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Research Article

IN SILICO STUDY OF ASIATIC ACID INTERACTION WITH *INDUCIBLE* NITRIC OXIDE SYNTHASE *(INOS)* AND *CYCLOOXYGENASE-2* (COX-2)

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ABSTRACT

The aim of the present study involves the application of in silico methods to elaborate the possibility of the interaction of asiatic acid into binding pocket of inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2) which have roles in inflammatory process, and its selectivity against both receptors at molecular level. The methods consist of docking moleculer by Autodock v.3.05 Program. The results show that the interaction of asiatic acid in the binding pocket of iNOS due to the hydrogen bonds on Gln 263 and Trp 372 with free-binding energy of –9.79 kcal/mol, while its interaction to COX-2 by hydrogen bonds to Arg 120 and Tyr 385, with free-binding energy of –1.73 kcal/mol. The selectivity of asiatic acid to iNOS receptor is higher than COX-2 receptor. The asiatic acid possesses anti-inflammatory activities, and its mechanism is suspected to inhibit iNOS enzymes.

Keywords: Asiatic acid, In silico study, iNOS, COX-2

INTRODUCTION

Pegagan (*Centella asiatica (L) Urban*) (*Umbelliferae*), also known as *Gotu Kola*, is a kind of plant that has a weak aroma and it can easily grow in damp and swampy areas. This plant is known for its benefits as anti-leprotic, anti-tumor, anti-stress, wound healing, anti-philaria, anti-feedan, and anti-bacteria. These pharmacological activities are connected to the four triterpen compounds in the alcohol extract of the pegagan foliage (*Centella asiatica (L) Urban*). The four compounds are asiatic acid, madecasic acid, asiaticoside, and madecasicoside. Among the four, asiatic acid is the most active compound in the biological activity[1].

Asiatic acid is a pentacyclic triterpen compound that has active features as the donor and acceptor of hydrogen bond. Asiatic acid has three hydroxyl at C(2), C(3) and C(23); it also has an olefin at C(12), and one carboxylic acid group function at C(28) (figure 1). Asiatic acid has been known as showing anti-inflammatory activity both in in vivo[2] and in vitro studies, and it is suggested that this activity is due to the inhibition of production of *inducible nitric oxide synthase* (iNOS), COX-2, IL-6, IL-1β, as well as the expression of TNF-α through the decrease of NF-kappa (k)B activation[3]. Others pentacylic triterpen such as oleanolic acid and ursolic acid have been reported their ability to suppress of two enzyme, inducible nitric oxide synthase (iNOS) and cyclooxgenase-2 as potential antiinflammatory by Honda et.al [4-5]. Based on that, asiatic acid was predicted to have a same activity as oleanolic acid and ursolic acid.

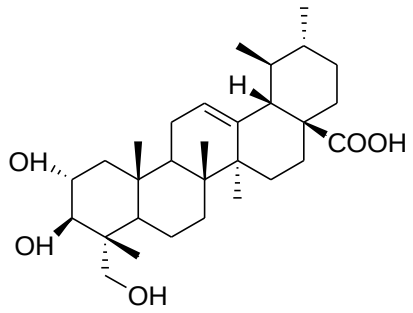

Fig. 1: The structure of asiatic acid

Inflammatory is a kind of respond from an organism against the invasion of unknown objects such as bacteria, parasites and viruses. In this context, the inflammatory respond is a protective reaction against irritation, wound, or infection in that the reaction shows signs of rashness, burning sensation, swollen parts, loss of function, and excruciating pain. The conceptual explanation concerning the cause of inflammatory is a complex and interwoven phenomenon, and until recently it has been the center of discussion of numerous studies[6]. Inducible enzymes like iNOS and COX-2 play an important role in the respond of a tissue against wound, infection, inflammatory, and carcinogenesis.

iNOS (*inducible nitric oxide synthase*) play a significant role in controlling vascular pressure, neurotransmission, microorganism inhibitor, tumor cells and homeostatic system. The high level of Nitric Oxide (NO) influences pathophysiological processes including shock on the blood-pressure circulation, inflammatory, and carcinogenesis. NO is produced from L-arginine by Nitric Oxide Syntase (NOS) enzyme. There are three isoforms of NOS: *neural* NOS, *endotelial* NOS –both are expressed constitutively–and *inducible* NOS (iNOS). The inducible forms of NOS are directly responsible for the number of NO in the inflammatory process[7].

