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Research Article

In silico exploration of anti-inflammatory activity of *Pseudarthria viscida* root

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ABSTRACT

Cyclooxygenase is the key enzyme in the biosynthesis of prostanoids, biologically active substances that are involved in several physiological processes but also in pathological conditions, such as inflammation. The non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly medication in the world. The mechanism of action of these drugs is the inhibition of cyclooxygenase-2 enzyme, which catalyse the biosynthesis of prostaglandins-G2 from arachidonic acid. Despite the success of NSAIDs to treat inflammatory disorders with decreased side effects is an ongoing effort. Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found brought to the clinical trials and eventually released to the marketplace. Computer-Aided Drug Design (CADD) is a specialized discipline that uses computational methods to stimulate drug-protein interaction. Discovery studio 2.1 provides a set of protocols for predicting and analyzing the interaction between protein and ligands. Molecular Docking experiments were carried out for the compounds identified from *the Pseudarthria viscida* root extract with cyclooxygenase-2 using Accelry's DISCOVERY STUDIO 2.1. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Out of 13 compounds characterized from *Pseudarthria viscida*, only three of them docked with cyclooxygenase-2. of that three, d-mannitol-1 – decyl sulfonyl alone can be considered to be lead compound since it satisfies all Lipinski's rule five.

Keywords: Inflammation; Cyclooxygenase-2; Pseudarthria *viscid;* d-mannitol-1 – decyl sulfonyl

1. INTRODUCTION

Inflammation is a part of the body's defense system, which acts to remove and repair damaged tissue or to neutralize harmful agents (Maslinka and Gajewski, 1998). This system can be activated by several stimuli, including mechanical and chemical injuries, and entities or events that invoke the defense against microorganisms. The stimuli activate various enzymatic pathways and the release of various mediators, including prostaglandin synthesis via Cox-2. Prostaglandin E2 (PGE2), one of the major metabolites, is an important mediator of inflammation, by contributing to induction of fever, increasing vascular permeability and vasodilatation, and enhancing pain and oedema caused by other agents such as bradykinin and histamine (Nathan, 2002).

The Cyclooxygenase family comprises three known members that catalyze prostaglandin biosynthesis, namely, Cyclooxgenase-1, 2 and 3 (COX-1, COX-2 and COX-3). The first identified enzyme in the COX family, COX-1, is normally constitutively expressed, and is present in almost all tissues at constant levels (Smith, 1992; Smith et al., 1996). The metabolites of arachidonic acid (AA), derived from COX-1, are responsible for maintaining basic physiological conditions in the body, as in the case of cytoprotection of the gastric mucosa (Vane et al.,1998). The COX-1 variant protein, named COX-3. Yet, it remains to be investigated where COX-3 is produced *in vivo*, and how it contributes to physiological and path physiological conditions (Chandrasekharan et al., 2002). COX-2 contrasts with COX-1, an inducible isoform triggered by various stimuli including inflammatory agents, growth factors and tumour promoters (Herschman, 1996). The induced enzyme is involved in production of prostaglandins that promote the inflammatory process (Vane and Botting, 2001).

The field of Molecular Docking has emerged during the last three decades and now is becoming an integral aspect in drug discovery and development area. Investigators often use docking computer programs to find the binding affinity of molecules that fit a binding site on the protein. Hence, here we have taken *in silico* molecular docking to analyze the compounds identified from *Pseudarthiria viscida* with Cyclooxygenase-2. This study also can explore the binding properties of the compounds with a potential molecular target for in-

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flammation, i.e., Cyclooxygenase-2, which might be led to development of novel ligand to treat inflammation.

2. MATERIALS AND METHODS

2.1. Compounds identified from *Pseudarthria viscida* root

The presence of compounds like 3-O-Methyl-d-glucose, Butane-1,1 Diethoxy-3-methyl, d-Mannitol-1-decyl sulfonyl, n-Hexadecanoicacid, Oleic acid, Oxirane tetra decyl, Tetradecanoic acid, Undecanoic acid was identified by GC-MS study. By HPLC analysis, the existence of phenolic compounds such as Rutin, Quercetin, Gallic acid, Ferulic acid and Caffeic acid was characterized. So in total 13 compounds identified in the root of *Pseudarthria viscida* was taken for binding analysis with Cyclooxygenase-2.

