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RESEARCH ARTICLE

INSILICO ANALYSIS OF BIOCHEMICAL COMPOUNDS USED AS HEPATO PROTECTIVE AGENTS

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ABSTRACT

"Hepatotoxicity is the capacity of a drug or chemical to produce injury to the liver diseases. The liver plays a central role in transforming and clearing chemicals which is susceptible to the toxicity from agents. Agents with recognized hepatotoxicity include carbon tetrachloride, alcohol". In the present study the functional properties of target proteins which are responsible for liver cancer were studied and comparative modeling of target proteins was done for high percent sequence identity for structural homology. The protein structure was modeled using Swiss-PDB as its corresponding structure not available in Protein Data Bank. Docking study of the modeled protein was done with natural compounds Beta sitosterol (*Justicia adhatoda*), Mimosine (*Mimosa pudica*), Berberine (*Vitex negundo*) and synthetic compounds Tiagabine, Vigabatrin, Zolpidem, Chloramphenicol, Gentamicin, Linezolid to treat Liver disease to find the preferred orientation and binding affinity of drug with target protein using scoring functions. Berberine was found to be effective drug selected on the basis of its binding affinity with the target protein and that can be potential target for Liver cancer.

INTRODUCTION

Hepatotoxicity is the quality of being destructive to the liver. In certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market (Friedman Scott *et al*., 2009). Chemicals often cause sub clinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Ostapowicz *et al*., 2002). *Justicia adhatoda* L. is a medicinal plant native to Asia. The leaves vary from 10 to 15cm in length, and are about 4cm broad. Antiasthmatic, antispasmodic, bronchodilator, expectorant, oxytocic activities have been reported. *Vitex negundo* L. is a large, aromatic shrub with quadrangular, densely whitish, tomentose branchlets. The plant possess anti-inflammatory (Dharmasiri *et al*., 2003), antibacterial (Perumal Samy *et al*., 1998), antifungal (Sathiamoorthy *et al.,* 2007 & Damayanti *et al*., 1996) and analgesic (Gupta *et al.,* 2005 Gupta *et al.,* 1999) activities. *Mimosa pudica* L. is a creeping annual or perennial herb often grown for its curiosity value. The degree of liver damage was correlated with the amount ofPiZ alpha 1-antitrypsin accumulated in the liver of the different pedigrees of mice (Lieberman *et al.,* 1976). GABA receptor subunits have been detected in many malignant tumors, such as π subunit in pancreatic cancer (Takehara *et al.,* 2007). PiZ protein and GABA are selected as receptor. Using bioinformatics tools the 3-Dimensional structure of the target protein was modeled. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengauer *et al.,* 1996). In the present study an attempt has made to develop specific anti-cancer agents using targets through automated docking methods. Several alkaloids are present in the leaves of *Justicia adhatoda* L. and the chief principle is a quinazoline alkaloid, vasicine (Abd *et al.,* 1998). The principal constituents the leaf juice of *Vitex negundo* L. are casticin, isoorientin, chrysophenol D, luteolin, p–hydroxybenzoic acid and D-fructose. *Mimosa pudica* contains an alkaloid called, mimosine which has been found to have potent antiproliferative and apoptotic effects (Restivo *et al.,* 2005). These three medicinal plants reported to possess potent antioxidant activity. Since many hepatic injuries are free radical mediated, it is possible that the extracts of these plants may show potent hepatoprotective activity. In this study a comparative hepatoprotective property of *Justicia adhatoda*, *Mimosa pudica* and *Vitex negundo* is being reported.

MATERIALS AND METHODS

The FASTA sequences of the target proteins GABA and PiZ protein is retrieved from the Swiss-Prot. Since the target

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proteins have no PDB entry, the target proteins were modeled through SPDBV. The sequence length of GABA is 118 amino acid residues and for PiZ protein 513 residues. We have aligned these two sequences using BLAST tool and got the high similarity of target proteins. These target proteins retrieved from protein databank and id is 1SZK for GABA and 3HWF for PiZ protein.

