MOLECULAR DOCKING STUDY
5.1 Introduction

Obesity is caused primarily by lipid metabolism disorder. The enzymes involved in lipid metabolism are usually used as targets in the development of new antiobesity drugs. Pancreatic lipase (PL) is the key enzyme in this process, hydrolyzing 50–70% of total dietary fats (Birari and Bhutani et al., 2007). Therefore, lipase inhibitors from plants are considered a good source for antiobesity drugs. In addition, identifying such inhibitors will help to disclose the mechanisms of the plants’ nutritious effects.

Study on the mechanism of molecular interaction between active compounds and enzymes can reveal pharmacokinetics as well as the relationship between the chemical structures and bioactivity of a drug (Yanagisawa et al., 2012). The molecular modeling study is another important tool for revealing the interactions between enzymes and their inhibitors, which could help to clarify directly the nature of binding between small molecules and enzymes (Park et al., 2008; Point et al., 2012).

Protein–ligand docking is a molecular modelling technique. The goal of protein–ligand docking is to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein receptor or enzyme. Pharmaceutical research employs docking techniques for a variety of purposes, most notably in the virtual screening of large databases of available chemicals in order to select likely drug candidates.

Drug design, sometimes referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it.
Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design. In addition to small molecules, biopharmaceuticals and especially therapeutic antibodies are an increasingly important class of drugs and computational methods for improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed.

Docking can be carried out by various methods. But, the most efficient method is Lamarckian genetic algorithm. AutoDock was run several times to get various docked conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates (Chang et al., 2010). Auto dock tools provide various methods to analyze the results of docking simulations such as, conformational similarity, visualizing the binding site and its energy and other parameters like intermolecular energy and inhibition constant (Park et al., 2006).

AutoDock tools was utilized to generate grids, calculate dock score and evaluate the conformers of inhibitors bound in the active site of enzymes pancreatic lipase and FTO as targets for antiobesity activity. Automated docking is a graphical user interface. AutoDock 4.2 was employed to get docking and binding scores; which is implemented by Lamarckian genetic algorithm method (Sudeep and Sahadevan, 2015).

Wheat (Triticum aestivum) germinated over a period of 6-10 days is called wheatgrass (Wg), because of its rich source of vitamins, antioxidants and minerals also familiar as “living food”. "Wheatgrass” refers to the young grass of the common monocot wheat plant Triticum aestivum. Its consumption in the Western world began in the 1930s. Today, wheatgrass is quickly becoming one of the most widely used supplemental health foods and is available in many health food stores as fresh produce, tablets, frozen juice, and powder. It is believed to have antioxidant enzymes like Superoxide Dismutase and Cytochrome Oxidase. It contains rich source of chlorophyll that is known to be responsible for deactivating the metabolic activation of carcinogens. In India, wheatgrass is consumed either tablet or as a juice to keep up
good health. Wheat grass juice is known to cure healing properties in many degenerative diseases and also very effective in the treatment of thalassemia, distal ulcerative colitis, and benefits other parts of the body.

The aim of this computational study is to find the potency of the isolated compounds from *T.aestivum* to treat metabolic disorder - Obesity. To process this study we have chosen two important drug target protein/enzyme/receptor to study the quorum sending mechanism using computational biology tools. The drug target chosen for this study is listed below

1. Structure of the human PTP1B catalytic domain
2. Structure of the pancreatic lipase

5.1.1 Specification of PTP 1B (drug target 1)

a. Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of the leptin and insulin signaling pathways
b. Coordinated tyrosine phosphorylation is essential for signaling pathways regulated by insulin and leptin.
c. Obesity are characterized by resistance to hormones leptin, Protein tyrosine phosphatase 1B (PTP1B) has been found to be a major regulator of body fat stores, energy balance, and insulin sensitivity in vivo.
d. Pharmacological agents capable of inhibiting possibly due to attenuated or diminished signaling from the receptors. the negative regulator(s) of the signaling pathways are expected to potentiate the action of leptin and therefore be beneficial for the treatment of obesity.
e. The isolated compound : 2,2-dimethyl-7-propyl-chroman -3-ol, is docked with drug target protein is shown below in figure 5.1.
Figure 5.1: The secondary structure of human PTP1B catalytic domain

Figure 5.2: The secondary structure of Structure of the pancreatic lipase
5.1.2 Specification of Pancreatic lipase (drug target 2)

a) Pancreatic, endothelial, hepatic, lipoprotein lipases are members of the human lipase super family and possess structural similarity. Other tissues like lungs, kidney, skeletal muscles, adipose tissue and placenta also secretes lipase enzyme

b) Pancreatic acinar cells secrete pancreatic lipase (triacylglycerol acyl hydrolase EC 3.1.1.3), an important enzyme of pancreatic juice responsible for digestion of dietary triglycerides in the small intestine

c) Pancreatic lipase is a common target for anti-obesity drug research. Pancreatic lipase (triacylglycerol acyl hydrolase) plays an essential role in the digestion of triacylglycerols. Lipase inhibitors may affect the amount of fat absorbed, yet they do not block the absorption of a particular type of fat.

d) Isolated compound : 2,2-dimethyl-7-propyl-chroman -3-ol, is docked with drug target protein is shown below in figure 5.2
5.2 Materials and Methods