2.2. Ligand preparation

The three dimensional structures of compounds taken for binding analysis were downloaded in .sdf format from PubChem database. Hydrogen bonds were added, and the energy was minimized using CHARMm force field. Lipinski properties such as Molecular weight, XLog P, number of hydrogen bond donors and acceptors for the compounds were obtained from PubChem (shown in Table: 1).

2.3. Protein preparation

The PDB is a key resource in areas of structural biology, is a key repository for 3D structure data of large molecules. The molecule which taken is Cyclooxygenase-2 for our consideration. The PDB ID is 6COX and a resolution factor is 2.80 and the method of incorporation is X-ray diffraction method. The ligand and crystallographic water molecules were removed from the protein; and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were connected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMm Force field.

2.4 Docking studies

The docking method used in this study is LigandFit. To perform docking process the modeled protein, a protocol called “Dockligands” (LigandFit) is selected among those listed under receptor-ligand interaction protocol cluster. Each ligand compound is given as input in the parameter meant for “input ligands” and the protocol was run for each of the inhibitors selected for the study. The various conformations for ligand in this docking procedure were generated by Monte Carlo trials. The final energy refinement of the ligand pose (or) pose optimization in ligandfit occurs by Broyden-Flecher Gold Farbshanno (BFGS) method. The Dock score of the best poses docked into the enzyme for all the 13 compounds is calculated.

3. RESULTS AND DISCUSSION

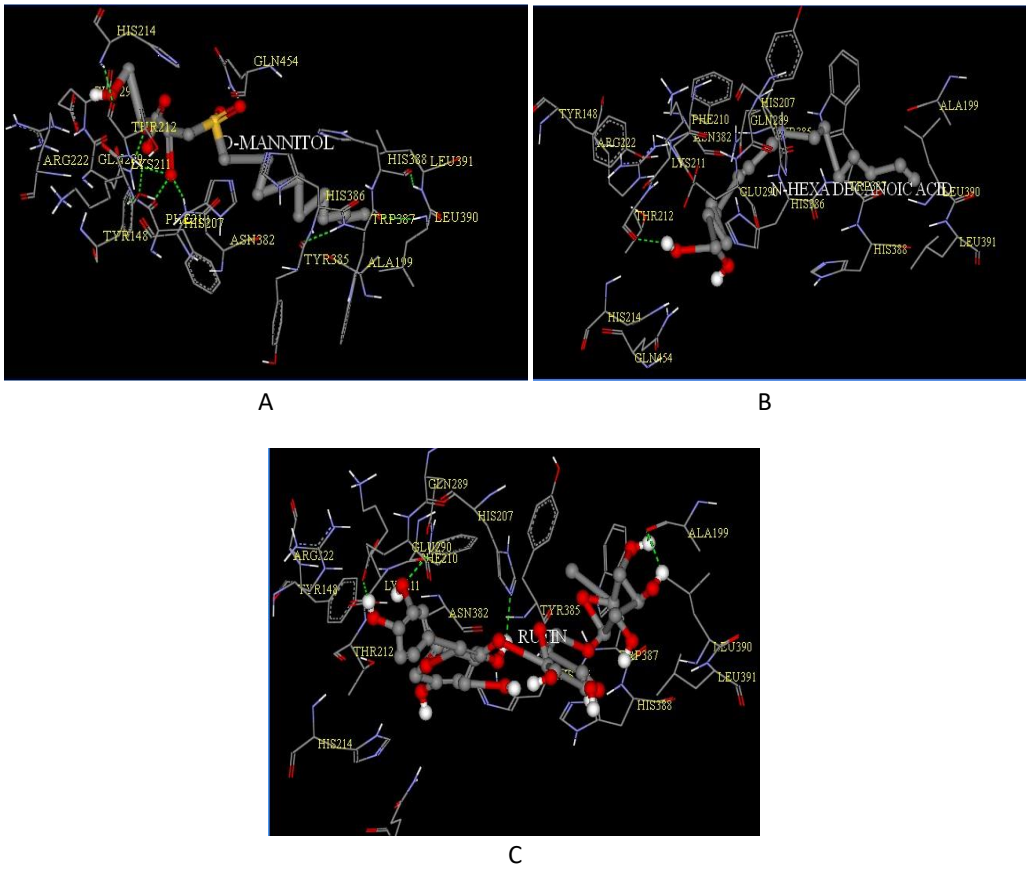
The crystal structure of Cyclooxygenase-2 (COX-2) with PDB ID 6COX having structure weight 138091.53 is retrieved from PDB. The resolution of COX-2 is 2.80 A^{o} and the method of incorporation is X-ray diffraction method. It has in total 2 chains of A and B. Both chains are sequence unique. Each chain has amino acid residues of length 587 residues. The structure of COX-2 has 33 helices and 24 stands. 41% of the structure comprising 244 amino acid residues belongs to helical part and 6% of the structure comprising of 36 amino acid residues belongs to strands.

