IN SILICO STUDIES ON PLANT DERIVED COMPONENTS OF CISSUS QUADRANGULARIS AGAINST COX-2 ENZYME

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ABSTRACT

Objective: Cissus quadrangularis, a perennial plant of grape family is traditionally used as an herbal medicine for treating inflammation caused by hemorrhoids, gastric ulcer and bone disorders. COX-2 is an oxidoreductase enzyme having a role in inflammatory responses. The objective of this study was to show the drug-likeness and the binding of Cissus quadrangularis derived biologically active compounds against the inflammation associated target COX-2 enzyme.

Methods: The 3D structure of COX-2 enzyme protein structure was taken from PDB database [PDB ID: 6cox]. The structures of plant derived compounds were retrieved from the PubChem database. The Lipinski's properties of about 16 compounds from Cissus quadrangularis were checked and those which satisfied the Lipinski’s rule of five were subjected to docking experiments. Docking studies had been carried out through AutoDock 4.2.

Results: About 6 compounds showed drug-likeness by satisfying Lipinski’s properties. The comparative molecular docking studies were done for 6 compounds which showed drug-likeness through AutoDock 4.2. The comparison reflected that flavonoid and stilbene derivatives bind in the active site region of COX-2 with good binding energy.

Conclusion: The in silico studies on compounds reported from Cissus quadrangularis showed that they possess potential medicinal values with anti-inflammatory properties which form insights to develop new leads for COX-2 inhibition.

Keywords: Cissus quadrangularis, Inflammation, COX-2, Lipinski’s rule of five, Molecular docking.

INTRODUCTION

Cissus quadrangularis Linn., is commonly known as Asthisamhari, a succulent plant belonging to family Vitaceae [1]. Cissus quadrangularis has medicinal properties and it is used in the management of weight loss, metabolic syndrome and in Ayurveda for complaints of back and spine [2,3,4]. The extract from Cissus quadrangularis can be used as analgesic, anti-inflammatory and antipyretic compound [5]. The extract contains triterpenes, flavonoids and stilbenes [6,7,8]. Several biologically active compounds were isolated and they have proved to be responsible for various pharmacological activities [9]. In this work, we have illustrated the binding potential of these compounds as anti-inflammatory molecules by studying their interaction with the COX-2 enzyme.

COX-1 and COX-2 are two isoforms of cyclooxygenase enzyme which is crucial for the production of prostaglandins [10]. COX-1 is involved in protecting the stomach lining and COX-2 is involved in the inflammatory pain found in Central nervous system and in inflammatory cells [11]. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX-2 enzymes but result in various side effects such as gastro-intestinal and renal functional suppression. Thus, there is a need to identify plant-derived compounds without any side effects. Plants form primary source of medicine for 65-75% of the world’s population for the treatment of various diseases. A series of new compounds can be produced by traditional synthesis but it is time consuming and at high cost. Hence screening of small molecule databases of novel compounds is an alternative process [12].

The drug-likeness of plant derived compounds can be predicted by Lipinski’s rule of five which refers to the similarity of compounds to oral drugs. Molecular docking plays an important role in the rational drug design. It predicts the binding orientation of small drug targets to their protein targets. The anti-inflammatory activity of Urosolic acid, a plant derived pentacyclic triterpenoid compound is revealed by binding mechanism of the compound with COX-2 enzyme [13]. Molecular docking studies on biologically active compounds from plant Litsaea Genus against COX-2 enzyme were also done which proves their anti-inflammatory property [14]. In the present study, we have studied the drug-likeness of compounds from Cissus quadrangularis using Lipinski’s rule of five and the binding mechanism of the compounds with COX-2 enzymes using molecular docking.

MATERIALS AND METHODS

Cissus quadrangularis derived compounds:

Compounds selected for this study are (a) Asarone, (b) Luteolin, (c) Quercetin, (d) Resveratol, (e) Piceatannol, (f) Kampferol, (g) Stigmasterol, (h) Lapeol, (i) Freidalin, (j) Quadrangularin, (k) Hexadecanoic acid, (l) Tetradecanoic acid, (m) Phytol, (n) Oleic acid, (o) Linoleic acid ethyl ester, (p) Octadecanoic acid ethyl ester. The structures and the physiochemical properties of these compounds were retrieved from the PubChem database (www.ncbi.nlm.nih.gov/pubchem) which is shown in Table 1.