5.2.1 Compound details

<table>
<thead>
<tr>
<th>Structure of Compound</th>
<th>Molecular composition</th>
<th>Molecular weight</th>
<th>No. Of atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2-dimethyl-7-propyl-chroman 3-ol</td>
<td>C:0.763, H:0.092, O:0.145</td>
<td>220.314</td>
<td>16</td>
</tr>
<tr>
<td>1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxyl-propyl)-benzyl)-urea</td>
<td>C:0.464, H:0.046, F:0.046, N:0.135, O:0.232, S:0.077</td>
<td>414.42</td>
<td>47</td>
</tr>
</tbody>
</table>

5.2.2 Retrieval of protein and its preparation

Drug target protein 1: Crystal Structure of the human PTP1B catalytic domain of uniprot number P18031 with unique (http://www.rcsb.org/) protein data bank ID: 1JF7 with 298 residues length of 33% helical (10 helices; 99 residues) 21% beta sheet (13 strands; 65 residues) and 2.2Å x-ray diffraction resolution is retrieved from pdbe database(www.ebi.ac.uk/pdbe).

Similarly, for drug target protein 2: Structure of the pancreatic lipase-colipase complex of uniprot number : P16233 with unique (http://www.rcsb.org/) protein data bank ID: 1N8S with 449 residues length of 22% helical (13 helices; 99 residues) 29% beta sheet (26 strands; 133 residues) and 3.04 Å x-ray diffraction resolution is
retrieved from pdbe database (www.ebi.ac.uk/pdbe). The common method of auto
dock protein preparation is followed by adding and applying the hydrogen bonds and
kollamaan charges respectively. Finally, the structure is saved in .pdb for docking

5.2.3 Auto Grid calculation

AutoGrid is a program that pre-calculates grid maps of interaction energies for
various atom types, such as aliphatic carbons, aromatic carbons, hydrogen-bonding
oxygens, and so on, with a macromolecule such as a protein, DNA or RNA.

These grid maps are then used by AutoDock docking calculations to determine
the total interaction energy for a ligand with a macromolecule. Doing this pre-
calculation saves a lot of time during the docking, primarily because we do not have
to update non-bonded lists during the calculation. Also, what was a calculation with
order N-squared complexity, is reduced to one that is order N, where N is the number
of atoms interacting.

The process of rigid docking was carried out for drug targets with isolated
compound in 3D grid center for drug target 1(Refer above for the name) of 19.38 X
12.611 Y 46.434 Z co-ordinates with grid spacing of 0.553 in 46x42x46 grid points.
However, for the drug target 1, the flexible residue are provided from the reference
structure PNU177836 that already bound with the protein as a inhibitor grid was
calculated (http://www.rcsb.org/pdb/explore/explore.do?structureId=1JF7).

Similarly, for drug target 2 (Refer above for the name) of 38.33X 22.27 Y
74.51 Z co-ordinates with grid spacing of 0.503 in equal 50x50x50 grid points, since
there is no observation of any bound structure, hence initially study was carried out
with orlistat the current used for treatment for obesity is docked and the residues are
identified and the same grid point is kept constant for isolated compound docking.

5.2.4 Molecular docking

Molecular docking is a key tool in structural molecular biology and computer-
assisted drug design. The goal of ligand—protein docking is to predict the
predominant binding mode(s) of a ligand with a protein of known three-dimensional
structure. Successful docking methods search high-dimensional spaces effectively and
use a scoring function that correctly ranks candidate dockings. Docking is processed
in autodock 4.0v in linux environment with genetic algorithm and lamarckian default setting of 150 generations and 10 runs for each protein and compound.

5.3 Results and Discussion

Bioinformatics and systems biology approaches are becoming increasingly important along with the above-mentioned chemoinformatics methods to study the therapeutic potential of medicinal plants (Barlow et al., 2012). They are used to select targets for docking and to identify relationships between the revealed actions of phytochemicals on targets and the known therapeutic effects of medicinal plants. Thus, the aim of this review is a critical consideration of the various available databases of medicinal plants and *in silico* tools for their utilisation in new drug discovery based on expanding the use of folk medicinal plants through the exploration of phytochemical diversity.

The concept of docking is important to determine the properties associated with protein-ligand interactions such as binding energy, electron distribution, hydrogen bond donor acceptor properties and hydrophobicity (Jayasimha Rayalu Daddam et al., 2013).

Structure-based computational methods, including molecular docking, have increasingly been used in the study of biomolecular structure and function, as well as in the design of structure-based rational drugs. In particular, molecular docking contributed to the development of several inhibitors and inhibitor candidates that have been advanced to clinical trials (Kufareva and Abagyan, 2008; Torktaz et al., 2013; Zhang et al., 2014), indicating that docking simulation is a useful tool for enriching a chemical library with active compounds.

Drug discovery is a complex process that involves the identification of active constituents from traditional medicines. Auto dock is a ligand docking program for predicting protein-ligand binding modes and for virtual screening. Auto Dock is an automated procedure for predicting the interactions of ligands with Macro molecular targets (Haripriya Doraiswamy et al., 2014).
Plant-derived flavonoids and phenolics are known as potential active compounds that possess a broad range of pharmaceutical properties, including antibacterial, antifungal, antiviral, and anticancer activity (Soto-Vaca et al., 2012). These compounds have also been used as templates for the development of new pharmaceuticals (Weston and Mathesius, 2013).

