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A COMPUTATIONAL DRUG DESIGNING FROM REPORTED ACTIVE PRODUCT OF *Andrographis Panicultata* TO CURE HEPATIC TOXICITY

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ABSTRACT

Andrographis panicultata (Kaalmegha) has long been used for hepatotoxicity according to ancient herbal practices. One of the major active products of the herbal plant is Silymarin. Silymarin consists of four flavonolignan isomers namely- silybin/silibinin, isosilybin, silydianin and silychristin. Here, the computational approach shows that silybin/silibinin binds to IGFBP3 and ALOX5, two major proteins that are upregulated as a body defense mechanism during liver damage. IGFBP3 is an IGF1 binding protein. IGF1 is involved in curing hepatotoxicity. On the contrary ALOX5 is an enzyme. Both the protein molecules can be potential targets of silibinin. Silibinin 3D structure was docked to the target proteins as a ligand. The scores for such docking was satisfactory. However, this conclusion should be subjected to further in vitro experiment as in biological system often that binds irreversibly is not the best effector. In final analysis, satisfactory binding scores explain a novel mechanism of how silibinin may act to cure hepatic toxicity. This research, although was focused to find the target of the afore-mentioned ligand, in the future may pave the way for a synthetic drug. It should also boost our confidence on herbal practices and thereby link a bridge between traditional and modern treatment techniques.

KEYWORDS

Andrographis panicultata, IGFBP3, ALOX5, Silymarin, Molecular Docking.

1 INTRODUCTION

Silymarin, a flavonolignan plant is used almost exclusively for hepatoprotection (Colturato, Constantin et al, Salamone, Galvano et al.). The use of silymarin may replace the polyherbal formulations and will avoid the major problems of standardization, quality control and contamination with heavy metals or bacterial toxins (Girish 2006). Silymarin consists of four flavonolignan isomers namely- silybin, isosilybin, silydianin and silychristin. Among them, silybin/silibinin is the most active and commonly used form. Silymarin is orally absorbed and is excreted mainly through bile as sulphates and conjugates. Silymarin offers good protection in various toxic models of experimental liver diseases in laboratory animals (Haddad, Vallerand et al. 2009). It acts by antioxidative, anti-lipid peroxidative, antifibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating mechanisms. Silymarin has clinical applications in

alcoholic liver diseases, liver cirrhosis, Amanita mushroom poisoning, viral hepatitis, toxic and drug induced liver diseases and in diabetic patients (Hruby, Csomos et al. 1983; Vogel, Tuchweber et al. 1984; Dehmlow, Erhard et al. 1996; von Schonfeld, Weisbrod et al. 1997). Though silymarin does not have antiviral properties against hepatitis virus (Ahmed-Belkacem, Ahnou et al. ; Dahari, Guedj et al. ; Eurich, Bahra et al. ; Neumann, Biermer et al.), it promotes protein synthesis, helps in regenerating liver tissue, controls inflammation (Zi, Zhang et al. 2000; Cheung, Taylor et al. 2007; Wang, Tashiro et al. 2008), enhances glucuronidation and protects against glutathione depletion (Lin, Sukarieh et al. 2009). Silymarin may prove to be a useful drug for hepatoprotection in hepatobiliary diseases and in hepatotoxicity due to drugs. The nontraditional use of silymarin may make a breakthrough as a new approach to protect other organs in addition to liver (Singh and Agarwal 2006; Cheung, Vesey et al. 2007; Singh, Deep et al. 2007; Jung, Park et al. 2009; Kim, Kim et al. 2009). As it is having a good safety profile, better patient tolerability and an effective drug at an affordable price, in near future new derivatives or new combinations of this drug may prove to be useful (Kim, Kim et al. 2003; Tyagi, Agarwal et al. 2003).

The Insulin-like growth factor-binding protein also known as IGFBP serves as a carrier protein for Insulin-like growth factor 1 (IGF-1). IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors. It is also reported to Interacts with XLKD1 and bind IGF2 more than IGF1 and thereby forms a ternary complex of about 140 to 150 kDa with IGF1 or IGF2 and a 85 kDa glycoprotein ALS named Insulin-like Growth Factor Acid-labile Subunit (IGFALS). It is expressed by most tissues and also present in plasma. One key observation is that IGFBP3 levels are higher during extra-uterine life and it elevates during puberty (Cubbage, Suwanichkul et al. 1990; Jasper, Pennisi et al. 1999).

IGFBP3 gene is a member of the insulin-like growth factor binding protein (IGFBP) family and encodes a protein with an IGFBP domain and a thyroglobulin type-I domain. In this form, it circulates in the plasma, prolonging the half-life of IGFs and altering their interaction with cell surface receptors. Alternate transcriptional splice variants, encoding different isoforms, have been characterized.

IGFBP3 protein levels decrease during the progression of prostate cancer from benign prostatic hyperplasia (BPH) to its metastatic form (Natsuizaka, Naganuma et al.; Regel, Eichenmuller et al. ; Wu, Buckner et al.). But, production of the protein does not cease completely. IGFBP3 is still made (at a lower level) by prostate cancer cells and secreted into the surrounding environment (Schildkraut, Demark-Wahnefried et al. 2005; Povelitsa 2007). However, instead of the full length, functional protein, IGFBP3 is found to be cleaved. This prevents IGFBP3 from binding and sequestering IGFs and the growth factors are free to bind the IGF-1R and promote cell survival

Approximately 98% of IGF-1 is always bound to one of six binding proteins (IGF-BP). IGFBP3, the most abundant protein, accounts for 80% of all IGF binding protein. IGF-1 binds to IGFBP3 in a 1:1 molar ratio. IGF-BP also binds to IGF-1 inside the liver, allowing growth hormone to continuously act upon the liver to produce more

IGF-1. This is important because proliferating IGF-1 + IGF-BP complex allow growth of the hepatic cells (Cubbage, Suwanichkul et al. 1990; Lovett-Racke, Bittner et al. 1998; Jasper, Pennisi et al. 1999).

Arachidonate 5-lipoxygenase, also known as 5-lipoxygenase or 5-LO, is an enzyme that in humans is encoded by the ALOX5 gene. Arachidonate 5-lipoxygenase is a member of the lipoxygenase family of enzymes (Kennedy, Diehl et al. 1991; Kim, Choi et al. 2005; Chen, Li et al. 2009). It transforms essential fatty acids into leukotrienes and is a current target for pharmaceutical intervention in a number of diseases. 5-LO catalyzes oxidation of AA (arachidonic acid) at the 5-position to yield 5-hydroperoxyeicosatetraenoic acid (5-HPETE). 5-LO then converts 5-HPETE to leukotriene A4. Two other lipoxygenases, 12-LO and 15-LO, act at the 12- and 15-positions, yielding 12- and 15-HPETE. These pathways lead to the leukotriene 12-hydroxyeicosatetraenoic acid (12-HETE) and to the lipoxins, respectively (KEGGPathwayDatabase).