PubChem is a public database which makes database search for a broad range of properties including compound structure, name, fragments, molecular weight, chemical formula, X Log P, hydrogen bond donor and acceptor count. It has own online editor with smiles format and our compounds are converted to .pdb format using this converter.

Lipinski’s properties such as molecular weight, log P and number of hydrogen bond donors and acceptors were taken from the PubChem database for Cissus quadrangularis derived plant compounds.

COX-2 enzyme protein structure:

The 3 dimensional structure of the COX-2 enzyme was taken from the Protein Data Bank (PDB) database (www.rcsb.pdb) which is given in Figure 1. The RCSB PDB is a repository for the 3D structural data of large biological macromolecules such as proteins and nucleic acids. It provides simple and advanced searches based on annotations related to sequence, structure and function. The PDB ID is 6cox which is a complex of COX-2 enzyme with selective inhibitor SC-558 [15].
The active site region of COX-2 enzyme is given in Figure 2. The docking process was done by submitting the pdb coordinates of protein and ligand to AutoDock 4.2. The binding energy was obtained for each ligand and the contact analysis of the docked complexes done using Discovery Studio 3.1 visualizer.

Docking Studies – AutoDock 4.2

Molecular docking studies were performed for the active plant components with COX-2 enzyme by AutoDock 4.2. It is an automated docking tool which works by Lamarckian Genetic Algorithm. It predicts how small molecules such as substrates or drug candidates bind to a receptor of known 3D structures.

The precise interaction of bioactive agents or candidate molecules with their targets is important in the drug development process. AutoDock combines two methods to achieve these goals: rapid grid-based energy evaluation and efficient search of torsional freedom.

AutoDock 4.2 using the Lamarckian Genetic Algorithm and empirical free energy scoring function will provide docking results for ligands with approximately 10 flexible bonds.

RESULTS

Molecular weight, Log P, Number of H bond donor and H bond acceptor are tabulated in Table 1. Compounds which obey Lipinski's rule of five were alone subjected to docking experiment.

Table 1: Lipinski properties of the active plant components

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Molecular weight (&lt; than 500 Da)</th>
<th>Log P (&lt; than 5)</th>
<th>No. of H bond donor (&lt; than 5)</th>
<th>No. of H bond acceptor (&lt; than 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Asarone</td>
<td>208.25</td>
<td>3</td>
<td>0</td>
<td>3</td>
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<tr>
<td>2.</td>
<td>Luteolin</td>
<td>286.24</td>
<td>1.4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>3.</td>
<td>Quercetin</td>
<td>302.235</td>
<td>1.5</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>4.</td>
<td>Resveratol</td>
<td>228.243</td>
<td>3.1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5.</td>
<td>Piceatannol</td>
<td>244.24</td>
<td>2.9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6.</td>
<td>Kampferol</td>
<td>286.23</td>
<td>1.9</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>7.</td>
<td>Stigmasterol</td>
<td>412.69</td>
<td>8.6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8.</td>
<td>Lupeol</td>
<td>426.72</td>
<td>9.9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9.</td>
<td>Quadrangularin</td>
<td>454.47</td>
<td>4.9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>10.</td>
<td>Freidalin</td>
<td>426.72</td>
<td>9.8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>11.</td>
<td>Hexadecanoic acid</td>
<td>256.42</td>
<td>6.4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>12.</td>
<td>Tetradecanoic acid</td>
<td>228.37</td>
<td>5.3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>13.</td>
<td>Phyto</td>
<td>296.53</td>
<td>8.2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14.</td>
<td>Oleic acid</td>
<td>282.46</td>
<td>6.5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15.</td>
<td>Linoleic acid ethyl ester</td>
<td>308.5</td>
<td>7.3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>16.</td>
<td>Octadecanoic acid ethyl ester</td>
<td>312.53</td>
<td>8.9</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Of all the 16 compounds taken from Cissus quadrangularis, six compounds (1-6) satisfy Lipinski's rule of five for drug-likeness which is shown in Table 1. The values of the Lipinski’s properties which deviated from the Lipinski's rule of five are highlighted in Table 1. Hence compounds which do not follow the Lipinski’s properties were not considered for further study.