Table: 2 shows the dock score values of top ranked ligands with the enzyme Cyclooxygenase-2. Docked pose of the compounds with enzyme (Cyclooxygenase -2) were presented in Figure 1. The dockscore values include Ligscore 1&2 (Mayo et al., 1990); Piecewise Linear Potential (PLP) - PLP1 (Krammer et al, 2005; Gehlhaar et al., 1995) and PLP 2 (Gehlhaar et al., 1995); Jain (Jain 1996); Potential of Mean Force- PMF (Mugge and Martin YC, 1999). PMF04 (Mugge, 2006), Ligand internal energy and dockscore obtained using LigandFit protocol of Discovery studio 2.1.

Out of the 13 ligands, only three ligands docked with protein Cyclooxygenase-2. They are d-Mannitol-1-decylsulfonyl, n-Hexadecanoic acid and Rutin. Out of this three, Rutin recorded higher dock score value followed by d-Mannitol-1- decylsulfonyl and n-Hexadecanoic acid. Hence Rutin can be considered as the better inhibitor for COX-2 out of 13 ligands screened for studies.

The formation of hydrogen bond between the ligand and enzyme is important for the protein-ligand interaction. It is understood from the Table: 3 that a maximum of five hydrogen bonds were formed between protein (Cyclooxygenase -2) and ligand Rutin followed by d-mannitol, 1 – decylsulfony which formed four bonds.

In between n-Hexadecanoic acid and protein, there is only one hydrogen bond is formed. It is also known from the table that amino acids Tyr148, Ala 199, His 207, Thr 212, Glu 290, Gln 289, Asn 382 plays an important role in binding mechanism. The molecular interactions that the ligands rutin and d-Mannitol, 1-decylsulfonyl showed with the cyclooxygenase -2 revealed better ligand-binding affinity. So it can be concluded that the plant extracts has the potential compound that can inhibit Cyclooxygenase- 2. Based on the Dockscore values it was predicted the ligand Rutin had a good binding affinity towards the protein followed by d.Mannitol, 1-decayl sulfonyl and n-Hexadecanoic acid. Rutin and n-Hexadecanoic fails to satisfy all the criteria of Lipinski’s rule. But d.-Mannitol, 1-decyl sulfonyl satisfies all the parameters such as molecular weight (370.50), 5 five hydrogen bond donor and 7 hydrogen acceptor. The Log P value is 0.9. There by it satisfies all the criteria of Lipinski’s rule.



A B

C

Figure 1: Summary of Docked Pose of the Compounds identified from Pseudarthria Viscida

Docked model of (A) d-Mannitol,1-decylsulfonyl (B) n-Hexadecanoic acid (C) Rutin with cyclooxygenase -2.

CONCLUSION

The present study indicates that the plant *Pseudarthria viscida* can be used in the treatment of inflammation, since the compounds identified from that plant shows a strng binding affinity towards cyclooxygenase-2. This brings strong focus towards this plant, when administered during the treatment of inflammation may block cyclooxygenase-2. Though Rutin showed highest dockscore value, but it fails to satisfy Lipinski's rule required to be drug. d-Mannitol, 1-decyl sulfonyl exhibited affinity towards Cox-2 and also satisfies all the criteria of Lipinski's rule of five. This creates a strong hypothesis that the effects of complex formation by COX-2 and d-Mannitol, 1-decyl sulphonyl will contribute in combating inflammation.

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