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RESEARCH ARTICLE

MOLECULAR DOCKING STUDIES OF THE CHEMICAL CONSTITUENTS OF HIPPO-08 WITH CYP2E1 AND CYP3A4

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ABSTRACT

In the present study, Molecular docking was done in order to find out whether the chemical compounds of Hippo-08 an ayurvedic formulation, were docked into the active sites of cytochrome p-450 2E1 and cytochrome p-450 3A4 using Accelrys Discovery studio2.1. These two enzymes were increased several folds after ethanol consumption, and these are the major source of free radicals or reactive oxygen species (ROS) causing oxidative stress in the liver. Based on the Dock score the compounds could be ranked for their activity against the protein. Highest Dock score means it has good interaction (inhibition) towards the selected target protein. Seven different chemical compounds present in Hippo-08 have good Dock score against CYP2E1 and five different chemical compounds present in Hippo-08 have good Dock score against CYP3A4. These lead compounds may possess the effectiveness against Alcoholic Fatty Liver Disorder.

INTRODUCTION

Long- term intake and abuse of alcohol results in various liver abnormalities ranging from simple fatty liver or steatosis to steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. Oxidation of ethanol mediated by alcohol dehydrogenase (ADH) produces acetaldehyde, a toxic and reactive metabolite. Acetaldehyde is further converted to a non-toxic form, aceticacid or acetate, by aldehyde dehydrogenase (ALDH). Both reactions reduce nicotinamide adenine dinucleotide (NAD) to its reduced form (NADH) Wu (2006). Another pathway of ethanol metabolism, the microsomal ethanol oxidizing system (MEOS), was shown to play an important role in the progression of liver diseases in baboons in 1974 (Takahashi, 1993). In MEOS, CYP2E1 is the primary enzyme involved in ethanol oxidation. Cederbaum (2006). CYP2E1 was induced by ethanol with corresponding 4 to 10 fold rise in mRNA in liver biopsy samples obtained from subjects who had recently drunk alcohol (Takahashi, 1993). In addition to CYP2E1, CYP3A4 also can break down alcohol Salmela (1998). Moreover, the amounts of various enyzmes of the cytochrome CYP3A family (including CYP3A4) can increase from human alcohol consumption Hoshino (1995); Niemela (1998). In view of severe undesirable side-effects of synthetic drugs used in the treatment of liver diseases, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. The Hippo-08 an oral formulation have been developed which is a combination of three different plant derived sources (*Entada pursaetha, Toddalia aculeata*, and *Ziziphus mauritiana*) encompasses a wide and natural medicinal source used in various diseases and ailments of disorders. In the present study, Molecular docking was done in order to find out whether the chemical compounds of Hippo-08 were docked into the active site of CYP 2E1 and CYP 3A4 using Accelrys Discovery studio2.1. Based on the Dock score the compounds could be ranked for their activity against the protein. Highest Dock score means it has good interaction (inhibition) towards the selected target protein.

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MATERIALS AND METHODS

The seeds of *Entada pursaetha*, the stem of *Toddalia aculeata*, and the fruits of *Ziziphus mauritiana* were used in the formulation of Hippo-08. The chemical constituents present in Hippo-08 were identified using GC-MS analysis. The chemical constituents present in Hippo-08 were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 2-Furancarboxaldehyde, 5-(hydrox ymethyl)-, Phenol, 2-methoxy-5-(1-propenyl)-(E)-, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, Caryophyllene, Cyclohe xane, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl- (S)-, 1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-syn:Spathulenol, Benzeneacetic acid, 2,5-dihydroxy-, Methyl tetradecanoate, Phenol,2-methyl-5-(1,2,2-trimethylcyclopentyl)-, (S)-, n-Hexadecanoic acid, Hexadecanoic

acid, ethyl ester, Tetradecanoic acid, 9,12-Octadecadienoic acid, methyl ester, (E,E)-, 9-Octadecenoic acid (Z)-, methyl ester, Oleic acid, 1,4,7-Androstatriene-3,17-dione, Pregna-5,16-dien-20-one,3-(acetyloxy)-16-methyl-(3β)-, (E)-2-Allyl-4-(2-(1,3-benzodioxol-5-yl)-1-methylvinyl)-4,5-dimethoxy-2,5-cyclohexadien-1-one, Galbelgin, and Galgravin. These compounds were used for the molecular docking study.