This gene encodes a member of the lipoxygenase gene family and plays a dual role in the synthesis of leukotrienes from arachidonic acid (Funk, Hoshiko et al. 1989; Hoshiko, Radmark et al. 1990; Samuelsson, Hoshiko et al. 1991). The encoded protein, which is expressed specifically in bone marrow-derived cells, catalyzes the conversion of arachidonic acid to 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid, and further to the allylic epoxide5(S)-trans-7,9-trans-11,14-cis-eicosatetrenoic acid (leukotriene A4)(Kim, Choi et al. 2005; Manev and Manev 2006).

Leukotrienes are important mediators of a number of inflammatory and allergic conditions. Mutations in the promoter region of this gene lead to a diminished response to antileukotriene drugs used in the treatment of asthma and may also be associated with atherosclerosis and several cancers. Alternatively spliced transcript variants encoding different isoforms have been found for this gene (Kim, Choi et al. 2005).

FIGURE 1: *Andrographis panicultata* plant. The plant is found in Sylhet district in Bangladesh. The herbaceous plant has distinctive white flowers.

Andrographis panicultata is a herbaceous plant in the family *Acanthaceae*, native to Bangladesh and India (Alagesaboopathi, Diwakaran et al. 1999; Zhang 2000; Alagesaboopathi, Dwarakan et al. 2001). It is widely used as a cardiac and (Awang, Abdullah et al. ; Nagalekshmi, Menon et al.) hepatoprotective agent (Nagalekshmi, Menon et al. ; Ojha, Bharti et al. ; Ojha, Bharti et al. ; Handa and Sharma 1990; Balu and Alagesaboopathi 1993; Kapil, Koul et al. 1993; Puri, Saxena et al. 1993; Panossian, Hovhannisyan et al. 2000; Singh, Banerjee et al. 2001; Trivedi and Rawal 2001; Sheeja and Kuttan 2006). Mostly the leaves and roots were used for medicinal Purposes as Hepatoprotective, cholinergic, antispasmodic, stomachic, anthelmintic, alterative, blood purifier and febrifuge agent.(Chao, Kuo et al. ; Chien, Wu et al. ; Chao, Kuo et al. 2009). It acts well on the liver, promoting secretion of bile. It is used in jaundice and torpid liver, flatulence and diarrhoea of children, colic, strangulation of intestines and splenomegaly; also for cold and upper respiratory tract infections (Sulaiman, Zakaria et al. ; Mandal, Dhara et al. 2001; Geethangili, Rao et al. 2008; Burgos, Hancke et al. 2009; Chandrasekaran, Thiyagarajan et al. 2009; Das, Gautam et al. 2009; Lin, Wu et al. 2009; Pekthong, Blanchard et al. 2009; Rattanachaikunsopon and Phumkhachorn 2009). It is also known as Kaalmegha locally.

Kaalmegha dried leaves and tender shoots yield not less than 1% andrographolide on dry-weight basis (Chakravarti and Chakravarti 1951; Rajani, Shrivastava et al. 2000). Several active constituents have been identified from the leaf and rhizome, including andrographolide, deoxyandrographolide and other diterpenes (Chao and Lin ; Jayaprakasam, Gunasekar et al. 2001; Rao, Harikishore et al. 2002; Kesava Reddy, Vijaya Bhaskar Reddy et al. 2003; Kesava Reddy, Vijaya Bhaskar Reddy et al. 2003; Qizhen, Jerz et al. 2003; Rao, Damu et al. 2003; Koteswara Rao, Vimalamma et al. 2004; Kumar, Sridevi et al. 2004; Pholphana, Rangkadilok et al. 2004; Chen, Qu et al. 2006). Andrographolide exhibited strong choleretic action when administered intraperitoneally (i.p.) to rats. It induces increase in bile flow together with change in physical properties of bile secretion. It was found to be more potent than silymarin. Andrographolide was found to be almost devoid of antihepatitis-B virus surface antigen-like activity (Chandrasekaran, Gupta et al; Maiti, Mukherjee et al. ; Singha, Roy et al. 2003). The leaf and stem extracts of Kaalmegha andrographolide given subcutaneously (s.c.) or orally did not change blood sugar level of normal or diabetic rats (Akbar; Pan, Abd-Rashid et al.; Parichatikanond, Suthisisang et al. ; Borhanuddin, Shamsuzzoha et al. 1994; Zhang, Tang et al. 1994; Zhang and Tan 2000; Zhang and Tan 2000).

Alcoholic extract of the plant exhibited antidiarrhoeal activity against *E. coli* enterotoxins in animal models (Mishra, Mishra et al. 2009). Clinical evidence of effectiveness of andrographis in humans is limited to the common cold. Preliminary evidence suggests that it might increase antibody activity and phagocytosis by macrophages, and might have mast cell-stabilizing and antiallergy activity. (Natural Medicines Comprehensive Database, 2007) The herb is contraindicated in bleeding disorders (Wang and Zhao 1994; Sheeja, Guruvayoorappan et al. 2007; Liu, Wang et al. 2008; Verma and Vinayak 2008), hypotension (Reyes, Bautista et al. 2006; Yoopan, Thisoda et al. 2007; Neogy, Das et al. 2008), as well as male and female sterility (exhibited activity against infertility in laboratory animals)(Mkrtchyan, Panosyan et al. 2005). Whole plant juice is taken at a dose of 5–10 ml;

50–100 ml decotion; 1–3 g powder (Li, Zhou et al. ; Xu and Wang).

AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure (Cosconati, Forli et al. ; Goodsell, Morris et al. 1996; Morris, Huey et al. 2008. Current distributions of AutoDock consist of two generations of software: AutoDock 4 and AutoDock Vina. AutoDock 4 actually consists of two main programs: autodock performs the docking of the ligand to a set of grids describing the target protein; autogrid pre-calculates these grids (Seeliger and de Groot ; Goodsell 2009). In addition to using them for docking, the atomic affinity grids can be visualised. This can help, for example, to guide organic synthetic chemists design better binders (Tiwari, Mahasenan et al. 2009). AutoDock Vina does not require choosing atom types and pre-calculating grid maps for them. Instead, it calculates the grids internally, for the atom types that are needed, and it does this virtually instantly.

PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock/) is a computational tool for determining protein ligand or protein protein interaction (Schneidman-Duhovny, Inbar et al. 2005). Its algorithm is based on object recognition and image segmentation techniques used in Computer Vision. It actually mimics human vision and extracts image data from protein structure files such as in .pdb file extension. Docking can be compared to assembling a jigsaw puzzle. When solving the puzzle it is tried to match two pieces by picking one piece and searching for the complementary one (Mashiach, Schneidman-Duhovny et al.). A puzzle-solver concentrates on the patterns that are unique for the puzzle element and look for the matching patterns in the rest of the pieces. PatchDock employs a similar technique. Given two molecules, their surfaces are divided into patches according to the surface shape. These patches correspond to patterns that visually distinguish between puzzle pieces. Once the patches are identified, they can be superimposed using shape matching (Schneidman-Duhovny D 2003).