Cyclooxygenase (COX) is an endogenous enzyme which catalyses the conversion of arachidonic acid into Prostaglandins (PGs) and thromboxane. PGs (prostaglandin) is a kind of inflammatory endogen mediator but also maintenance of the lining of the stomach and prevention of ulceration. The enzyme exists in two isoforms, constitutive enzyme COX-1 which responsible to the supply of prostaglandins to maintain the gastric mucosa and stabilize adequate vascular homeostatis, and inducible enzyme COX-2 which is induced by inflammatory factors. COX-1 is found mainly in the gastrointestinal lining, and COX-2 at sites of inflammation[8,9,20].

The excessive expression of iNOS or COX-2 can be found in a lot of disease pathogenesis of a variety of inflammatory disiases. The prostaglandin production by COX-2 and NO by iNOS are regulated at the transcriptional level by the transcription factor NF-KB that contributes to the induction of early gene expressions accurately[10].

The molecular modelling through docking simulation technique is a method to investigate the interaction between the ligand and bio-macromolecular target. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions[11]. The docking principle is done by placing ligand into the binding receptor pockets; further, the molecules based on its form-similarity, and on its characteristics like its electrostatic nature[12,13]. Generally, the binding between drugs and receptors is categorized as weak and non-covalent interaction so that the

produced effect is reversible. This inhibition can happened if the compound active features interact with the target-binding pockets through several ways. These are Van der Waals interaction, hydrophobic interaction, and hydrogen bond formation in which the last one has the strongest affinity with distance between 2.5-3.2 Å[14].

We are interest to study the possible interactions of asiatic acid with those reported protein, especially iNOS dan COX-2. We are perform an in silico study on affinity properties of asiatic acid on iNOS and COX-2 by applying docking study methods. Even though the anti-inflammatory of asiatic acid has been known, but its selectivity against both iNOS and COX-2 receptors at molecular level has not been reported.

MATERIAL AND METHODS

Material

This research used 3D LBD (ligand binding domain) *inducible Nitric Oxide Synthase* (PDB: 1NSI)[15] structure with 2.55 Å resolution, and 3D LBD (ligand binding domain) *cyclooxygenase*-2 (PDB: 3NT1)[16] structure with 1.73 Å resolution. The crystal structure were selected should have best resolution or lower resolution value, and also have R-free and R-value lower than 0.25. The 3D structures of asiatic acid were constructed using Hyperchem 7, then were optimized using Austin Model 1 (AM1).

Molecular docking

MGL tools program package 1.5.4. (*Molecular Graphics Laboratory, The Scripps Research Institute*) is used to prepare protein structures, ligand structures, *grid* parameter *file* and *docking* parameter *file*; furthermore, the AutoGrid v 3.05 program (*The Scripps Research Institute*) is used to prepare the grid, the Autodock v 3.05 (*http://autodock.scripps.edu*) is employed to simulate the docking process through Cygwin program (*www.cygwin.com*). These two PDB are chosen by considering that the R-value and R-free are close to 0.20, and that the resolution is less than 3 Å[14].The asiatic acid compound docking parameter against iNOS (PDB: 1NSI) is the grid box 40x40x40 with the space 0.375 Å and grid center x,y,z respectively 9.740; 64.640; 15.986. The number of *run* 50, population 150, and evaluation energy 2500000. The asiatic acid compound docking parameter against COX-2 (PDB: 3NT1) is the 60x60x60 grid box with space 0.375 Å, grid center x,y,z respectively -40.699; 51.500; -22.400 , with the number of *run* 50, population 150, and evaluation energy 2500000. Binding affinity was indentified by free-binding energy (ΔG) and hydrogen bonds between ligands and the enzymes.

RESULTS AND DISCUSSION

Validation of docking method

Prior to dock of asiatic acid into target, the re-docking of L-arginin as a substrate of iNOS (PDB: 1NSI), and naproxen as an inhibitor to COX-2 (PDB: 3NT1) were conducted to ensure whether the method is valid. The amino acid residues that bind compounds produced by the re-docking process are then compared with amino acid residues that bind crystal molecules, with RMSD that was less than or equal to 2.0 Å was defined as reasonable posess[17]. In present study shows the value of RMSD < 2 Å (iNOS: 1.962 Å and COX-2: 0.922 Å), indicating that the parameter set for docking is suitable of reproducing the x-ray structure. In addition, both of ligand from iNOS (L-arginine crytsal) and that from poduced by the re-docking interact to the same residue of iNOS, i.e., Tyr347, Asp 382, Glu 377, Gln 263 (table 1). Figure 2 shows the conformation superposition of L arginine from x-ray crystal structure of L-arginine–iNOS complex and that from re-docking. As well as the ligand of COX-2 in Table 2 shows that re-docking result naproxen can also invade COX-2 receptor-binding pockets, and that hydrogen bonds occur between carboxylic group with amino acid residue of Arg 120 dan Tyr 355, and this results are consistent with those reported before[16,18,20]. There is pi interaction (orange line) between COOH-Arg 120 and ring aromatic of naproxen (Figure 3). The visualization of the results were conducted by Discovery Studio 3.0 program. This methods is then used to simulate asiatic acid compound simulation into both receptor binding pockets.