Ligand Preparation

The three dimensional structures of compounds were downloaded in sdf format from Pubchem database. Hydrogen Bonds were added and the energy was minimized using CHARMm force field. Molecular weight, log *P* and number of Hydrogen-bond donors and acceptors for the active principles were noted.

Protein Preparation

The structure of the proteins CYP2E1 and CYP3A4 were viewed in Accelrys Discovery Studio 2.1 with force field applied on the protein in order to get a stable structure The ligands and crystallographic water molecules were removed from the protein; and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMm force field.

Docking Studies

The active site of the protein was first identified and it is defined as the binding site resulted in a cavity size. Thus Binding sites were defined based on the ligands already present in the PDB file which were followed by site sphere definition. Here site 4 was chosen as the binding site and the site sphere size was set. The determination of the ligand binding affinity was calculated using LigScore and PLP1, JAIN and Dock score were used to estimate the ligand-binding energies. A higher score indicates a stronger receptor-ligand binding affinity. The determination of the ligand binding affinity was calculated using the shape-based interaction energies of the ligand with the protein.

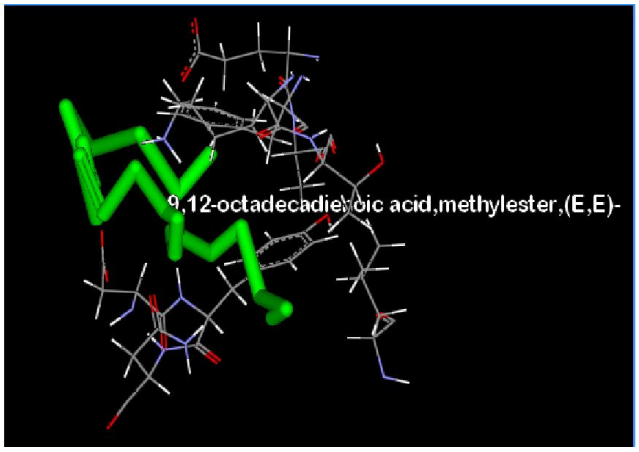

Fig.1. Docking of 9, 12-octadecadienoic acid, methylester, (E,E)- with protein Structures of human cytochrome P-450 2E1

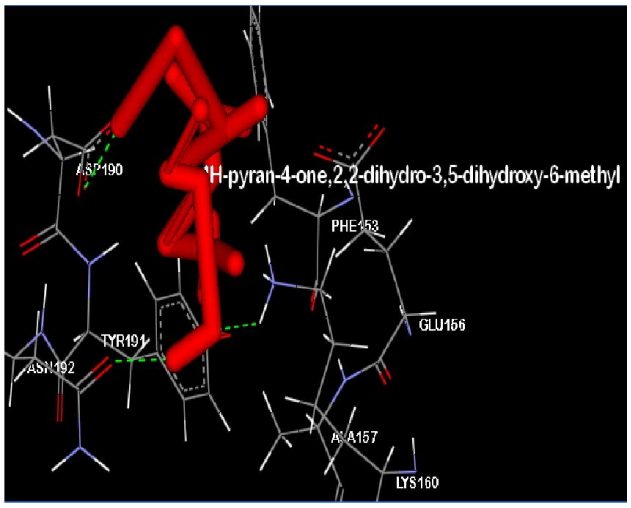

Fig.2. Docking of 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl with protein Structures of human cytochrome P-450 2E1

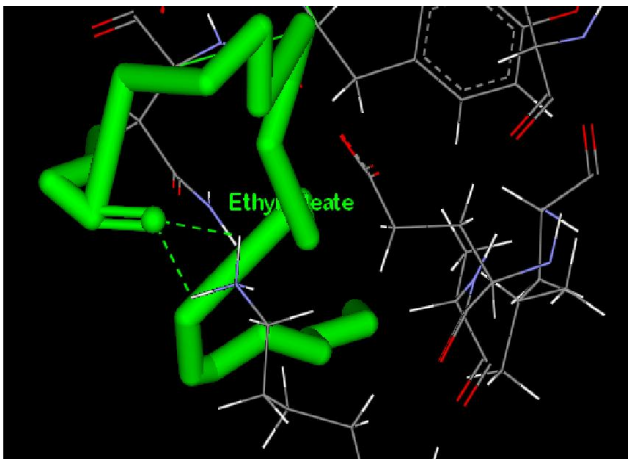

Fig.3. Docking of Ethyl Oleate with protein Structures of human cytochrome P-450 2E1