Pocket-Finder (http://www.modelling.leeds.ac.uk/pocketfinder/), an online computational tool to predict active site within a structure, is based on the Ligsite algorithm written by Hendlich *et al.(Huang and Zou 2006; Huang and Zou 2006)*. Pocket-Finder was written to compare Geometric shape complementarity pocket detection with our new ligand binding site detction algorithm Q-SiteFinder.

REACTOME (http://www.reactome.org/) is an open-source, open access, manually curated and peer-reviewed pathway database (Croft, O'Kelly et al.; Stein 2004). Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. These include NCBI Entrez Gene, Ensembl and UniProt databases, the UCSC and HapMap Genome Browsers, the KEGG Compound and ChEBI small molecule databases, PubMed, and Gene Ontology. The rationale behind Reactome is to convey the rich information in the visual representations of biological pathways familiar from textbooks and articles in a detailed, computationally accessible format. The core unit of the Reactome data model is the reaction. Entities (nucleic acids, proteins, complexes and small molecules) participating in reactions form a network of biological interactions and are grouped into pathways. Examples of biological

pathways in Reactome include signaling, innate and acquired immune function, transcriptional regulation, translation, apoptosis and classical intermediary metabolism.

STRING 9.0 (Search Tool for the Retrieval of Interacting Genes/Proteins) database (http://string-db.org/) (Szklarczyk, Franceschini et al.) is an online public database where data are deposited from experimental as well as predicted sources. For a system understanding annotation of all the proteins are an absolute necessity . However, public efforts to collect and present protein interaction information have struggled to keep up with the pace of interaction discovery, partly because protein-protein interaction information can be error-prone and require considerable effort to annotate. STRING provides uniquely comprehensive coverage and ease of access to both experimental as well as predicted interaction information. Interactions in STRING are provided with a confidence score, and accessory information such as protein domains and 3D structures is made available, all within a stable and consistent identifier space. New features in STRING include an interactive network viewer that can cluster networks on demand, updated on-screen previews of structural information including homology models, extensive data updates and strongly improved connectivity and integration with third-party resources.

ChemDB (http://cdb.ics.uci.edu/)(Jonathan H. Chen 2007) is a chemical database with readily downloadable structures in pdb file format. It contains millions of commercially available small molecules, important for use as synthetic building blocks, probes in systems biology and as leads for the discovery of drugs and other useful compounds. The data is publicly available over the web for download and for targeted searches using a variety of powerful methods. The chemical data includes predicted or experimentally determined physicochemical properties, such as 3D structure, melting temperature and solubility. Recent developments include optimization of chemical structure (and substructure) retrieval algorithms, enabling full database searches in less than a second. A text-based search engine allows efficient searching of compounds based on over 65M annotations from over 150 vendors. When searching for chemicals by name, fuzzy text matching capabilities yield productive results even when the correct spelling of a chemical name is unknown, taking advantage of both systematic and common names. Finally, built in reaction models enable searches through virtual chemical space, consisting of hypothetical products readily synthesizable from the building blocks in ChemDB.

Swiss-PdbViewer (DeepView) (http://spdbv.vital-it.ch/) (Guex and Peitsch 1997) is an application that provides a user friendly interface allowing to analyze several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface.

PROCHECK (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) (Laskowski, Rullmannn et al. 1996) checks the stereochemical quality of a protein structure, producing a number of PostScript plots analysing its overall and residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR.

I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/)(Roy, Kucukural et al. ; Zhang 2008) server is an Internet service for protein structure and function predictions. 3D models are built based on multiple-threading alignments by LOMETS and iterative TASSER assembly simulations; function inslights are then derived by matching the predicted models with protein function databases. CPHmodels 3.2 is a protein homology modeling server. The template recognition is based on profile-profile alignment guided by secondary structure and exposure predictions. (Ambrish Roy 2011) (http://www.cbs.dtu.dk/services/CPHmodels/)(Nielsen, Lundegaard et al. 2010)

2. METHOD

2.1 Determining Interacting macromolecule

Interacting macromolecule has been predicted by using Small molecule-Protein interaction database named STITCH 3.1 (Search Tool for Interactions of Chemicals) (http://stitch.embl.de/) (Kuhn, von Mering et al. 2008). Protein to Protein interaction network was established using **STRING 9.0** (Search Tool for the Retrieval of Interacting Genes/Proteins) database (http://string-db.org/) (Szklarczyk, Franceschini et al.)

Protein to Protein interaction can further be confirmed by screening for binding informations in Database of Interacting Protein (DIP). Pathways associated to the proteins were viewed at Reactome database (http://www.reactome.org/).

Binding motifs can be searched by MotifFinder online tool. The tool retrieves interaction motifs in the proteins.

Toxnet database gives us gene up or downregulation information which facilitate choosing the target macromolecule.

2.2 Computational docking

The macromolecule structures were simulated ab initio using Protein 3D structure is the best indicator of the function as only this gives good account of how protein in native conformation behaves in vivo in biologically significant microenvironment. 3D structure was generated using **I-TASSER** (Roy, Kucukural et al. 2010) and **CphModel3.0** (Nielsen, Lundegaard et al. 2010).

The 3D Ligand structure was downloaded from online database ChemDB (http://cdb.ics.uci.edu/) and was subsequently docked to protein 3D structure of the macromolecule.

Ligand and macromolecule was docked using two programs- AutoDock Vina (Download link: http://vina.scripps.edu/download.html) and online tool PATCHDOCK (bioinfo3d.cs.tau.ac.il/PatchDock/).

2.3 Prediction analysis

Prediction of the structures were confirmed by online tool QMEAN, Swiss PDB Viewer (SPDV 4.0) and PROCHECK. The best 3D structure was selected on the basis of Ramachandran plot and Model quality Z-score. The best macromolecule structure was selected for docking with the downloaded ligand molecule.

The ligand bound protein structures provided by PATCHDOCK were viewed by RasWin and PyMol. The AutoDock results were analyzed using AutoDock tools -1.5.6rc3 and PyMol.

Protein pockets, candidates for ligand binding sites, were found by Pocket-Finder. The Pocket-

Finder tool results were screened with similar results found from binding. Thus this step can be viewed as a confirmatory step for the binding results.

3. RESULTS

3.1 Detarmination of Interacting macromolecule

The STITCH 3.1 database predicted multiple interaction macromolecules for Silibinin. From the interaction diagram we can see that it is somehow associated with a special type of Cytochrome P-450 (CYP3A4), Tumor suppressor protein P53, Cell signaling protein CDK4 and CDK2 and some more **(FIGURE 4 and 5)**. The result indicates that this small molecule may have important roles as a cancer drug (Fan, Qi et al. ; Fan, Yu et al. ; Wang, Ye et al. ; Zhang, Li et al. ; Agarwal, Singh et al. 2003; Tyagi, Agarwal et al. 2003) and rightly so as it has been proved to be very effective against hepatocellular carcinoma (HCC).

However the interaction does not gives us clear information regarding the binding pattern of the small molecule and subsequent signal transduction **(FIGURE 3)**. To estimate binding of the molecules we needed to perform the docking experiment.