Homology Modeling

Swiss-pdb Viewer 4.0.1 is used to model the 3D structure of the GABA and PiZ protein, for which there were no structure in PDB. Fasta sequence of the target proteins were retrieved from the NCBI. Since the target proteins have no PDB entry from protein databank. Template proteins were identified using fasta sequence by Swiss Model Workspace (Guex *et al.,* 1997). The energy minimization and loop building was done for the modeled structures and the refined models were obtained. The refined modeled protein's structures were validated using SAVS server (Ramachandran *et al.,* 1963). A Verify_3D result has shown good results that means the quality of the modeled structure is PASS. The 3D structure of the modeled protein was visualized using RasMol (Roger *et al.,* 1995).

Preparation of ligand structures

The 2D structures of the ligands obtained from Pubchem Compound and using ACD/Chemsketch software (Li *et al.,* 2004). We have retrieved the structures of natural and synthetic compounds from the pubchem compound database. The compound names are Tiagabine, Vigabatrin and Zolpidem for GABA protein and Chloramphenicol, Gentamicin and Linezolid for PiZ protein. Natural compound used for this study are Berberine, Mimosine and Beta sitosterl from three plants viz *Justicia adhatoda, Mimosa pudica and Vitex negundo*. The 2D structure converted into 3D structure using further docking analysis.

Identification and size characterization of surface pockets and occluded cavities are initial steps in protein structure-based ligand design. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of

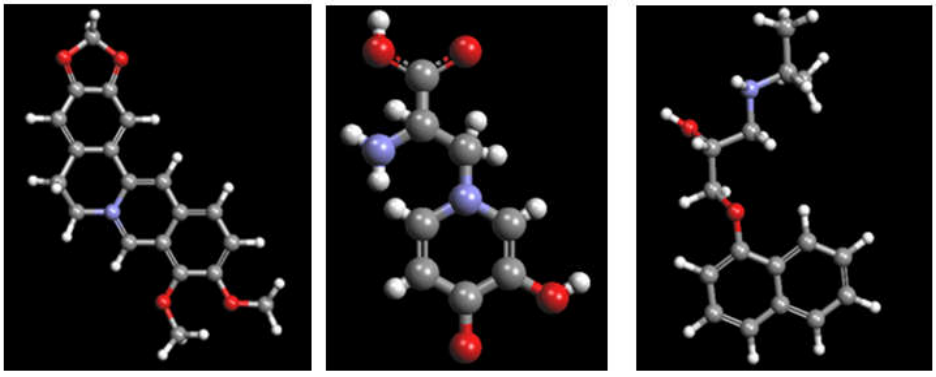


Fig. 1. Graphical representation of the 3D structure of natural compounds

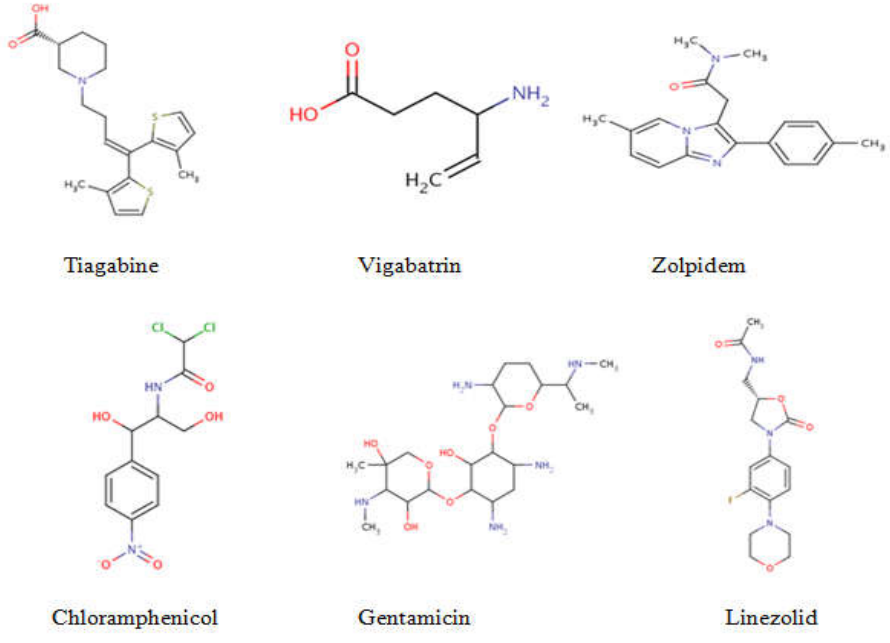


Fig. 2: 2D structure of synthetic compounds drawn using Chemsketch software

pockets and cavities; and the area and circumference of mouth openings (*Liang et al.,* 1998).