PTP1B has been found to be a major regulator of body fat stores, energy balance, and insulin sensitivity in vivo. Pancreatic acinar cells secrete pancreatic lipase, an important enzyme of pancreatic juice responsible for digestion of dietary triglycerides in the small intestine. Pancreatic lipase is a common target for anti-obesity drug research. Pancreatic lipase (triacylglycerol acyl hydrolase) plays an essential role in the digestion of triacylglycerols. Lipase inhibitors may affect the amount of fat absorbed, yet they do not block the absorption of a particular type of fat.

Protein tyrosine phosphatase (PTP) 1B is the superfamily of PTPs and a negative regulator of multiple receptor tyrosine kinases (RTKs) (Ostman and Bohmer, 2001). Also PTP1B is involved in the down regulation of insulin and leptin signaling. Thus, inhibitors of PTP1B have potential as therapeutics for treating Type II diabetes and obesity (Li et al., 2005). Tyrosine phosphorylation is controlled by protein tyrosine kinases (PTKs) and phosphatases (PTPs). Protein tyrosine phosphatase 1B (PTP1B) has been implicated as one of the key regulators of insulin and leptin signal transduction pathways.

In silico molecular docking provided additional explanation for the experimental inhibition activity of Drug I and II with PTP 1B and PL.

5.3.1 Interaction of 2,2-dimethyl-7-propyl-chroman-3-ol (Drug I) with PTP 1B and Pancreatic lipase

5.3.1.1 PTP 1B phosphatase interaction with 2,2-dimethyl-7-propyl-chroman-3-ol (1JF7)

The isolated compound: 2,2-dimethyl-7-propyl-chroman-3-ol docked with PTP 1B phosphatase. The various energy parameters of intermolecular energy, electrostatic energy and vanderwaals energy and binding mode with specific residues
of flexible docking is shown in the figure 5.3 and 5.4 for compound 1. The interaction with active site amino acid residues in for compound is stated in table 5.2.

Table 5.1 summarizes results of the docking study based on binding conformations and energies. The lowest energy conformation, representing the best binding conformation of inhibitors to these receptor protein molecules, was identified by the molecular docking procedure. The complex formed by protein molecules with ligands yielded a plethora of information highlighting the conclusive role portrayed by numerous factors namely hydrogen bonds, salt bridges, metal interactions, lipophilic interactions, π-π, and π-cation interactions in the Protein–Ligand interaction profile. The data obtained from these Protein–Ligand docking molecule interactions, including their G score, hydrogen bond, electrostatic bond, and residues, are depicted in Table 5.1.

The docking of *T.aestivum* compound drug I into active site of PTP1B was carried out using Autodock tool 4.2. The final docking score in Kcal/mol for each docking experiment was calculated and represented in Table 5.1. From, figure 5.3 docking of Drug I into active site of PTP1B, we observed three H bonds with protein amino acid residues that are Ser 216, Arg 221, Aln 217.

Lower binding energy of the ligands indicates better inhibition affinity and thus low Ki value.
Table 5.1 Energy parameters of best docking of 2,2-dimethyl-7-propyl-chroman-3-ol with PTP 1B phosphatase

|   | Estimated Free Energy of Binding | -6.06 kcal/mol  
                             | \[=(1)+(2)+(3)-(4)\] |
|---|----------------------------------|-------------------|
| 2 | Estimated Inhibition Constant, $k_i$ | 35.97 uM (micromolar) |
| 3 | Temperature                       | 298.15 K          |
| 4 | Final Intermolecular Energy       | -6.96 kcal/mol    |
| 5 | $vdW + Hbond + desolv$ Energy     | -6.85 kcal/mol    |
| 6 | Electrostatic Energy              | -0.11 kcal/mol    |
| 7 | Final Total Internal Energy       | -0.87 kcal/mol    |
| 8 | Torsional Free Energy             | +0.89 kcal/mol    |
| 9 | Unbound System's Energy \[=(2)\]  | -0.87 kcal/mol    |

According to the docking result, the ligand was selected with best dock score and low bound energy. In that, the Drug I with PTP 1B phosphatase had the good binding score and energy (-6.04 kcal/mol). The docking interactions between the selected natural compound and the known inhibitor of each target were compared and from the result it was revealed that the drug I (Compound I) possess better score.
Figure 5.3: Active site of PTP 1B Phosphatase amino acid interaction with 2,2-dimethyl-7-propyl-chroman-3-ol with PTP 1B phosphatase

Figure 5.4: Binding of 2,2-dimethyl-7-propyl-chroman-3-ol with cavity of active site of PTP 1B phosphatase protein
Table 5.2: PTP 1B phosphatase interaction with isolated compound 2,2-dimethyl-7-propyl-chroman-3-ol its free energy binding and Inhibition Constant, Ki

<table>
<thead>
<tr>
<th>RECEPTOR and LIGAND</th>
<th>Compound</th>
<th>Amino acid Binding</th>
<th>Distance in Å</th>
<th>Free Energy of Binding kcal/mol</th>
<th>Inhibition Constant, Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure of the human PTP1B catalytic domain</td>
<td>2,2-dimethyl-7-propyl-chroman-3-ol</td>
<td>SER B:216</td>
<td>3.3</td>
<td>-6.06</td>
<td>35.97 uM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ARG B:221</td>
<td>4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALA B:217</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From the table 5.2, the bioactive compound also interacted the protein with more number of hydrogen bonds than known inhibitors, six hydrogen bond interactions (Ser216, Arg221 and Ala217). The Ki value is 35.97kcal/mol for Drug I also confirmed the result with good binding energy.