CTD data for silibinin retrieved up and downregulation gene information but failed to put insight into protein binding information (**FIGURE 2**). Following are the genes with maximum confidence whose expression is changed due to silibinin administration-
ABCB11, AKR1C3, ATM, BAX, BCL2, BID, CASP2, CASP3, CASP8.

Related Genes:

Gene:	ABCB11 **[Link to main CTD database]**
Organism:	Rattus norvegicus
Interaction Type:	affects activity | affects localization
Interaction:	**silybin** affects the localization of and affects the activity of ABCB11 protein
Synonyms:	ABC16 | ABC member 16, MDR/TAP subfamily | ATP-binding cassette, subfamily B (MDR/TAP), member 11 | ATP-binding cassette sub-family B member 11 | ATP-binding cassette, sub-family B, member 11 | bile salt export pump | BOS_1671 | BRIC2 | Bsep | BSEP/SPGP | Lith1 | liver bile salt export pump | PFIC2 | PFIC-2 | PGY4 | progressive familial intrahepatic cholestasis 2 | RP23-451M16.2 | sister of P-glycoprotein | sister p-glycoprotein | SPGP
PubMed References:	(1)15763547

FIGURE 2: Screenshot of the CTD result. CTD retrieves gene expression information. Given above is an example screenshot.

Binding motifs can be searched by MotifFinder online tool. The tool retrieves interaction motifs in the proteins. Based on literature information and MotifFinder results 2 proteins were selected as potential target. They are- Arachidonate 5-lipoxygenase (ALOX5) and Insulin-like growth factor-binding protein 3 (IGFBP3).

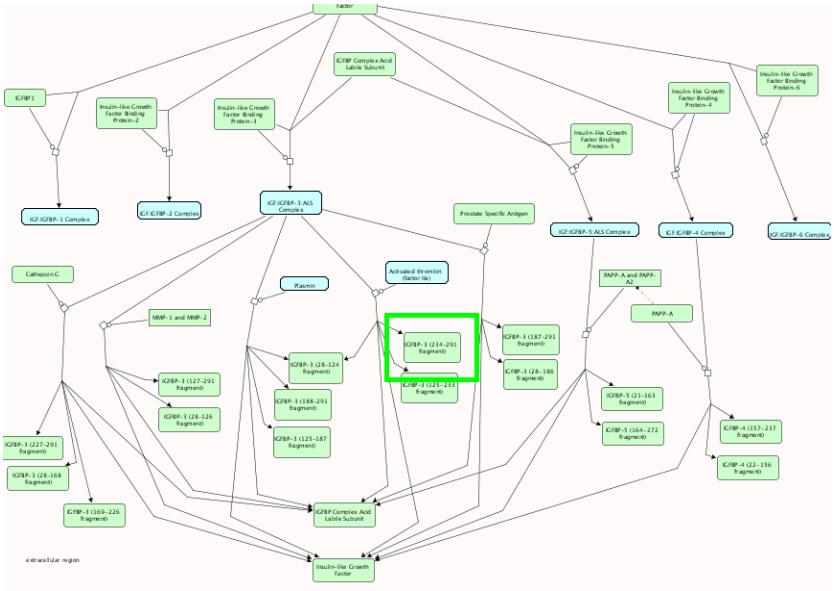


FIGURE 3: IGFB3 Reactome result: The working mechanism of the protein IGFBP3 was retrived from interactome database Reactome. The marked in green box represents IGFBP3's position amidst the cellular system. The network seen here has the parent node named 'Diabetes disease'.

From the network databases it was clear that these two proteins play important roles in relieving hepatocellular toxicity. Insulin like Growth Factor (IGF-1) is produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion. However, the hypothesis that IGF-1 stimulation is triggered by the drug could only be proven by docking experiment.

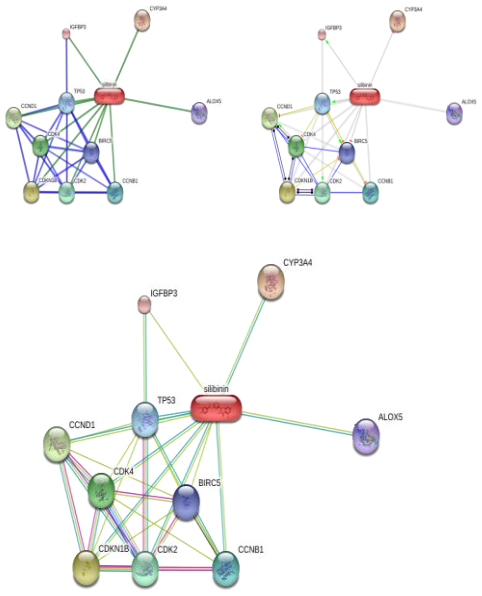
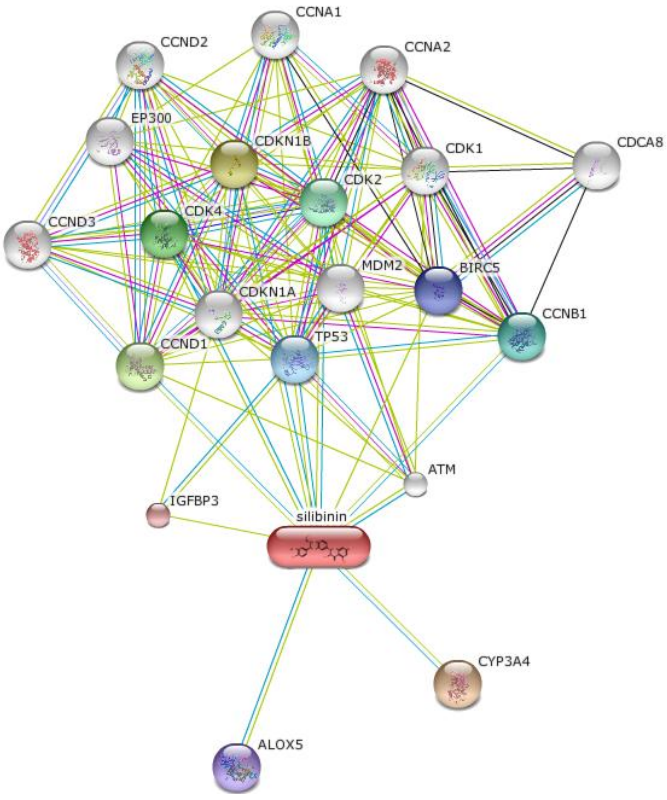


FIGURE 4: STITCH 3.1 results: The interactions are viewed in three different modes. Top left one is the confidence view and the top right one represents the action view. The bottom one represents the evidence view. For IGFBP3 some activation evidences are available. However ALOX5 relationship was left out unannotated.

IGFBP3 seems to interact with few more proteins whereas ALOX5 is more concerned with enzymatic reactions instead of binding. Both the roles are important in case of curing hepatotoxicity.