ADME toxicity

ADME is an acronym in pharmacokinetics and pharmacology for absorption, distribution, metabolism, and excretion and describes the disposition of a pharmaceutical compounds within an organism. The four criteria all influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drugs. The ADME/Tox properties of the ligands which we have selected from plants viz Berberine, Mimosine and Beta sitosterol were shown in the Table 1. The stability of a drug is the length of time a drug retains its properties without loss of potency; usually referred to as shelf life. Toxicity studies of the bioactive compounds were done using ADME/Tox Web (Balani *et al.,* 2005).

Table 1. ADME/Tox properties of Drugs

Drug Name	Stability	Absorption Rate	Vol. of Distribution	Solubility
Berberine	pH< 2	K_a = 0.001 min^{-1}	2.54 L/kg	LogS at: pH = 1.7 (Stomach): -6.56 pH = 4.6(Duodenum):-6.56 pH = 6.5 (Jejunum &Ileum): -6.56 pH = 7.4 (Blood): -6.56 pH = 8.0 (Colon): -6.56
Mimosine	pH < 2	K_a = 0.001 min^{-1}	0.39L/kg	LogS at: pH = 1.7 (Stomach): -0.11 pH = 4.6 (Duodenum): -0.65 pH = 6.5 (Jejunum & Ileum): -0.65 pH = 7.4 (Blood): -0.65 pH = 8.0 (Colon): -0.65
Beta sitosterol	pH < 2	K_a = 0.100 min^{-1}	6.29 L/kg	LogS at: pH = 1.7 (Stomach): -5.98 pH = 4.6 (Duodenum): -5.98 pH = 6.5 (Jejunum & Ileum): -5.98 pH = 7.4 (Blood): -5.98 pH = 8.0 (Colon): -5.98

BINDING SITES

CASTp software predicted the active site of the proteins GABA and PiZ with a higher average precision. CASTp takes its input file as pdb and its results are predicted as binding sites. The active site of GABA comprises of amino acid residues such as GLY 20, GLN 21, ILE 22, ILE 50, GLN 79, VAL 80, LEU 106, LEU 107, VAL 108, THR 109, THR 110, GLY 11, SER 112, GLU 113, VAL 115, GLU 116, ASN 117, LYS 120, ARG 123, LYS 127, ARG 128, SER 129, SER 135, GLY 136, ALA 137, TYR 138, HIS 139, GLY 140, ARG 141, THR 149, GLY 150, LYS 151, VAL 152, ASN 153, PRO 154, TYR 155, SER 156, ALA 157, GLY 158,MET 159, GLY 160, LEU 161, MET 162, PRO 163, GLY 164, HIS 165, VAL 166, TYR 167, ARG 168, ALA 169, LEU 170, TYR 171, CYS 173, HIS 176, GLY 177, ILE 178, ASP 181, ASP 182, ILE184, ALA 185, SER 186, HIS 188, ARG 189, ILE 190, PHE 191, LYS 192, ASN 193, ASP 194, ALA 195, ALA 196, PRO 197, GLU 198, ASP 199, GLU 206, VAL 208, GLN 209, GLY 210, SER 211, GLY 212, TYR 215, ARG 224, LEU 228, GLU 231, HIS 232, ASP 239, VAL 241, GLN 242, SER 243, ALA 267, LYS268, ALA 290, PRO 291, GLY 292, LEU 294, GLY 222, GLY 296, THR 297, TYR 298, ALA 299, GLU 358, PHE 360, GLU 361, HIS 365, ASN 366, PRO 368, ASP 369, ALA 370, LYS 371, THR 373, ALA 374, VAL 377, LEU 388, SER 389, CYS 390, GLY 391, PRO 392, TYR 393, TYR 394, ASN 395, VAL 396, ARG 398 etc and active site of PiZ protein comprises of amino acid residues such as LEU 18, GLN 19, GLN 20, ASN 21, VAL 34, ILE 41, LEU 42, ARG 43, GLU 44, ASP 45, PRO 48, GLN 49, MET 51, VAL 111, SER 112, THR 113, ASN 114, TYR 115, ASN 116, GLN 117, HIS 118, ALA 119, MET 120, LEU 137, TYR 138, GLY 139, ARG 140, THR 141, GLU 143, LEU 144, THR 145, SER 146, GLU 147, LEU 148, LYS 149, GLU 150, ASN 151, ILE 153, ARG 154, LYS 157, SER 158, GLY 160, LEU 161, PRO 162, GLU 163, ASN 164, ILE 166, VAL 167, PHE 168, PRO 169, VAL 170, PRO 171 etc. Most of the amino acid residues in the active site were hydrophobic so they are the main contributors to the receptor-ligand interaction.

