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Molecular docking and binding study of harpagoside and harpagide as novel anti-inflammatory and anti-analgesic compound from *Harpagophytum procumbens* based on their interactions with COX-2 enzyme

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ABSTRACT

Objective: To clarify the molecular inhibition mechanism of harpagoside and harpagide with cyclooxygenase-2 (COX-2) as natural potent anti-inflammatory and anti-analgestic compounds derived from the *Harpagophytum* species and evaluate the drug-likeness and pharmacokinetic characteristics of both compounds.

Methods: The X-ray crystal structure of the COX-2 enzyme and harpagoside and harpagide were retrieved and energetically minimized by SPDV viewer and ChemAxon softwares, respectively. Then, all nonpolar hydrogens were merged, and partial atomic charges were assigned using the Gasteiger-Marsili method. The binding sites and surfaces of enzymes were detected. In addition, docking studies were performed by AutoDock 4.2 using the Lamarckian genetic algorithm. Finally, the drug-likeness and molecular pharmacokinetic properties of the compounds were calculated.

Results: Molecular docking showed that both harpagoside and harpagide interact with COX-2, and their binding energies were –9.13 and –5.53 kcal/mol, respectively. Furthermore, interactions were stabilized for harpagoside and harpagide within the active site of COX-2 by 7 and 10 hydrogen bonds, respectively. These results suggested that harpagoside and harpagide complied with most of Lipinski's rules. Bioactivity scores revealed that harpagoside was a moderate G protein-coupled receptor ligand, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. Harpagide is a suitable enzyme inhibitor and moderate G protein-coupled receptor ligand, ion channel modulator, nuclear receptor ligand, and protease inhibitor.

Conclusions: Results clearly revealed that harpagoside and harpagide act as potential highly selective COX-2 inhibitors. They are safe anti-inflammatory/analgesic compounds compared with classical non-steroidal anti-inflammatory drugs and could be considered as promising inflammatory inhibitors of a natural origin.

1. Introduction

Inflammation is a common human body response to injuries and infection[1]. In harmful immune response status, it causes the development of different inflammatory, allergic, and autoimmune diseases[2,3]. The arachidonic acid pathway plays key roles in the inflammation mechanism, in which arachidonic acid is oxygenated by the enzymes cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) and then, by transforming into a variety of products, it modulates inflammatory reactions[4-6]. Therefore, COX-2 and LOX are responsible for inflammation and pain. Furthermore, these

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inhibitor enzymes could be used as powerful drugs to treat these complications in different diseases and surgery[7-9].

Harpagophytum procumbens (*H. procumbens*) (common names: devil's claw, grapple plant, wood spider, and harpago) belongs to the Pedaliaceae family and is native to the African continent[10,11]. It has medicinal benefits and has become an established traditional treatment for some inflammatory diseases[12,13]. Active constituents of *H. procumbens* are defined as iridoid glycosides (primarily harpagoside, harpagide, and procumbide), sugars, triterpenoids, phytosterols, aromatic acids, and flavonoids[14]. Major chemical components in tuberous roots are harpagoside (a monoterpene glucoside), harpagide, and procumbide, all thought to be responsible for anti-inflammatory and analgesic activities[15-19]. *In vitro* tests have shown that harpagoside and harpagide may inhibit lipopolysaccharide-induced inducible nitric oxide as well as COX-2 and 5-LOX expression, thus inhibiting inflammation[20-22]. Dual inhibitors of COX-2/5-LOX enzyme are known as effective, promising regimes for controlling the molecular processes of inflammation and causing minimal adverse effects compared with non-steroidal anti-inflammatory drugs[23,24]. To the best of the authors' knowledge, this study is the first investigation using molecular docking to clarify the molecular mechanism of inhibition in harpagoside and harpagide as natural potent anti-inflammatory compounds derived from the *Harpagophytum* species for the inhibition of COX-2 enzymes. Moreover, the drug-likeness and molecular pharmacokinetic properties of harpagoside and harpagide were evaluated for application as a novel drug in the treatment of inflammatory diseases.

2. Materials and methods

2.1. Retrieving structures and energy optimization

The X-ray crystal structures of COX-2 (protein data bank ID: 4PH9) and 5-LOX enzyme (protein data bank ID: 3vv9) were downloaded from the protein data bank. Then, water molecules were deleted and the COX-2 and 5-LOX were cleaned from any unwanted interactions. These structures were energetically minimized using the SPDB viewer software tool with a GROMOS96 implementation. The 3D chemical structures of harpagoside (PubChem ID: 5281542) and harpagide (Pubchem ID: 10044294) were retrieved through the PubChem-compound database at the NCBI web server, USA (www.pubchem.ncbi.nlm.nih.gov). Ligand geometry was minimized by Avogadro software.