Your Input:

		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
silibinin	Silibinin, also known as silybin, is the major active constituent of silymarin, the mixture of flavonolignans extracted from milk thistle (Silybum marianum) consisting of silibinin A and B, isosilibinin A and B, silicristin and silidianin. Both in vitro and animal research suggest that silibinin has hepatoprotective (antihepatotoxic) properties that protect liver cells against toxins. (482.4 g/mol) *(Homo sapiens)*									

Predicted Functional Partners:

CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4; Cytochromes P450 are a group of heme-thi [...] (503 aa)						•	•		0.991
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1); Important regulator of cell cycle progression [...] (198 aa)						•	•		0.970
CCND1	cyclin D1; Essential for the control of the cell cycle at the G1/S (start) transition (295 aa)						•	•		0.965
CDK4	cyclin-dependent kinase 4; Probably involved in the control of the cell cycle (303 aa)						•	•		0.961
CDK2	cyclin-dependent kinase 2; Involved in the control of the cell cycle. Interacts with cyclins A, [...] (298 aa)						•	•		0.958
CCNB1	cyclin B1; Essential for the control of the cell cycle at the G2/M (mitosis) transition (433 aa)						•	•		0.952
TP53	tumor protein p53; Acts as a tumor suppressor in many tumor types; induces growth arrest or apo [...] (393 aa)						•	•		0.945
BIRC5	baculoviral IAP repeat-containing 5; Component of the chromosomal passenger complex (CPC), a co [...] (165 aa)							•		0.935
ALOX5	arachidonate 5-lipoxygenase; Catalyzes the first step in leukotriene biosynthesis, and thereby [...] (674 aa)						•	•		0.932
IGFBP3	insulin-like growth factor binding protein 3; IGF-binding proteins prolong the half-life of the [...] (297 aa)							•		0.919

FIGURE 5: STRING 9.0 Results: The interactions are viewed expanding the protein network for better insight into the drug action. ALOX5 did not parented any nodes even in the expanded mode. That gives rise to two possibilities; one is that it is a poor target, the other one is that it is a special case target that works as a 'magic bullet' during special conditions such as liver toxicity. The later one seemed to be the case after the final analysis.

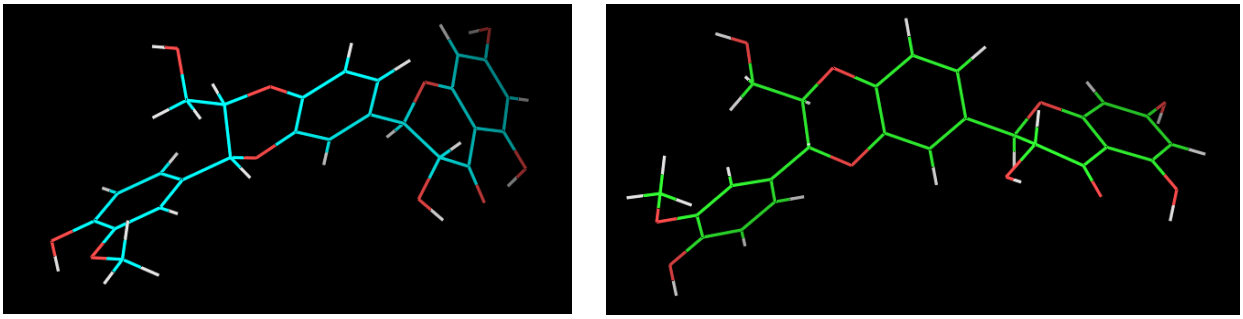


FIGURE 6: Ligand Structures as downloaded from the Databases: 3D structures of the ligands- the structure of Silibinin in two different conformations.

3.2 Computational Prediction

The ligands **(FIGURE 6)** were docked to the proteins using two docking tools AutoDock and PATCHDOCK. PATCHDOCK gave the following results for solution 1 and 4 for ALOX5- **(Table 1)**

Table 1: PATCHDOCK results for docking with ALOX5

Solution	Score	Area	ACE	Transformation
1	6354	719.70	-131.89	-1.86 -0.87 -0.30 0.91 -18.09 -9.73
4	5842	745.60	-232.93	-2.52 -0.37 -1.08 -1.15 -8.99 -4.89

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE	HB
1	4	-63.26	-31.66	8.31	-14.11	0.00
2	3	-59.35	-24.58	4.28	-16.37	0.00

Following are the results for IGFBP3- **(Table 2)**

Table 2: PATCHDOCK results for docking with IGFBP3

Solution	Score	Area	ACE	Transformation
1	7990	1084.60	-407.31	-0.23 1.08 -1.59 -8.54 8.17 -14.99
2	7836	944.00	-277.01	0.35 -0.62 2.50 59.33 10.92 -10.04

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE	HB
1	1	-68.90	-31.48	22.23	-25.30	0.00
2	3	-62.80	-27.70	10.03	-18.94	0.00

Following are the Solutions and their corresponding binding scores (Binding affinity in Kcal/mol, RMSD upper and lower cut) for AutoDock for ALOX5- **(Table 3)**

Table 3: AutoDock results for Silybin-ALOX5 binding

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Silibinin (conformation1)	-9.2	0	0
Silibinin (conformation2)	-8.9	9.662	5.865
Silibinin (conformation3)	-8.8	9.985	5.5
Silibinin (conformation4)	-8.3	39.865	34.269
Silibinin (conformation5)	-8.2	41.031	37.613
Silibinin (conformation6)	-8	37.21	34.141
Silibinin (conformation7)	-7.8	40.883	35.545
Silibinin (conformation8)	-7.8	28.25	24.548
Silibinin (conformation9)	-7.8	42.605	36.524

Following are the Solutions and their corresponding binding scores (Binding affinity in Kcal/mol, RMSD upper and lower cut) for AutoDock for IGFBP3- **(Table 4) (FIGURE 7, 8, 9 and 10)**

Table 4: AutoDock results for Silybin-IGFBP3 binding

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Silibinin (conformation1)	-8.1	0	0
Silibinin (conformation2)	-7.7	5.531	2.907
Silibinin (conformation3)	-7.4	62.518	59.188
Silibinin (conformation4)	-7.3	3.222	1.689
Silibinin (conformation5)	-7.1	30.248	26.748
Silibinin (conformation6)	-7.1	9.971	1.806
Silibinin (conformation7)	-7.1	29.515	25.831
Silibinin (conformation8)	-7	10.839	3.095
Silibinin (conformation9)	-6.9	31.057	27.974