Molecular Docking

Docking studies carried out in Argus Lab 4.0.1 (Taylor *et al.,* 2002). The protein and the ligand were loaded and docking studies were performed. While performing docking the protein and ligand appeared in a grid as shown below with its various binding configurations are analyzed and finally the list of best poses are given as output and saved as ArgusLab.agl files. Inhibition was measured by the binding energy of the best ligand pose measured in kcal/mol.

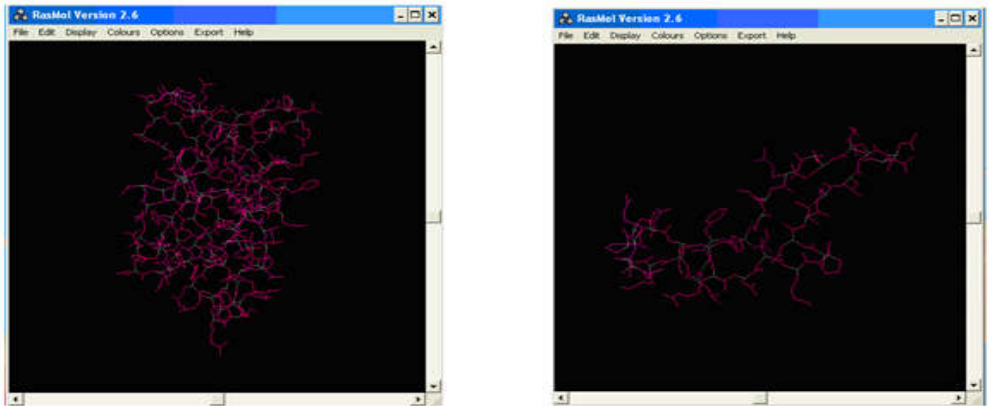
RESULTS AND DISCUSSION

Molecular Modeling

The 3D structure of the target proteins has been modeled by SWISS MODEL Workspace using the template proteins 3HWF and 1SZK. In this model protein, energy minimization work done by Swiss pdb. The modeled proteins were selected based on the value from Ramachandran plot.