2.2. Preparation of receptors for docking

The retrieved enzyme structures were prepared by removing water molecules, ligands, and any unwanted structures. Then, hydrogen bonds and a molecular charge were added using the Gasteiger-Marsili method.

2.3. Molecular docking

Molecular docking was done to evaluate a possible binding mode with COX-2 and 5-LOX active sites, separately. Molecular docking in virtual screening revealed the possible biological activities of ligands and significantly reduced the cost and time involved in drug discovery. Furthermore, it evaluated the strength of the binding, the energy of the formed complex, and computed binding affinities using scoring functions.

The docking studies were carried out using AutoDock 4.2. The Lamarckian genetic algorithm was selected for the molecular docking studies[25]. COX-2 and 5-LOX conformational flexibility was neglected by choosing rigid receptor docking. All probable rotatable bonds were assigned for the ligands[25,26]. To estimate the perfect grid, grid box values were obtained by trial and error and from previous research using AutoGrid. Grid maps with 126-126-126 points (the x, y, and z directions) were made, and the grid-point spacing was considered as 0.375 Å. Each map was centered so that it covered the entire protein, especially all possible binding sites. The number of independent docking runs was set at 50 with step sizes of 0.2 Å and 5°.

2.4. Drug-likeness of the ligands

The molinspiration server (www.molinspiration.com) was applied to estimate the harpagoside and harpagide drug-likeness and molecular pharmacokinetic properties (absorption, distribution, metabolism, and excretion properties). Said server offers free tools for calculating the important molecular properties based on Lipinski's rule of five (such as LogP, polar surface area, number of hydrogen bond donors and acceptors). Compounds with more than one violation of these rules should be eliminated from the docking study because of bioavailability problems. The molinspiration server also evaluates drug-likeness, including G protein-coupled receptors (GPCR) ligand, kinase inhibitor, ion channel modulator, nuclear receptor and enzyme inhibitor ligand, and eventually the number of violations of Lipinski's rule for ligands.

3. Results

3.1. Molecular docking

Molecular docking showed that both harpagoside and harpagide interact with the COX-2 enzyme. The ΔG (binding energy) of these compounds were computed as –9.13 and –5.53 kcal/mol, respectively. Clearly, harpagoside interacts more effectively with the COX-2 enzyme. Furthermore, harpagide and harpagoside interactions were stabilized by different hydrogen bonds within the amino acid active site of COX-2. The interactions mode between harpagoside and harpagide in the allosteric binding site pocket of the COX-2 enzyme were shown in Figure 1, while in

Figure 2, the hydrogen bonds and hydrophobic interaction were depicted.

In case of harpagoside 7 hydrogen bonds (Gly527, Ser531, Ala152, Glu381, Thr150, Ser380, Asn383) and 5 hydrophobic interactions (Ile378, Ala203, Hem602, Arg151, Tyr149), were reported. Also in case of harpagide 10 hydrogen bonds (Gln290, Phe211, Hem602, 2 Thr213, 2 Arg223, 3 Tyr410) and 6 hydrophobic bonds (His208, Ile275, Leu295, Lys212, Val292, His215) were reported (Figure 1). The strongest hydrogen bands between harpagoside and harpagide with COX-2 were Gly527 (2.25 Å) and Hem602 (2.50 Å), respectively.

3.2. Drug-likeness and toxicity analysis

Molecular pharmacokinetic parameters were calculated by the molinspiration server (represented in Table 1). The results suggested that harpagoside and harpagide complied with most Lipinski's rules (as described in the methods section). However, the polar surface area and number of hydrogen bond acceptors of harpagoside and polar surface area in harpagide are within a