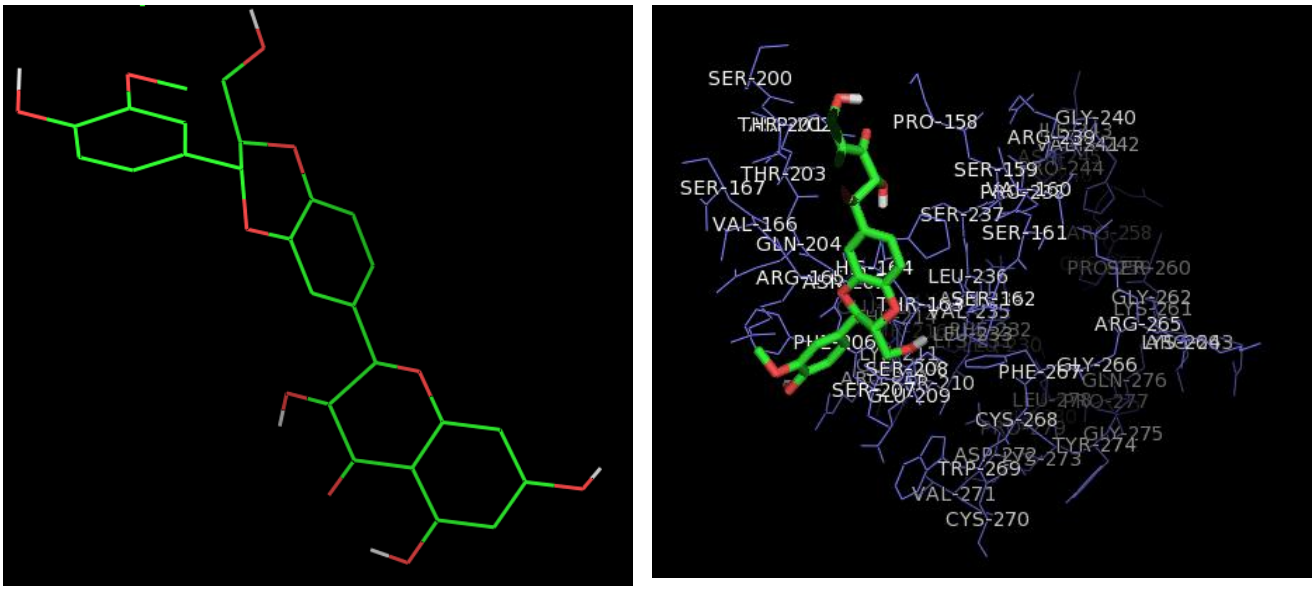


FIGURE 7: Best binding conformation of the ligand: At the left the binding conformation of the silibinin ligand after binding to ALOX5 is portrayed. At the right the binding conformation of the ligand is represented with IGFBP3 protein in a wireframe structure with residue lebelling.

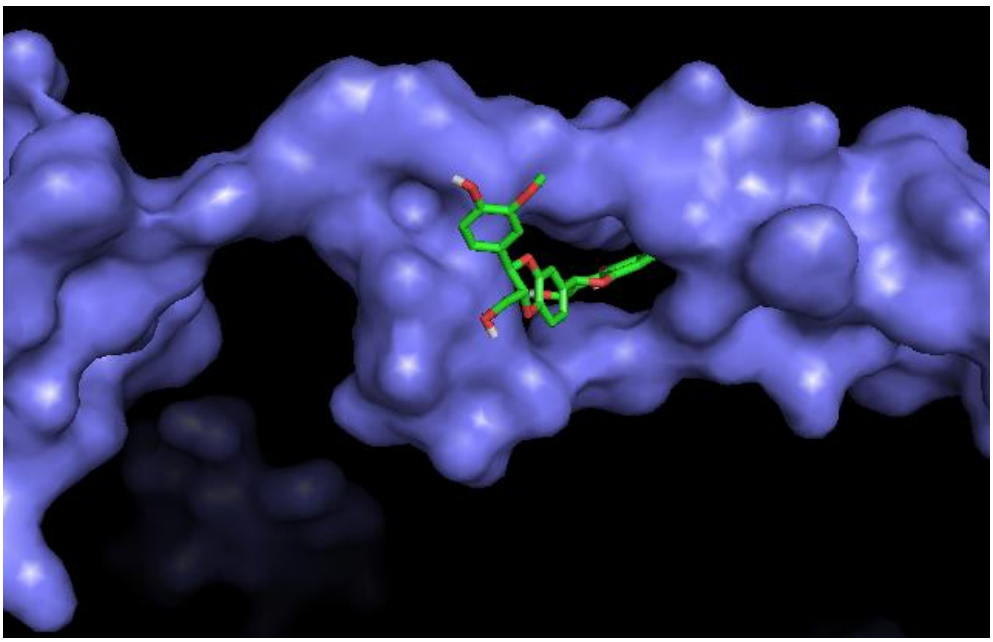


FIGURE 8: Ligand bound to the binding site in IGFBP3: Molecular surface of the protein IGFBP3 is marked in blue. The ligand (marked in green and red) is bound to the binding site.

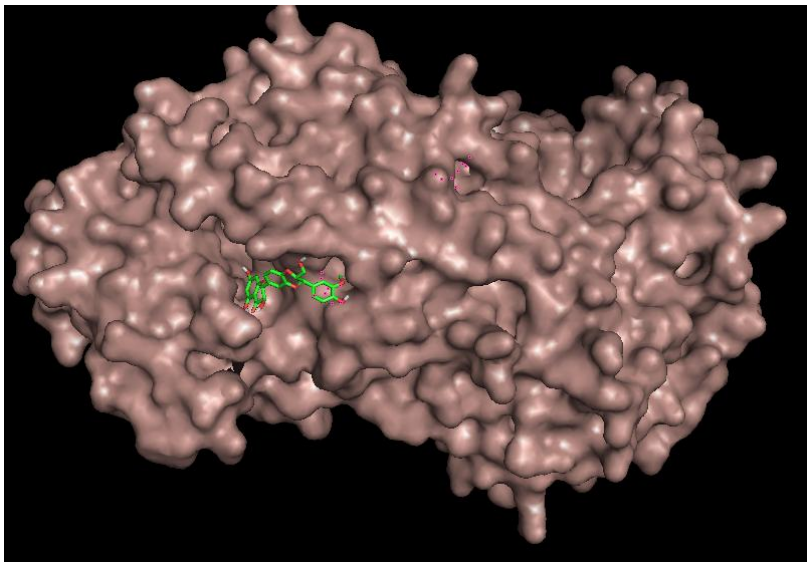


FIGURE 9: **Ligand bound to the binding site in ALOX5:** Molecular surface of the **protein** ALOX5 is marked in cocoa color. The ligand (marked in green and red) is bound to the binding site.

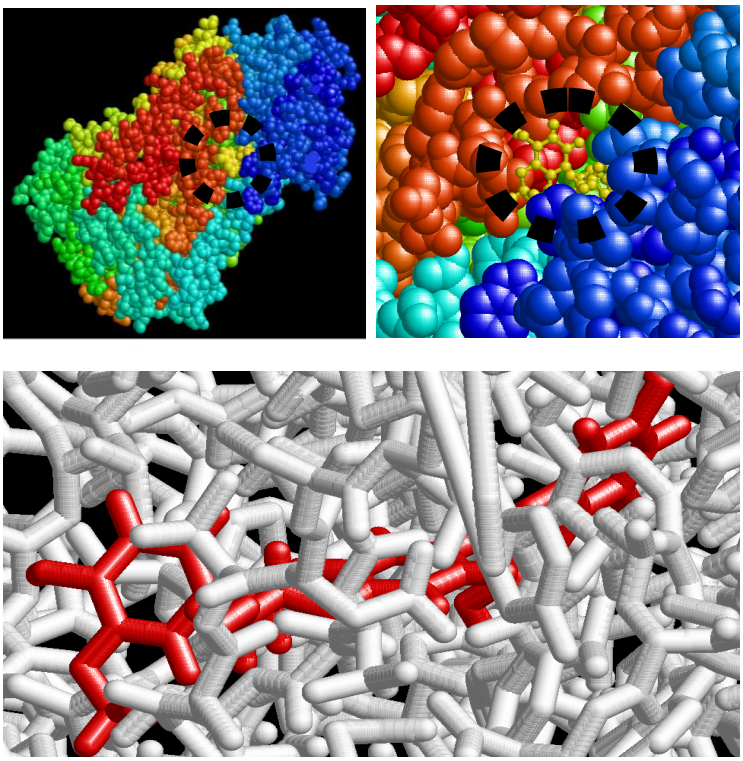


FIGURE 10: Ligand Binding to ALOX5 as viwed by RasWin. Ligand bound to the binding site is marked in Stick representation in yellow (top right and left, circled in black dotted lines). Ligand (marked in red, bottom) protrudes the protein structure to a good extent.