5.3.1.2 Pancreatic lipase interaction –2,2-dimethyl-7-propyl-chroman-3-ol (1N8S)

Pancreatic lipase is a common target for anti-obesity drug research. Pancreatic lipase (triacylglycerol acyl hydrolase) plays an essential role in the digestion of triacylglycerols. Excreted in the duodenum, pancreatic lipase hydrolyzes 50-70% fat (at a maximum rate of 140 grams per minute) in the presence of colipase and Ca2+. Several classes of substances exhibit pancreatic lipase inhibitory activity, including flavonoids, saponins (De la Garza et al., 2011; Yun et al., 2010) and substances of microbial sources such as lipstatin, pancelincins, etc (Birari and Bhutani, 2007).

The isolated compound : 2,2-dimethyl-7-propyl-chroman-3-ol with Pancreatic lipase. The various energy parameters of intermolecular energy, electrostatic energy and vanderwals energy and binding mode with specific residues of flexible docking is shown in the figure 5.5 and 5.6 for compound. The interaction with active site amino acid residues in for compound is stated in table 5.4.
The pancreatic lipase possesses two domains. Its N-terminal domain is an α/β hydrolase fold, which contains the catalytic sites of Ser216, Arg221, and Ala217. The crystal structure of the ternary porcine lipase-colipase-tetraethylene glycol mono-octyl ether showed that the overall position of the three catalytic sites is conservative in different species. The crystal structure of PL was determined at a resolution of 2.80 Å, and the lipase active site was fully accessible so that docking experiments with the two inhibitors could be carried out. The in silico docking experiment showed that Drug I (Compound I) mostly interacted with PL through hydrogen bonding of Cys304 (Figure 7A).

The estimated binding energies were −6.06 kcal mol⁻¹ for PTP 1B Phosphatase and −5.79 kcal mol⁻¹ for Pancreatic lipase respectively.

**Table 5.3 Energy parameters of best docking of 2,2-dimethyl-7-propyl-chroman-3-ol with Pancreatic lipase**

<table>
<thead>
<tr>
<th></th>
<th>Estimated Free Energy of Binding</th>
<th>-5.79 kcal/mol [=(1)+(2)+(3)-(4)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Estimated Inhibition Constant, Ki</td>
<td>57.09 uM (micromolar)</td>
</tr>
<tr>
<td>3</td>
<td>Temperature</td>
<td>298.15 K</td>
</tr>
<tr>
<td>4</td>
<td>Final Intermolecular Energy</td>
<td>-6.68 kcal/mol</td>
</tr>
<tr>
<td>5</td>
<td>vdW + Hbond + desolv Energy</td>
<td>-6.62 kcal/mol</td>
</tr>
<tr>
<td>6</td>
<td>Electrostatic Energy</td>
<td>-0.07 kcal/mol</td>
</tr>
<tr>
<td>7</td>
<td>Final Total Internal Energy</td>
<td>-0.84 kcal/mol</td>
</tr>
<tr>
<td>8</td>
<td>Torsional Free Energy</td>
<td>+0.89 kcal/mol</td>
</tr>
<tr>
<td>9</td>
<td>Unbound System's Energy</td>
<td>-0.84 kcal/mol</td>
</tr>
</tbody>
</table>
Figure 5.5: Active site of Pancreatic lipase (amino acid) interaction with 2,2-dimethyl-7-propyl-chroman-3-ol

Figure 5.6: Binding of 2,2-dimethyl-7-propyl-chroman-3-ol with cavity of active site of Pancreatic lipase protein
Drug I (Compound I) strongly inhibited PL. The three-dimensional and pharmacophore structure of taraxerol is shown in Figure 5.5. The isolated compound displayed potent inhibition of target protein. Conformity with Lipinski’s rule of five for the molecular properties important for potential drugs is shown in Table 5.3. Drug I (Compound I) showed minimum binding of $-5.59 \text{ kJ mol}^{-1}$, with acceptable affinity for the active pocket. Thus, Drug I can be considered a potent inhibitor of Pancreatic lipase. The protein–ligand interaction and the binding mode docking of Drug I showed in Fig. 5.6. Protein–ligand interaction are presented in Fig.5.5. Molecular simulations were performed on the crystal structure of the docked complex structure and the distribution of potential energy and hydrogen bond distance is presented in Fig.5.5.