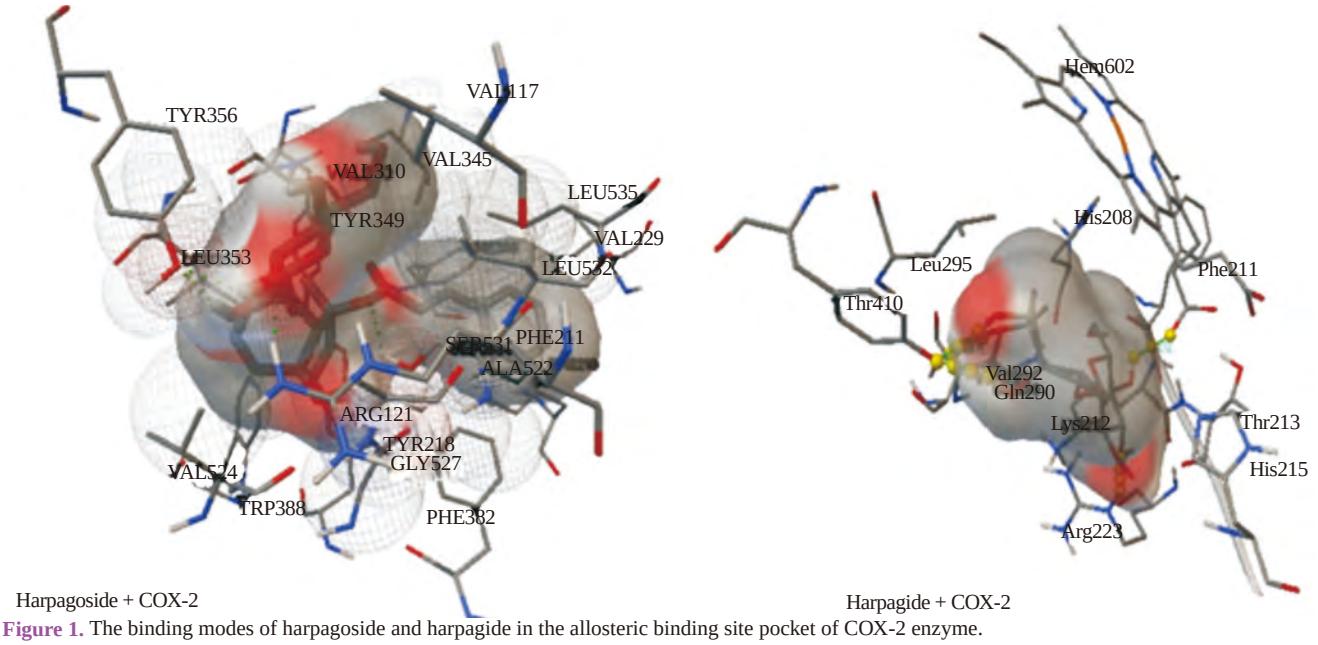

Figure 1. The binding modes of harpagoside and harpagide in the allosteric binding site pocket of COX-2 enzyme.