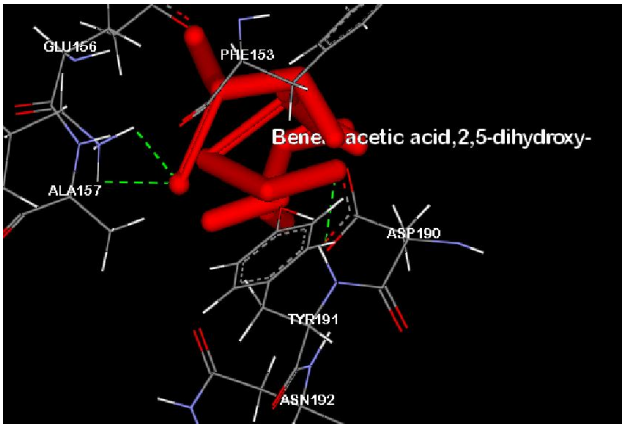

Fig.4. Docking of Benzene acetic acid, 2, 5-dihydroxy- with protein Structures of human cytochrome P-450 2E1

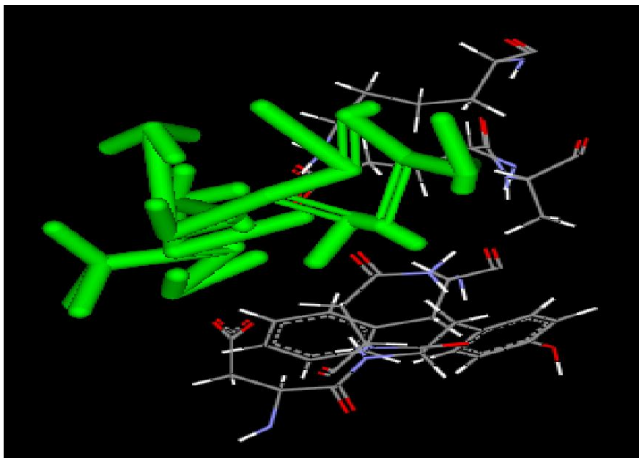

Fig.5. Docking of Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- with protein Structures of human cytochrome P-450 2E1

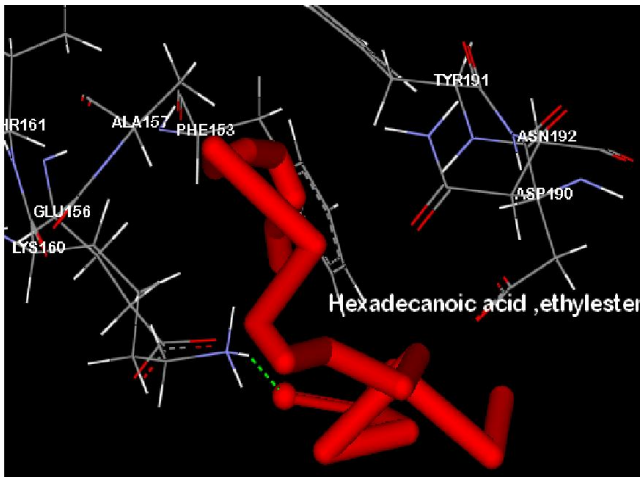

Fig.6. Dokcing of Hexadecanoic acid, ethylester with protein Structures of human cytochrome P-450 2E1

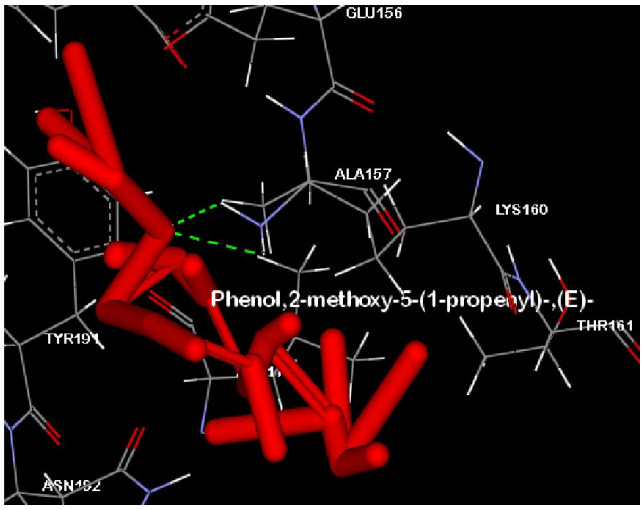

Fig.7. Docking of Phenol,2-methoxy-5-(1-propenyl)-,(E)- with protein Structures of human cytochrome P-450 2E1