**Table 5.4: Pancreatic lipase interaction with 2,2-dimethyl-7-propyl-chroman-3-ol its free energy binding and Inhibition Constant, $K_i$**

<table>
<thead>
<tr>
<th>RECEPTOR and LIGAND</th>
<th>Compound</th>
<th>Amino acid Binding</th>
<th>Distance in Å</th>
<th>Free Energy of Binding kcal/mol</th>
<th>Inhibition Constant, $K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure of the pancreatic lipase</td>
<td>2,2-dimethyl-7-propyl-chroman-3-ol</td>
<td>CYS A:304</td>
<td>4.4</td>
<td>-5.79</td>
<td>57.09 uM</td>
</tr>
</tbody>
</table>

In order to investigate the binding capacity of bioactive compounds in *T.aestivum* on proteins related to Obesity in humans, we docked the compound to the target proteins. Results showed that the dock score ranged from -6.04 for PTP 1B Phosphatase and -5.79 (kcal/mol) represented in Table 5.1 and 5.3. High binding affinity of the ligand to the receptor was explained clearly by interaction analysis in Figures 5.3 and 5.5. PTP 1B Phosphatase is the key enzyme in the leptin signalling pathway and plays an important role in Obesity. In our study, docking of novel compound with PTP 1B Phosphatase showed binding interaction established the hydrogen bond with Ser216, Arg221,Aln217 which is the major interaction of the ligand with PL Cys304 residues which are presented in the active site form strong polar interactions with the compound.
5.3.2 Interaction of 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1-hydroxyl-propyl)-benzyl)-urea (Drug II) with PTP 1B and Pancreatic lipase

a) PTP 1B Phosphatase – 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1-hydroxyl-propyl)-benzyl)-urea (1JF7)

The isolated compound: 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1-hydroxyl-propyl)-benzyl)-urea docked with PTP 1B Phosphatase. The various energy parameters of intermolecular energy, electrostatic energy and vanderwals energy and binding mode with specific residues of flexible docking is shown in the figure 5.7 and 5.8 for compound. The interaction with active site amino acid residues in for compound is stated in table 5.6.

Table 5.5 Energy parameters of best docking of 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1-hydroxyl-propyl)-benzyl)-urea docked with PTP 1B Phosphatase

<table>
<thead>
<tr>
<th></th>
<th>Estimated Free Energy of Binding</th>
<th>-5.56 kcal/mol [=(1)+(2)+(3)-(4)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Estimated Inhibition Constant, Ki</td>
<td>84.50 uM (micromolar)</td>
</tr>
<tr>
<td>3</td>
<td>Temperature</td>
<td>298.15 K</td>
</tr>
<tr>
<td>4</td>
<td>Final Intermolecular Energy</td>
<td>-7.94 kcal/mol</td>
</tr>
<tr>
<td>5</td>
<td>vDW + Hbond + desolv Energy</td>
<td>-7.77 kcal/mol</td>
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<tr>
<td>6</td>
<td>Electrostatic Energy</td>
<td>-0.18 kcal/mol</td>
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<td>7</td>
<td>Final Total Internal Energy</td>
<td>-0.24 kcal/mol</td>
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<td>8</td>
<td>Torsional Free Energy</td>
<td>+2.39 kcal/mol</td>
</tr>
<tr>
<td>9</td>
<td>Unbound System's Energy [=(2)]</td>
<td>-0.24 kcal/mol</td>
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</tbody>
</table>
Chemical Information of docking calculations were carried out using Docking Server that interaction parameters for drug target II and its structure shown in Table 5.6 and Figure 5.7 respectively. The docking energy values were calculated as the sum of the electrostatic, Van der Waals energies and the flexibility of the ligand itself. Low docking energy indicates high binding ability. Based on the results obtained, Drug II with PTP 1B Phosphatase $K_i = 84.50 \, \text{uM}$ showed the highest inhibitory. From the present kinetic data, we conclude that the tannin inhibitor can bind to the active site of the free enzyme to give rise to Ei complexes. Since tannins play such varied biological roles, and because of the enormous structural variation among plant compounds, it is difficult to develop models that allow an accurate prediction of effects in enzyme systems.

**Table 5.6 PTP 1B Phosphatase interaction with isolated compound its free energy binding and Inhibition Constant, $K_i$**

<table>
<thead>
<tr>
<th>RECEPTOR and LIGAND</th>
<th>Compound</th>
<th>Amino acid Binding</th>
<th>Distance in Å</th>
<th>Free Energy of Binding kcal/mol</th>
<th>Inhibition Constant, $K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure of the human PTP1B catalytic domain</td>
<td>1-(4,6-Dimethoxy-pyrimidin-2-yl)-3-(2-(2-fluoro-1-hydroxyl-propyl)-benzyl)-urea</td>
<td>ASP B:48</td>
<td>3.8</td>
<td>-5.56</td>
<td>84.50 uM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALA B:217</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>GLN B:262</td>
<td>5.7</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Pi-Pi stack</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>TYR B: 46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ARG B: 24</td>
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</tbody>
</table>
Figure 5.7: PTP 1B Phosphatase active site amino acid interaction with 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxyl-propyl)-benzyl)-urea

Figure 5.8: Binding of 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxyl-propyl)-benzyl)-urea with cavity of active site of PTP 1B Phosphatase protein
b. Pancreatic lipase –1-(4,6-Dimethoxy-pyrimidin-2-yl)-3-(2-(2-fluoro-1-hydroxyl propyl)-benzyl)-urea (1N8S)

The isolated compound: 1-(4,6-Dimethoxy-pyrimidin-2-yl)-3-(2-(2-fluoro-1-hydroxyl-propyl)-benzyl)-urea docked with Pancreatic lipase. The various energy parameters of intermolecular energy, electrostatic energy and vanderwalls energy and binding mode with specific residues of flexible docking is shown in the figure 5.9 and 5.10 for compound. The interaction with active site amino acid residues in for compound is stated in table 5.8.