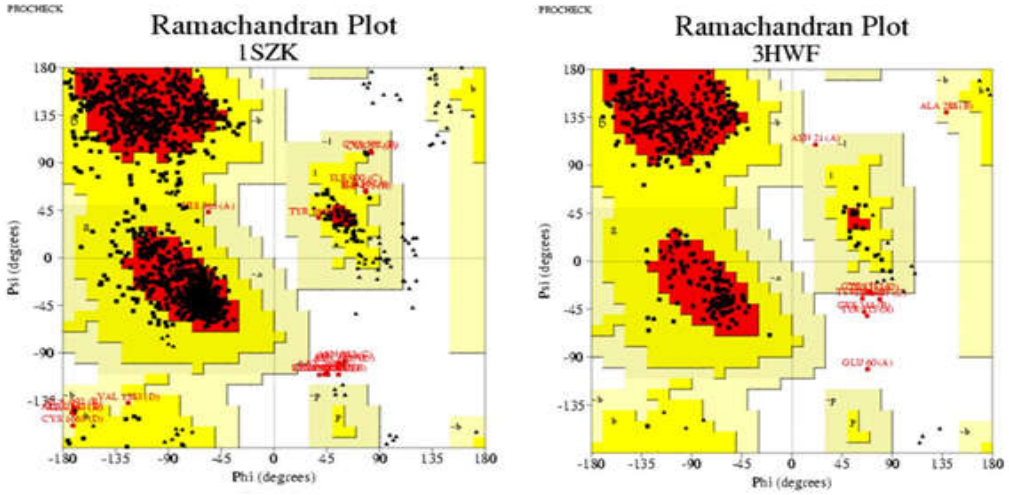
Structure verification

Ramachandran plot is a way to visualize dihedral angles phi against psi of amino acid residues in protein structures. Ramachandran plot generated from our predicted model shows the GABA protein 87.1% most favored regions and 11.9% allowed regions. PiZ protein shows 86.8% most favored regions and 11.2% allowed regions. In Good quality model the



(A) (B)

Fig. 3. Visualization of 3D structure of GABA and PiZ protein using Swisspdb software



(A) (B)

Fig. 4. Ramachandran plot of GABA and PiZ protein

Table 2. Ramachandran Plot Statistics

	GABA Protein		PiZ Protein	
Residues in most favoured regions (A,B,L)	1258	87.1%	389	86.8%
Residues in additional to allowed regions (a,b,l,p)	166	11.9%	50	11.2%
Residues in generously allowed regions (-a,-b,-l,-p)	12	0.8%	3	0.7%
Residues in disallowed regions	8	10.6%	6	1.3%
Number of non – Glycine and non – Proline residues	1444	100.00%	448	100.0%
Number of end – residues (excl. Gly and Pro)	713		42	
Number of Glycine residues (Shown as triangles)	168		27	
Number of Proline residues	80		33	
Total Number of residues	2405		550	

most favored regions and allowed regions combines and it should come above 90%. The Ramachandran plot shows the phi-psi torsion angles for all residues in the structure. Glycine residues are separately identified by triangles as these are not restricted to the regions of the plot appropriate to the other side chain types. The colouring/shading on the plot represents the different regions: the darkest areas (here shown in red) correspond to the "core" regions representing the most favorable combinations of phi-psi values. Ideally, one would hope to have over 90% of the residues in these "core" regions. The percentage of residues in the "core" regions is one of the better guides to stereo chemical quality.

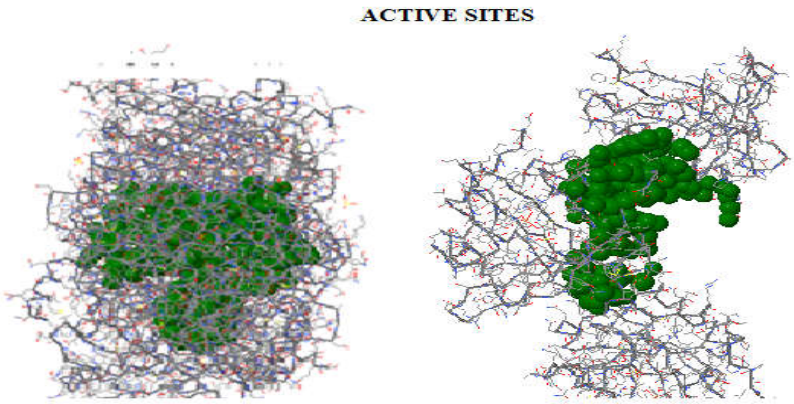


Fig. 5. Binding site of GABA and PiZ protein. In this picture grey colour depicts the whole protein structure and green colour is the binding site of protein.