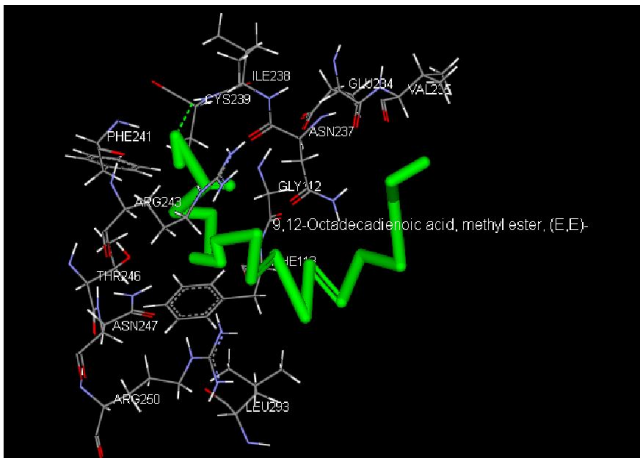

Fig. 8. Docking of 9, 12-Octadecadienoic acid, methyl ester, (E,E)- with protein Structures of human cytochrome P-450 3A4

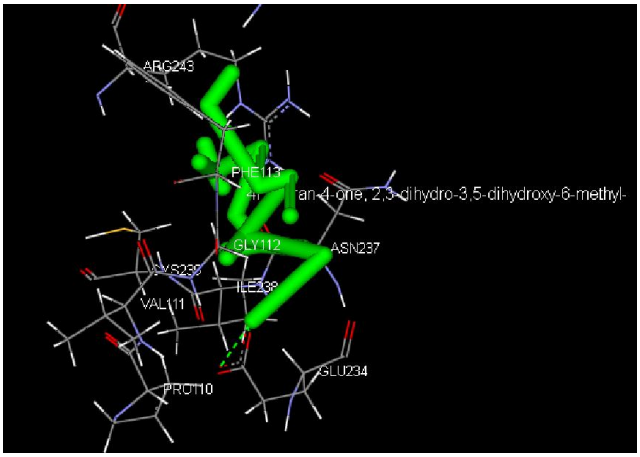

Fig.9. Docking of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- with protein Structures of human cytochrome P-450 3A4

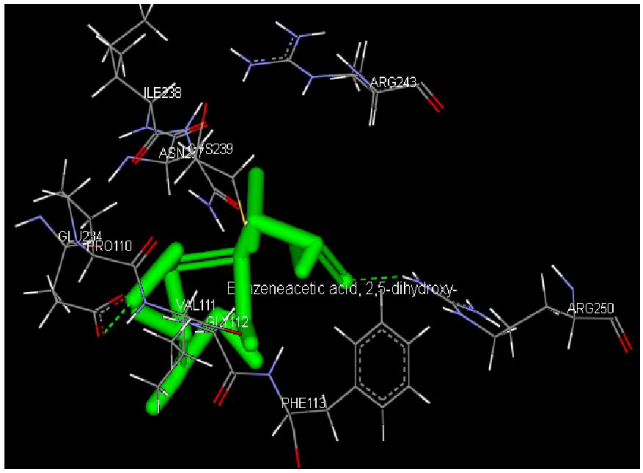

Fig.10. Docking of Benzeneacetic acid, 2,5-dihydroxy- with protein Structures of human cytochrome P-450 3A4

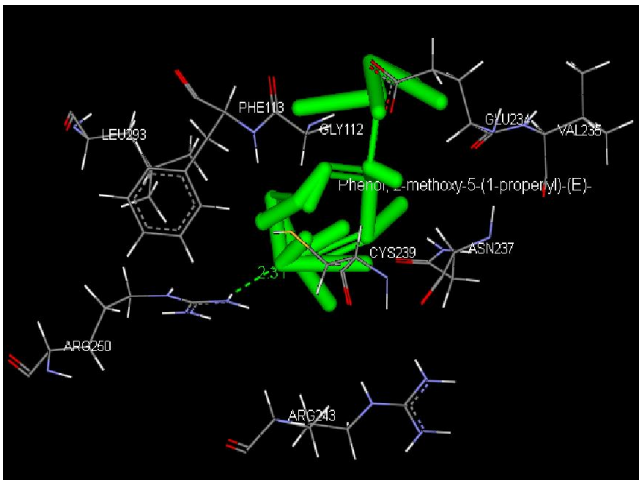

Fig.11. Docking of Phenol, 2-methoxy-5-(1-propenyl)-(E)- with protein Structures of human cytochrome P-450 3A4