Docking of Drug II to PL also showed an apparent hydrogen-bonding network resulting from the interaction of the PL catalytic residue Asn425. Additionally, the carboxyl and hydroxyl moieties on the sugar residue together had strong Pi-Pi stack bonding interactions with the side chain of the PL catalytic residue Lys428 (Figure 5.9).

Table 5.7 Energy parameters of best docking of 1-(4,6-Dimethoxy-pyrimidin-2-yl)-3-(2-(2-fluoro-1-hydroxyl-propyl)-benzyl)-urea docked with PL

<table>
<thead>
<tr>
<th></th>
<th>Estimated Free Energy of Binding</th>
<th>-5.57 kcal/mol [= (1)+(2)+(3)-(4)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Estimated Inhibition Constant, Ki</td>
<td>82.08 uM (micromolar)</td>
</tr>
<tr>
<td>3</td>
<td>Temperature</td>
<td>-7.96 kcal/mol</td>
</tr>
<tr>
<td>4</td>
<td>Final Intermolecular Energy</td>
<td>-6.77 kcal/mol</td>
</tr>
<tr>
<td>5</td>
<td>vDW + Hbond + desolv Energy</td>
<td>-7.77 kcal/mol</td>
</tr>
<tr>
<td>6</td>
<td>Electrostatic Energy</td>
<td>-1.19 kcal/mol</td>
</tr>
<tr>
<td>7</td>
<td>Final Total Internal Energy</td>
<td>-1.91 kcal/mol</td>
</tr>
<tr>
<td>8</td>
<td>Torsional Free Energy</td>
<td>+2.39 kcal/mol</td>
</tr>
<tr>
<td>9</td>
<td>Unbound System's Energy [= (2)]</td>
<td>-1.91 kcal/mol</td>
</tr>
</tbody>
</table>
Figure 5.9: Pancreatic lipase active site amino acid interaction with \(1-(4,6\text{-Dimethoxy- pyrimidin -2-yl})-3-(2-(2\text{-fluoro -1- hydroxyl-propyl})-benzyl)-urea\)

Figure 5.10: Binding of \(1-(4,6\text{-Dimethoxy- pyrimidin -2-yl})-3-(2-(2\text{-fluoro -1- hydroxyl-propyl})-benzyl)-urea\) with cavity of active site of Pancreatic lipase protein
Molecular modeling (docking) study was carried out for series of Drug II figures 5.9 and 5.10 for PL. The potential active site amino acids of PL were predicted using Auto dock showed in Fig 5.7. Thus, the protein was targeted against pocket 1. Given the three-dimensional structure of a target receptor molecule usually a protein; chemical compounds having potential affinity towards it are designed rationally, with the aid of computational methods. Detailed bioinformatics analysis offers a convenient methodology for efficient in silico preliminary analysis of possible function of new drug. The target protein and inhibitors were geometrically optimized. The Drug II was docked against active site of the target protein using Auto Dock which gives an insight into the binding modes for the various inhibitors. The drug II (Compound II) has showed best binding energy of – 5.57 Kcal/mol and the ki value 82.08 uM against the target protein. The binding energy of all the inhibitors was shown in Table 5.7. Fig: 5.9 represent the docked complex (1I) of the inhibitors to that of target protein.

Table 5.8 Pancreatic lipase with 1-(4,6-Dimethoxy-pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxyl-propyl)-benzyl)-urea its free energy binding and Inhibition Constant, Ki

<table>
<thead>
<tr>
<th>RECEPTOR and LIGAND</th>
<th>Compound II</th>
<th>Amino acid Binding</th>
<th>Distance in Å</th>
<th>Free Energy of Binding kcal/mol</th>
<th>Inhibition Constant, Ki</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure of the pancreatic lipase</td>
<td>1-(4,6-Dimethoxy-pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxyl-propyl)-benzyl)-urea</td>
<td>ASN A:425</td>
<td>3.9</td>
<td>-5.57</td>
<td>82.08 uM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pi-Pi stack</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LYS A: 428</td>
<td>4.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pancreatic lipase (PL) is a key enzyme of Triglycerols biosynthetic pathway, potentially leading to raised fatty acid concentrations in Obesity. The docking studies of Drug target II with the PTP 1B phosphatase makes reveals the best dock score of -5.56 kcal/mol and interact with hydrophobic residues such as Asp48, Ala217, Gln262 are involved in the interaction with the compound. These interactions clearly suggest that the compound fits very well into the binding pocket of PTP enzyme. PTP 1B Phosphatase plays a crucial role in glucose utilization and lipid metabolism. In diabetes, PTP 1B phosphatase activity is markedly upregulated and this enzyme has been an emerging therapeutic target for type 2 diabetes as well as a candidate. The novel compound interacts in the similar manner like PL inhibitor fits neatly in the hydrophobic active site and induces unique and dramatic conformational changes.