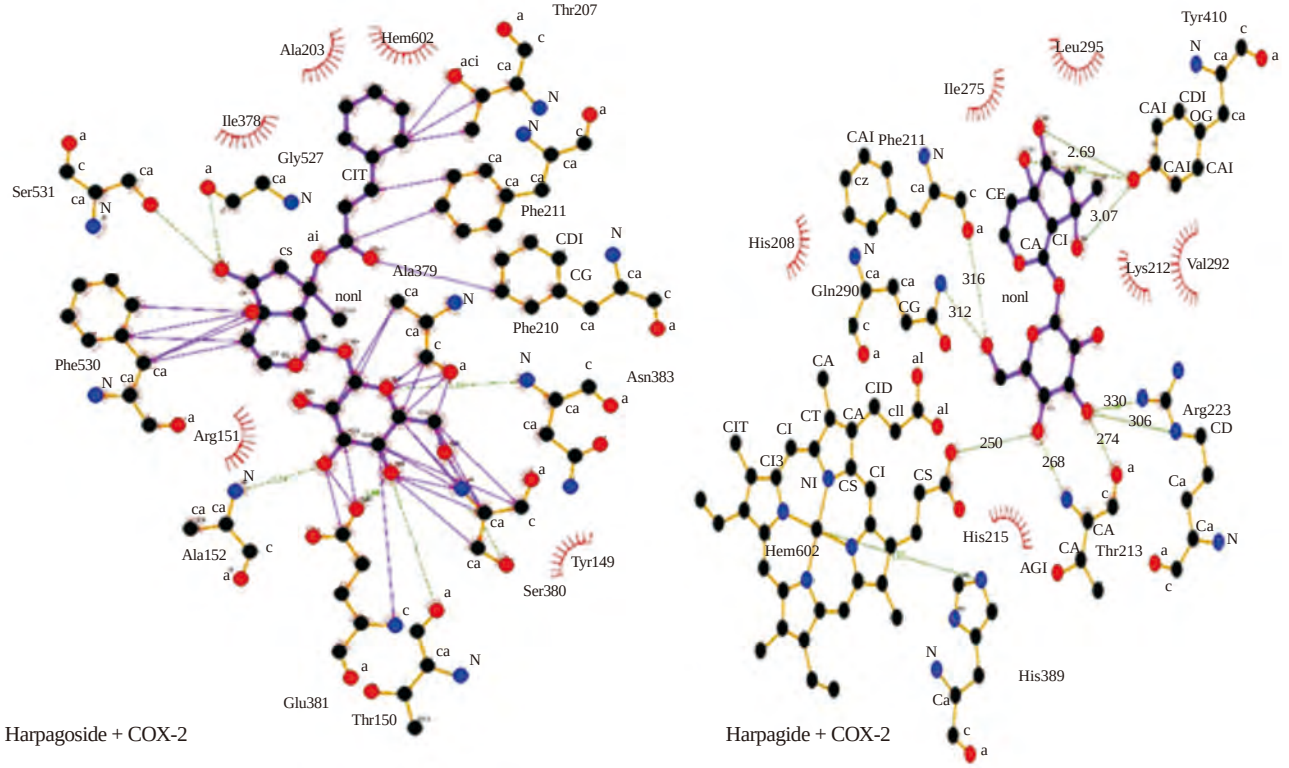

Figure 2. Hydrogen bonds along with their distances between the harpagoside and harpagide and active sites of COX-2 enzyme.

Table 1
Molecular pharmacokinetic properties of harpagoside and harpagide compounds.

Ligand	miLogP[a]	TPSA[b]	MW[c]	natoms[d]	nON[e]	nOHNH[f]	Nviolations[g]	Nrotb[h]	Volume[i]
Harpagoside	–0.10	175.38	494.49	35	11	6	2	7	427.17
Harpagide	–2.94	169.30	364.35	25	10	7	1	3	308.40

[a]: The logarithm of octanol/water partition coefficient; [b]: Topological polar surface area; [c]: Molecular weight; [d]: Number of non hydrogen atoms; [e]: Number of hydrogen bond acceptors (O and N atoms); [f]: Number of hydrogen bond donors (OH and NH groups); [g]; Number of rule of 5 violations; [h]: Number of rotatable bonds; [i]: Molecular volume.

more than optimum range. The term of LogP value shows the hydrophilicity character (lipophilicity) of the compound and greatly affects absorption or permeation. In both compounds, this parameter is very impressive. Harpagoside showed two violations in Lipinski's rule of five criteria, so its drug-likeness is somewhat lower than that of harpagide.

4. Discussion

Historically, *H. procumbens* has been known as an analgesic, a remedy for fever, allergies, and as a stimulant for gastro-intestinal complexities[27-29]. Moreover, it has been recommended as a diuretic and sedative as well as for dyspepsia, appetite stimulation, and degenerative disorders of the musculoskeletal system (British Herbal Pharmacopoeia, and German Food and Drug Administration)[14,20]. Therapeutic drugs for the treatment of pain and inflammation have shown limited effectiveness and safety. Moreover, repetitive and long-term use of non-steroidal anti-inflammatory drugs may cause adverse effects such as gastrointestinal lesions or renal and liver failure. Herbal compounds are frequently used to treat different inflammatory conditions[29,30]. This use has caused a significant decrease in the use of anti-inflammatory drugs[31].

Numerous studies have shown that the efficacy and safety of *H. procumbens* is significant and progressive in reducing pain and inflammation, and its use has resulted in a significant decrease in the use of anti-inflammatory drugs and other pain-relieving and inflammatory medications[15,31,32]. Clinical evidence suggests that *H. procumbens* is an inhibitor of COX-2[15,20].

In the present study, molecular docking was used to evaluate harpagoside and harpagide compounds from *H. procumbens* as inhibitors of COX-2 for the treatment of inflammatory and analgesic diseases.

Results showed that both of these chemical compounds can inhibit this enzyme, and both have suitable LogP. Interactions were stabilized by different hydrogen bonds within the active site of COX-2. Although harpagoside has a more effective interaction (binding energy = –9.13 kcal/mol), harpagide is somewhat more compatible than harpagoside with Lipinski's rule of five (bioavailability). Harpagoside might pass through the Lipinski's rule of five criteria if some structural modifications occur to improve its polar surface area and number of hydrogen bond acceptors levels. However, some successful commercial drugs do not follow some of Lipinski's rules.

The bioactivity scores of harpagoside and harpagide against GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitor, and enzyme inhibitory activity were evaluated. Harpagoside was found to be a moderate GPCR ligand, nuclear receptor ligand, protease inhibitor, and enzyme inhibitor. Harpagide, however, is a suitable enzyme inhibitor and moderate GPCR ligand, ion channel modulator, nuclear receptor ligand, and protease inhibitor.

To date, a limited number of studies have reported harpagoside and harpagide molecular interactions with the COX-2 enzyme. In the current study, these interactions were clarified, and both compounds were suggested for use as future anti-inflammatory and anti-analgesic drugs, because of their natural origins.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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