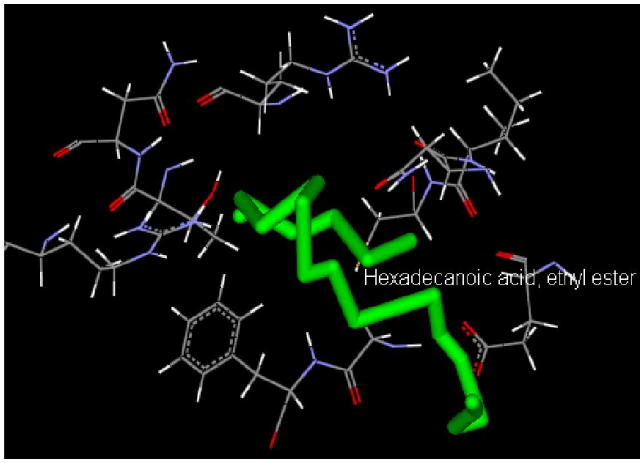

Fig.12. Docking of 3 Hexadecanoic acid, ethyl ester with protein Structures of human cytochrome P-450 3A4

RESULTS AND DISCUSSION

In the present study, the molecular docking studies of the chemical constituents present in Hippo-08 were carried out with CYP2E1. The compounds like 9,12-octadecadienoic acid, methylester,(E,E)-, 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl, Ethyl Oleate, Beneneaceticacid 2,5-dihydroxy-, Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-, Hexadecanoic acid ,ethylester, and Phenol,2-methoxy-5-(1-propenyl)-,(E)- have better dock score with CYP2E1, of which, 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl showed highest dock score (71.365) followed by Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- (50.726) against CYP2E1. The lig score 1&2, PLP1&2, Jain scoring function, PMF and the Dock score of the compounds were given in Table- 1. The docking results of the compounds with CYP3A4 are given in Table-2. The componds 9,12-octadecadienoic acid, methylester,(E,E)-, 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl, Beneneacetic acid,2,5-dihydroxy-, Phenol,2-methoxy-5-(1-propenyl)-,(E)-, and 3 Hexadecanoic acid, ethyl ester have better dock score with CYP3A4, of which, Beneneacetic acid,2,5-dihydroxy-, revealed highest dock score (75.117) followed by 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl,(58.377).

Actually, natural agents inhibiting CYP2E1, including diallyl sulphide (from garlic), phenyl ethyl isothiocyanate and sulforaphane (present in cruciferous vegetables) and bergamottin (found in the essential oils of grapefruit and certain oranges) have been proposed as possible candidates for minimizing the ethanol-induced hepatotoxicity(Mc Carty, 2001). In addition, trans 1, 2-dichloroethylene was reported to be a selective inhibitor of CYP2E1. Mathews (1998). YH439 is a novel synthetic compound inhibiting CYP2E1 that is being evaluated as a hepatoprotective agent. Jeong (1996). CYP3A4 activity was known to be inhibited by a protease inhibitor nelfinavir which is used in the treatment of malaria, tuberculosis and cancer Bruning (2010). Hyperforin which is an antidepressant inhibited the activity of CYP3A4 Lee (2006).

Table 1. Docking results of the chemical compounds present in Hippo-08 with CYP2E1

S.No	Compound	Lig Score1	Lig Score2	PLP1	PLP2	JAIN	PMF	Dock score
1	9,12-octadecadienoic acid,methylester,(E,E)-	0.57	2.52	31.14	34.56	-3.01	29.15	25.515
2	4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl	3.96	3.18	13.11	14.13	-0.19	38.28	71.365
3	Ethyl Oleate	2.29	3.98	40.43	43.38	-3.91	49.34	47.255
4	Benzene acetic acid,2,5-dihydroxy-	2.49	0.93	-11.55	-5.97	-2.42	27.69	38.91
5	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	-0.33	1.18	2.2	9.49	-1.21	21.88	50.726
6	Hexadecanoic acid ,ethylester	2.43	3.28	29.94	33.37	-2.03	33.76	34.395
7	Phenol,2-methoxy-5-(1-propenyl)-,(E)-	1.47	2.52	26.26	26.03	1.78	18.71	27.089

Table 2. Docking results of the chemical compounds present in Hippo-08 with CYP3A4