The docking of Triticum aestivum compounds namely 2,2-dimethyl-7-propylchroman-3-ol and 1-(4,6-Dimethoxy- pyrimidin -2-yl)-3-(2-(2-fluoro -1- hydroxy-propyl)-benzyl)-urea into active site of PTP1B and PL were carried out using Autodock tool 4.2. The protein PTP 1B and PL were targeted against Drug I and II. Figures 5.7 to 5.10 given the three-dimensional structure of a target receptor molecules with drug I and II. Usually a protein; chemical compounds having potential affinity toward sit are designed rationally, with the aid of computational methods. Detailed bioinformatics analysis offers a convenient methodology for efficient in silico preliminary analysis of possible function of new drug. The target protein and inhibitors were geometrically optimized. The isolated compounds used as inhibitors were docked against active site of the target protein using Auto Dock which gives an insight into the binding modes for the inhibitors.

Drug I When docked in the SER-ARG-ALA catalytic pocket of PTP 1B, the result shows a binding energy value of -6.06 kcal/mol and Ki value of 35.97 μM represented in Table 5.1. While Drug II (Compound II) (ASP-ALA-GLN) shows binding energy value and ki with values -5.56 and 84.50 kcal/mol respectively. Drug I When docked in the CYS catalytic pocket of PL, the result shows a binding energy value of -5.79 kcal/mol and Ki value of 57.09 uM shows in table 5.3. While Drug II (Compound II) shows binding energy value and ki with values -5.57 and 82.08 kcal/mol respectively. According to the inhibition constant values, Drug I and II shows lower binding energy. Lower binding energy of the ligands indicates better
inhibition affinity and thus low Ki value. From the docking analysis it is observed that
the both two compounds could be used for the inhibition of obesity. A docking
analysis of plant derived compounds into active site of PTP1B has been studied in the
present work, to identify the inhibitor binding position and affinity to PTP1B using
Autodock tool (Radhika and Sudarsanam, 2012).

Wheatgrass compounds leads to stronger inhibition with PTP1B and PL. According to the report, amentoflavone, naturally occurring bioflavonoids derived
from Selaginella tamariscina, inhibited activity of PTP1B (Lee and Young, 1996).
Anh Nguyen et al., reported that the Chalcone derivatives are proven to have the anti-
pancreatic lipase, applying into obesity treatment.

For the purpose of selecting some of wheat grass derivatives by using docking
model running on computer, this research is hoped to find out potential anti obesity
compounds which are able to resist pancreatic lipase and PTP 1B activities. Based on
docking outcomes, it is found that isolated compounds with many hydroxy or amino
substituents not only lead to good cohesion results but also be capable of creating
bonds with some critical amino acids. Among the derivatives used in the docking
model, some of them were studied for many other effects such as anti-inflammatory,
anti-bacterial, anti- tumor abilities (S19, S14, S18…) (Minh-Tri Le and Thi-Ngoc-
Phuong Huynh, 2011; Tran et al., 2009). A new point of this research is that in the
list of used compounds, there are some derivatives containing two hydroxy
substituents at meta-position (resorcinol derivatives). These derivatives bring about
favorable docking results.

Conclusion

Hence, form this study it was concluded that, isolated compound has potency
to bind with active amino acid residues of PTP1B catalytic domain. Similarly it has
tendency binds with active amino acid residues of Pancreatic lipase. With drug target
1 the isolated compounds shows three interactions in addition the scaffold of protein
structure is more favor for Compound 2 to make Pi-Pi stack interaction. With drug
target 2 (PL) the isolated compounds shows one interaction in addition the scaffold of
protein structure is more favor for compound 2 to make Pi-Pi stack interaction with
three atom binding with ASN 425 residue of active site. Thus, the isolated compounds
have potency of obesity activity and can serve as a drug candidate. The supportive Ki values help the compounds to improve the activity in wet lab to prove the same. This is theoretical study with molecular level mechanism reveals initial screening and explains about how the mechanism will take place in biological system. Thus, further study, is need to be carried out in \textit{invitro} and \textit{invivo}. 
SUMMARY

Natural products, which come out from medicinal plants are important for pharmaceutical research and for drug development as a sources of therapeutic agents. At present the demand for herbal or medicinal plant products has increased significantly. In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects (Naik et al., 2003). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube, 2008).

With this wide potential and therapeutic value, the present work has been undertaken and the inferences drawn are summarized as follows.

- Phytochemical qualitative screening exhibited a good range of primary metabolites and a wide range of secondary metabolites (alkaloids, tannins, phenols, saponins and glycosides) present in *Triticum aestivum*. In the quantitative analysis confirms the presence of Alkaloids, Phenols and flavonoids.

- The quantification of Alkaloids (Methanol 32.9 ± 0.35mg/g), Phenols (Methanol 15.4 ± 0.80mg/g) and flavonoids (Methanol 28.7 ± 0.40mg/g) indicates the quantum store of valuable secondary metabolites compounds in *Triticum aestivum*.