PocketFinder computational tool was used to find active site within the protein strudture. Although the tool gives positive results for few potential active site, only the predicted site 1 in both the cases (**FIGURE 11 AND 12**) shows significance within confidence interval. The active site correlates excellently to the one cleft where ligand binds. Thus, it confirms tight binding of the ligand in the exact groove where predicted by docking tools as both the results correlates well to each other.

Site 1 ☑
Site 2 ☐
Site 3 ☐
Site 4 ☐
Site 5 ☐
Site 6 ☐
Site 7 ☐
Site 8 ☐
Site 9 ☐
Site 10 ☐

Help!
Download
Start Again

Site Info:

Predicted site 1

Site Volume: 2788 Cubic Angstroms

Protein Volume: 64976 Cubic Angstroms

Binding Box Around Selected Sites

Min Coords: (-24, -21, -36)
Max Coords: (17, 13, 10)

Residues:

75	CA	GLY	A	11
76	C	GLY	A	11
78	N	SER	A	12
80	CB	SER	A	12
82	C	SER	A	12
84	N	GLN	A	13
87	CG	GLN	A	13
88	CD	GLN	A	13
89	OE1	GLN	A	13
90	NE2	GLN	A	13
520	CD2	LEU	A	67
641	OD1	ASP	A	80
657	C	TRP	A	81
658	O	TRP	A	81
659	N	TYR	A	82
660	CA	TYR	A	82

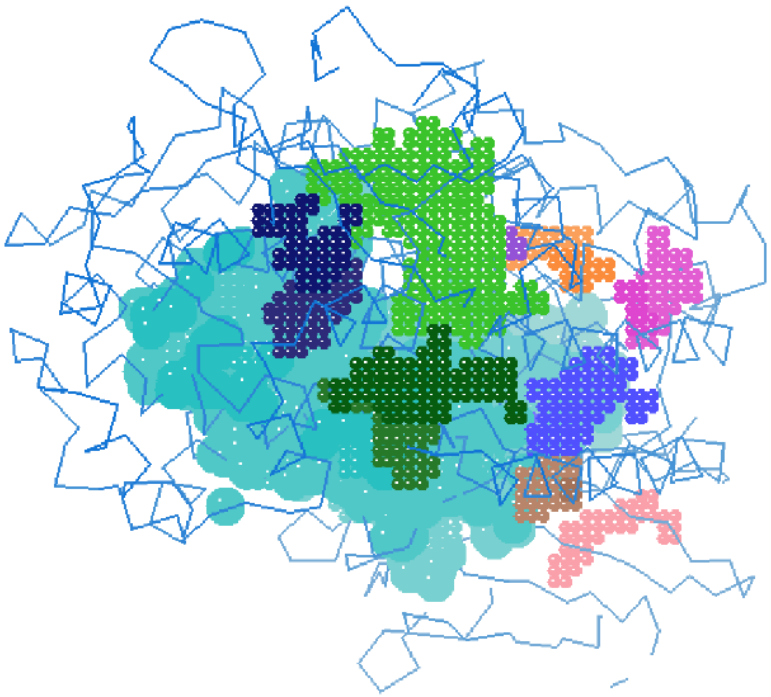
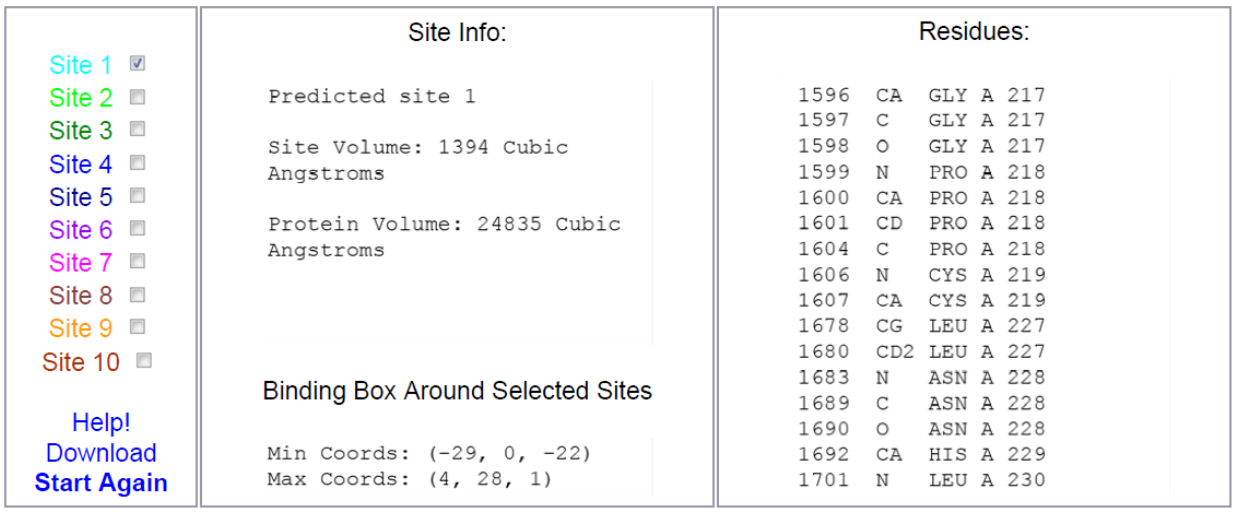


FIGURE 11: Pocket-Finder results for surface cleft identification of ALOX5. Binding site of the ligand), as predicted by the tool. The cleft colored in cyan shows a significant pocket. This pocket correlates well with the binding site as predicted by the docking tools.