The plant components which showed drug-likeness from Cissus quadrangularis were a) Asarone, b) Luteolin, c) Quercetin, d) Resveratol, e) Piceatannol and f) Kampferol. The structures of the selected compounds are shown in Fig. 3.

The binding energy for each chosen compound with the COX-2 enzyme using AutoDock 4.2 is given in Table 2. Docking studies show that the ligands bind to the active site region of COX-2 enzyme with good binding energy. Also all the ligands bind in the same hydrophobic pocket which is the active site region of COX-2 enzyme.

The docking models of the plant compounds a) Asarone, b) Luteolin, c) Quercetin, d) Resveratol, e) Piceatannol, f) Kampferol in 3D and 2D view are shown in Figure 4, 5, 6, 7, 8 and 9. The hydrogen contacts of the ligands are tabulated in Table 3.

Hydrogen bonds indicate the strength of contact between the ligand and the receptor and the catalytic activity of an enzyme can be predicted by the hydrogen bonds between them. The arrow in the 2D view denotes the hydrogen bond interactions and the other aminoacids form hydrophobic contacts with the compounds.
Cyclooxygenase (COX), also known as Prostaglandin (PG) H synthase enzymes aid in the conversion of arachidonic acid to prostaglandins [16]. Various phytopathological processes such as inflammatory and cardiovascular responses are regulated by prostaglandins. Mammalian cells contain COX-1 which is a constitutive enzyme whereas COX-2, an inducible enzyme is abundant in macrophages and inflammatory sites [17].

Table 2: Dock scores of the compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Dock score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Asarone</td>
<td>-5.88</td>
</tr>
<tr>
<td>2</td>
<td>Luteolin</td>
<td>-8.62</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>-8.69</td>
</tr>
<tr>
<td>4</td>
<td>Resveratol</td>
<td>-7.57</td>
</tr>
<tr>
<td>5</td>
<td>Piceatannol</td>
<td>-7.58</td>
</tr>
<tr>
<td>6</td>
<td>Kampferol</td>
<td>-3.59</td>
</tr>
</tbody>
</table>

DISCUSSION

COX-2 enzyme plays an important role in carcinogenesis and their levels are up-regulated in various carcinomas. Thus, apart from inflammation, suppressing levels of COX-2 will be a better method for inhibiting carcinogenesis [18]. It has been estimated that NSAIDs can cause 3500 hospitalizations and 400 deaths from ulcer bleeding per annum in the UK in those aged 60 years and above. The cardiac risk for NSAIDs has received a lot of attention both in media and within the medical profession. About 30-60% of NSAID users have gastrointestinal effects such as dyspepsia and some abdominal discomfort [19]. The current study dealt with the in silico investigation of *Cissus quadrangularis* plant compounds for inflammation inhibitor to avoid any undesirable side effects. The docking studies using AutoDock 4.2 reveals that flavonoids such as Luteolin and Quercetin bind strongly to COX-2 enzyme with good binding energy of -8.69 and -8.62 respectively. Both form more hydrogen bonds on interaction with best cavity.

The catalytic activity of an enzyme molecule can be assessed by their hydrogen bonds in a docking study [20]. Recent studies have shown that some flavonoids attenuate inflammatory responses by acting as modulators of pro inflammatory gene expression [21]. In addition to flavonoids, stilbene derivatives such as Resveratol and Piceatannol also proved to be good inhibitors whose hydrogen bonds are with COX-2 enzyme.
radical scavenging, antiulcer, bone healing, analgesic and diuretic. In drugs for treating inflammation. The insights gained in this work can
be further used in experimental studies to develop leads of drugs against COX-2 enzyme.

CONFLICT OF INTERESTS
Declared None

REFERENCES

