table-1]. ADME-TOX analysis of these two compounds showed reliable pharmacokinetics and pharmacodynamics features. Toxicity box results showed that these two compounds has lower ames test value when compared to commercially used drugs for hepatitis treatment such as Lamivudin, Adefovir Dipivoxil, Entecavir, Telbivudin and Tenofovir [table-2]**.**

Conclusion

It can be concluded Pterocarpol and Cryptomeridiol hold a strong potential for acting as drug candidates that target HBx to inhibit the biological processes leading to hepatitis. Further animal studies needs to be done to confirm the exact role and mechanism of these two compounds as a chemo preventive agent for Hepatitis.

Table 1: Compounds from *P.santalinus* having minimum energy values.

Sl. No.	NAME OF THE COMPOUND	ΔG kcal/mol
1.	Cryptomeridiol	-119.52
2.	Pterocarpol	-108.42

Table 2: Comparision of Ames test values of lead compounds found through insilico analysis with commertially used drugs to treat hepatitis

Sl. No.	Name of the Compound	Compound Type(lead/drug)	Ames Test Value
1.	Cryptomeridiol	lead	**0.008**
2.	Pterocarpol	lead	**0.000**
3.	Lamivudine	drug	**0.421**
4.	Entecavir	drug	**0.071**
5.	Adefovir Dipivoxil	drug	**0.073**
6.	Telbivudine	drug	**0.154**
7.	Tenofovir	drug	**0.173**

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