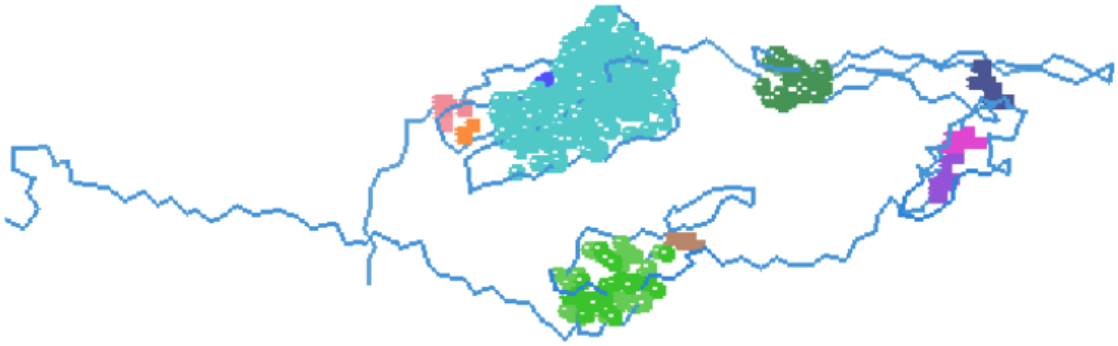


FIGURE 12: Pocket-Finder results for surface cleft identification of IGFBP3. Binding site of the ligand), as predicted by the tool. The cleft colored in cyan shows a significant pocket. This pocket correlates well with the binding site as predicted by the docking tools.

3.3 Prediction analysis

Ramachandran plot analysis was done to measure the robustness of the structure. Both the structure were robust. However, IGFBP3 had a higher percentage of loop structure making it a bit less stable prediction **(FIGURE 13 AND 14)**.

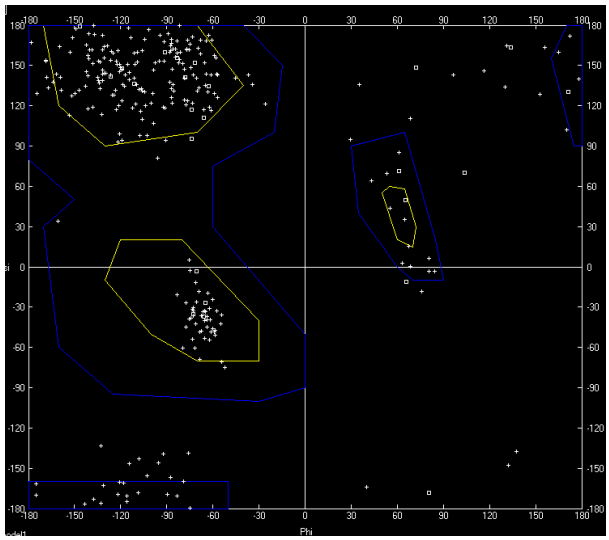


FIGURE 13: Ramachandran Plot for ALOX5. This plot shows that most of the scattered plots (phi vs psi) are located in the permissive region proving that the structure is a stable one.

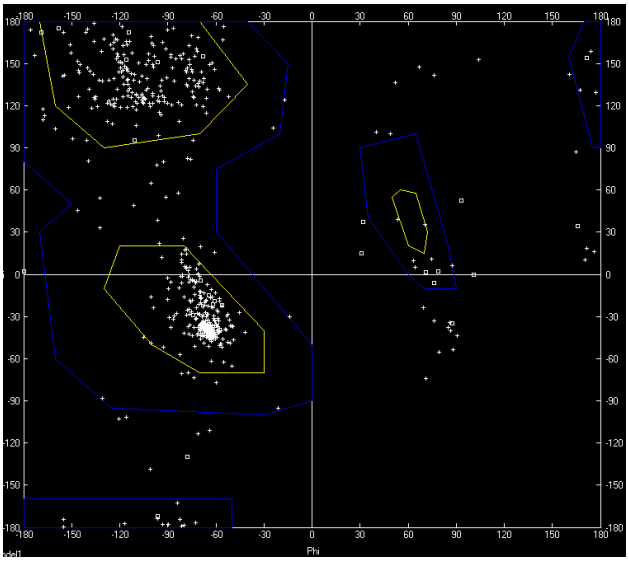


FIGURE 14: Ramachandran Plot for IGFBP3. Although a significant amount of dots have fallen into the non permissible zone the structure is still a stable one. This may indicate that the protein has a too open conformation which is often characteristic to proteins that are designed to bind a counterpart constitutively.

4. DISCUSSION

Andrographis panicultata is reported to contain high level of silibinin. Silibinin, an active compound affects gene expression of many genes involved in cell cycle regulation, apoptosis and tumor suppression. However the immediate binding partners were not predicted by it.

A search through STITCH 3.1 database gave a gross estimation of interaction partners. Using that data further literature search was carried

out keeping binding motifs in the focus. Few potential targets were selected and based on the docking score two protein ALOX5 and IGFBP3 showed significant binding potential with the ligand.

AutoDock used binding affinity (kcal/mole) as a binding parameter whereas PATCHDOCK has their own binding score which can often be fine-tuned using FIRDOCK on the basis of free energy information. The PATCHDOCK score for docked ligand and the macromolecules were significant enough. The high 'Area' of interaction also reveals tight binding. The ligand was bound well inside the active site cleft and no nonspecific interaction was found. Therefore, the binding of silibinin to the macromolecule definitely indicates potential of a predictive drug. PocketFinder results scoring confirmed that macromolecule forms pockets in the binding sites of the ligand and therefore rules out possibility of false positives (**FIGURE 11 AND FIGURE 12**).

AutoDock predicted binding affinity was above -8.0 and -9.0 which is quite a satisfactory one. Studies with control showed that a random binding might show around -5.o to -6.0 binding affinity score (data not given here). This indicates that the binding was not by chance. However further confirmatory simulation was run with better exhaustiveness (default value 8.0). PocketFinder results correlated well with the docking results proving the accuracy of the model.

Important residues of ALOX5 that forms the pocket are-
GLY11, SER12, GLN13, LEU67, ASP80, CYS100, TYR101, ARG102, TRP103, ILE 104, THR 105, ASP 107, VAL108, GLU 109, VAL110, VAL111, LEU112, ARG113, HIS131, LYS134, GLU135, TRP148, PHE152, ARG166, PHE 170, ASP171, ASn181, HIS361, GLN364, HIS368, TYR 384, ARG402, ARG412 and more.

Important residues of IGFBP3 that forms the pocket are -
GLY217, PRO218, CYS219, LEU227, ASN228, HIS229, LYS231, PHE232, ASN234, VAL235, LEU236, ARG239, GLY240, HIS242, PRO244, CYS246, GLN256, ARG257, ARG258, SER260, LYS261, ARG265, GLY266, PHE267, TYR 274 and more.

SUBCOMP search tool was used to identify structurally similar compounds.The C value of those compounds were plotted against their binding affinity and the found result indicate the potential of designing a synthetic drug more efficient as the lower c values had better binding efficiency. However this comment has to be analyzed further with concrete lab studies before concluding so.

Modern rational drug designing belongs to a twofold process. At first, potential hit are generated using approaches as carried out here. However, irrespective of values hits have to be analyzed furthermore in the molecular level physically. Despite our significant scoring values we believe the drug protein interaction will have to be further investigated physically.

Our study here should give us better insight about how the traditional use of *Andrographis panicultata* is riveted well inside science rather than folklore. We hope, this study would help in designing a more efficient rational drug in the future and also would help us collect more natural products from the plant concerned. Hence, we hope the study will assist us to understand the beauty underneath natural products, to unlock the mystery of nature itself.

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