- The antibacterial activity in the aqueous, methanolic, ethyl acetate and chloroform extract of *Triticum aestivum* is exhibited in pour plate method. Zone of inhibition developed against *Staphylococcus aureus* (gram positive) and *E.coli* (gram negative) bacterial strains signifies antibacterial effect.

- The antifungal activity in the aqueous, methanolic, ethyl acetate and chloroform extract of *Triticum aestivum* is exhibited in pour plate method. Zone of inhibition developed against *Candida albicans* and *Aspergillus niger* fungal strains signifies antifungal effect.
- In Free radical scavenging activity by DPPH, methanolic extract of *T.aestivum* has good activity.
- In ABTS activity *T.aestivum* has high activity when compared to ascorbic acid.
- In hydroxyl radical scavenging activity *T.aestivum* was dose dependent and *T.aestivum* has high scavenging activity.
- The methanolic extract of *T.aestivum* has high activity of hydrogen peroxide scavenging activity.
- The total antioxidant activity of *T.aestivum* has best activity.
- In metal chelating activity *T.aestivum* has high antioxidant activity and antiradical activity.
- Methanolic extract of *T.aestivum* has high reducing power when compared to ascorbic acid. Antioxidant property is directly proportional to the UV absorption. Higher the absorbance of the reaction indicated greater the reducing potential.
- Acetone extract was found to possess significant pharmacological activity when compared to other extracts it was subjected to column chromatography for the isolation of phytoconstituents. Three compounds were isolated from the extract and their structures were identified by spectral studies.
- The name of the Compound I is 2,2-Dimethyl-7-Propyl-Chroman-3-Ol.
- The name of the Compound II is 1-(4,6-Dimethoxy- Pyrimidin -2-Yl)-3-(2-(2-Fluoro -1- Hydroxyl-Propyl)-Benzyl)-Urea.
- **Compound I** and **Compound II** are not cytotoxic to 3T3-L1 cells at physiological or supraphysiological concentrations. During 3T3-L1 adipocyte differentiation in the presence of Compound I or Compound II, average cell viability was 94.84% and 97.63%, respectively. Compared to control 3T3-L1 cells, the following results were obtained.
- Compound II suppressed lipid accumulation more than Compound I during 3T3-L1 cell differentiation. Compound II down regulated lipid accumulation by 41.75% and 43.29% at 10 μM and 25 μM, respectively.
- Compound I at 25 μM down regulated lipid droplet accumulation only by 8.64 ± 9.54%.
- C/EBP-β levels decreased dramatically by 72.68 ± 1.54% and 69.31 ± 1.22% using 10 μM and 25 μM Compound II, respectively. C/EBP-β levels decreased by 68.73 ± 2.66% and 54.48 ± 2.60% respectively using 25 μM Compound I compared to control.

- Compound I decreased PPAR-γ expressions by 45.03 ± 3.17% and 27.58 ± 12.39%, while Compound II decreased PPAR-γ expressions by 41.48 ± 9.51% and 2.01 ± 32.46%, at 10μM and 25μM respectively. These results suggest that Compound II at low concentrations can inhibit lipid accumulation in 3T3-L1 cells.

- These findings show how Compound I and Compound II at physiological and supraphysiological concentrations affect the regulation of fat cell volume and number, and further suggest that Compound II at physiological concentrations might inhibit lipid droplet accumulation during adipocyte differentiation by down-regulating the expression of C/EBP-α and PPAR-γ.

- It was concluded that, isolated compounds (Compound I and II) have potency to bind with active amino acid residues of PTP1B catalytic domain. Similarly, it has tendency binds with active amino acid residues of pancreatic lipase.

- With PTP 1B (Drug target I) the isolated compounds shows three interaction in addition the scaffold of protein structure is more favor for compound 2 to make Pi-Pi stack interaction.

- With PL (Drug target II) the isolated compounds shows one interaction in addition the scaffold of protein structure is more favor for compound 2 to make Pi-Pi stack interaction with three atom binding with ASN 425 residue of active site.
The presence of wide store of phytoconstituents in *Triticum aestivum* grass upholds the medicinal value which has been explored by a detailed voluminous preliminary standardisation protocol followed by the determination of antioxidant status of the *T.aestivum* some potentiates the effectiveness indulged in it to serve as antiobese component. The isolated and purified Compounds (Compound I and Compound II) from *T.aestivum* was subjected with reference to adipose tissue markers (PPARγ and CEBPβ) whereby the ascertained effect is very clearly exemplified with the results states the antiobesity property of *T.aestivum*.

The effectiveness of *T.aestivum* serving as a potential drug to curtail the obesity (excess fat) is further justified by molecular docking study. The druggability of the isolated/purified compounds (I and II) from *T.aestivum* grass has been documented with their binding effectiveness on PTP 1B and Pancreatic lipase. The *insilico* scaffold of receptor-ligand explicit the binding efficiency of *T.aestivum* (grass). Hence forth by this detailed systematic study on the herbal option selected has comprehensively and conclusively validates the antiobesity potential of *T.aestivum* (grass).

Further detailed *invivo* and clinical trials are warranted in order to establish its therapeutic efficacy of *T.aestivum*. 