S.No	Compound	Lig Score1	Lig Score2	PLP1	PLP2	JAIN	PMF	Dock score	H-Bond Distance
1	9,12-octadecadienoic acid,methylester,(E,E)-	2.16	2.97	51.29	48.15	-2.05	17.57	21.657	2.334000
2	4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl	1.86	1.07	5.01	12.29	0.15	-4.22	58.377	2.47 0.43
3	Benzene acetic acid,2,5-dihydroxy-	2.74	2.35	27.59	32.95	-0.06	5.56	75.117	2.46 0.44 2.15
4	Phenol,2-methoxy-5-(1-propenyl)-,(E)-	1.12	2.46	16.68	20.66	-0.2	25.59	16.882	2.31
5	3 Hexadecanoic acid, ethyl ester	1.37	1.99	25.22	27.55	-3.32	27.28	14.943	0

Ethanol intoxication enhanced the level of CYP2E1 and CYP3A4 several folds. The several fold increase of these enzymes may be due to the detoxification of ethanol by these enzymes. The free radicals generated during this process may lead to oxidative damage. The compounds present in the Hippo-08 formulation showed the inhibitory effect against CYP2E1 and CYP3A4 and there by suppressing the free radical mediated damage to the liver.

Conclusion

The present study revealed the five compounds namely 9,12-octadecadienoic acid, methylester (E,E)-, 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl, Benzene aceticacid,2,5-dihydroxy-, Phenol,2-methoxy-5-(1-propenyl)-,(E)-, and 3 Hexadecanoic acid, ethyl ester which are present in Hippo-08 were successfully docked with both the proteins namely CYP2E1 and CYP3A4, of which, 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl showed highest dock score (71.365) against CYP2E1 and Benzene aceticacid,2,5-dihydroxy- exhibited highest dock score (75.117) followed by 4H-pyran-4-one,2,2-dihydro-3,5-dihydroxy-6-methyl (58.377) against CYP3A4.

REFERENCES

Bruning A, Gingelmaier A, Friese K Mylonas I. 2010. New prospects for nelfinavir in non-HIV related diseases. *Curr Mol Pharmacol*., 3P: 91-97.

Cederbaum AI. 2006. Cytochrome P450 2E1- dependent oxidant stress and upregulation of anti-oxidant defense in liver cells. *J Gastroenterol Hepatol*.,21: S22-5.

Hoshino U , Kawasaki H. 1995. Urinary 6-β- hydroxycortisol excretion in patients with alcoholic liver disease. *Res Commun Alcohol Sub Abuse.*,16: 116-124.

Jeong KS, Lee IJ, Roberts BJ, Soh Y, Yoo JK, Lee JW, Song BJ. 1996. Transcriptional inhibition of CYP4502E1 by a synthetic compound, YH439. *Arch Biochem biophys*., 326: 137-144.

Lee JY, Duke RK, Tran VH, Hook JM Duke CC. 2006. Hyperforin and its analogues inhibit CYP3A4 enzyme activity. *Phytochemistry.*, 67: 2550-2560.

Mathews JM, Etheridge AS, Raymer JH, Black SR, Pulliam DW, Bucher JR. 1998. Selective inhibition of CYP2E1 in vivo and in vitro with trans1, 2-dichloro ethylene". *Chem. Res. Toxicol.*, 11: 778-785.

Mc Carty MF. 2001. Inhibition of CYP4502E1 with natural agents may be a feasible strategy for minimizing the hepatotoxicity of ethanol. *Med. Hypothesis.,* 56:8-11.

Niemela O, Parkkila S, Pasanen M, Iimur Y, Bradford B, Thurman RG. 1998. Early alcoholic liver injury: Formation of protein adducts with acetaldehyde and lipid peroxidation products, and expression of CYP2E1 and CYP3A. *Alcoholism: Clinical and Experimental Research.*,22:2118-2124.

Salmela KS, Kessova IG, Tsyrlov IB, Lieber CS. 1998. Respective roles of human cytochrome P-4502E1, 1A2 and 3A4 in the hepatic microsomal ethanol oxidising system. *Alcoholism: Clinical and Experimental Research.*, 22: 2125-2132.

Takahashi T, Lasker JM, Rosman AS, Lieber CS. 1993. Induction of cytochrome P-450 2E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. *Hepatology.*,17: 236-45.

Wu D, Zhai, Q Shi X. 2006. Alcohol – induced oxidative stress and cell responses. *J Gastroenterol Hepatol.,*21: S26-9.

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