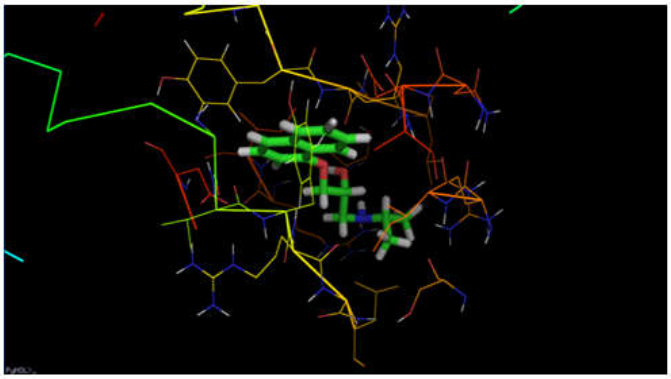


Fig. 6. Docking analysis of GABA protein with Beta sitosterol (protein represents wire frame and ligand is stick model)

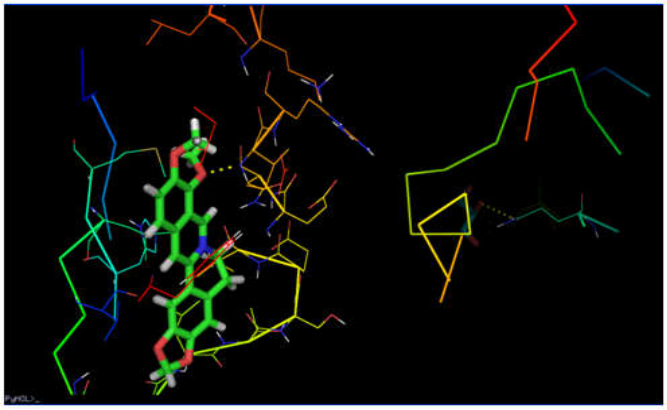


Figure 7. Docking analysis of PiZ protein with Berberine (protein represents wire frame and ligand is stick model

Table 3. Docking Energy Scores

Receptor		Ligands	Docking Energy
GABA	Synthetic compounds	Vigabatrin	-7.37569 kcal/mol
		Zolpidem	-7.98269 kcal/mol
	Natural Compounds	Berberine	-7.90784 kcal/mol
		Mimosine	-7.08339kcal/mol
		Beta sitosterol	-8.96553 kcal/mol
PiZ protein	Synthetic compounds	Gentamicin	-7.30579kcal/mol
		Linezolid	-6.88117kcal/mol
	Natural Compounds	Berberine	-9.66677 kcal/mol
		Mimosine	-7.16121 kcal/mol
		Beta sitosterol	-9.41023 kcal/mol

Docking Analysis

The docking studies were analysed for natural compounds like Beta sitosterol, Mimosine, Berberine and synthetic compounds like Tiagabine, Vigabatrin, Zolpidem, Chloramphenicol, Gentamicin and Linezolid.The interactions were stronger (energetically lesser) for two out of ten compounds which are used for docking simulation. The docking scores were thehighest for Beta sitosterol with -8.96553 kcal/mol docking energy in GABA protein and Berberine is showing the highest score for PiZ protein with -9.66677 kcal/mol docking energy. Figure 6&7 shows the crucial interaction between best 2 compounds with GABA and with PiZ protein. Analysis of ligand binding interaction with the GABA and PiZ proteins can be useful for new preventive and therapeutic drugs for cancer. Docking results (Table 3) show the better binding energy scores with synthetic and natural compounds. Finally concluded the result GABA protein binds with Beta sitosterol, the best binding energy with other compounds with binding energy score -8.96553 kcal/mol. The best binding energy for PiZ protein is with Berberine compound, binding energy score is -9.66677 kcal/mol. The results obtained from this study would be useful in understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of new inhibitors on the basis of docking scores.

CONCLUSION

The results of our present study can be useful for the design and development of novel compounds having better inhibitory activity against Liver cancer. The comparative docking studies against these proteins revealed that the *Justicia adhatoda, Vitex negundo and Mimosa pudica's* chemical compounds used as drugs to treat liver diseases. Thus with the least binding energy and with no toxicity risk at all ensures that the ligands to be a good drugs that can act in an effective way against the GABA and PiZ proteins. The potential drug candidates can further be analyzing in wet lab studies. Thisstudy suggests that these proteins can be used as a potential targets for further studies on Liver cancer and these three plant chemical compounds as a natural drug compounds for the identification of new drugs for cancer in future. On the basis of results obtained in the present study, it can be concluded that *Justicia adhatoda* has high hepatoprotective activity than *Mimosa pudica* and *Vitex negundo*.

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