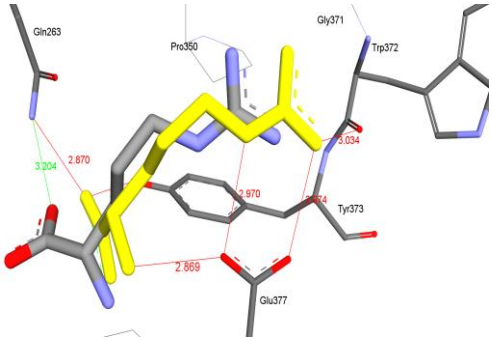

Fig. 2: This figure shows the crystal L-arginine interaction results (shown in grey), and the L-arginine re-docking results (shown in yellow) in the iNOS receptor-binding pocket (1NSI).

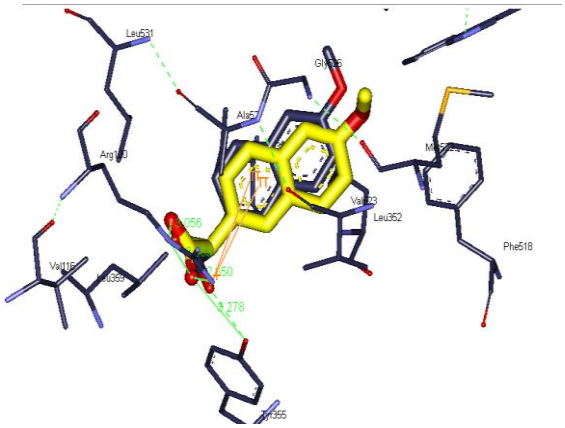

Fig. 3: Interaction of naproxen crystal (shown in grey), and docking result naproxen (shown in yellow) in COX-2 receptor binding pockets (*3NT1)*

Table 1: The amino acid residue interaction of L-arginine crystal and the redocking results in iNOS receptor binding pockets (*PDB : 1NSI*)

Substrate (crystals)	Substrate *re-docking* Result	Hydrogen Bond
Gln 263, Tyr 347, Pro 350, Val 352, Gly 371, Trp 372, Tyr 373, Glu 377, Asp 382	Gln 263, Tyr 347, Pro 350, Val 352, Gly 371, Trp 372, Tyr 373, Glu 377, Asp 382	Tyr347, Asp 382, Glu 377, Gln 263

Table 2: The amino acid residue interaction of naproxen crystal and the re-docking results in COX-2 receptor-binding pockets (*PDB: 3NT1*)

Amino Acid Residue in Crystal Molecules	Amino Acid Re-docking Result Residue	Hydrogen Bond
Val 116, Arg 120, Val 349, Leu 352, Tyr 355, Leu 359, Tyr 385, Trp 387, Val 523, Gly 526, Ala 527, Ser 530, Leu 531	Val 116, Arg 120, Val 349, Leu 352, Tyr 355, Leu 359, Lue 384, Trp 387, Phe 518, Met 522, Val 523, Gly 526, Ala 527, Leu 531	Arg 120, Tyr 355

Molecular docking of asiatic acid

Asiatic acid is reported possesing as antiinflammatory activity due to its ability to down regulate iNOS and COX-2 expression through the decrease of NF-kappa (k)B activation[3], but its selectivity and the possibility of interaction into both receptors is not yet reported. In this study was perform simulation docking molecular of asiatic acid into binding pocket of iNOS and COX-2 by Autodock v.3 program.

The active site of iNOS devided into four pockets, i.e. the substrate binding S pocket, the midlle M pocket, the C1 pocket and C2 pocket in the substrate access have been reported before [15,21]. Asiatic acid was docked into the S pocket of iNOS that have been found the main residues (Trp 372 and Glu 377) with which substrates (L-arginin) form hydrogen bond. Our results showed that the asiatic acid could compete against iNOS to potentially replace of L-Arg role. Hydrogen bond interaction of COOH-asiatic acid with Gln-iNOS (distance: 2.769 Å) might proves that this compound has potent against iNOS. There are differences amino acid residue of iNOS taking part in the interaction between L-arginine and asiatic acid, however interaction OH-asiatic in ring A (C23) with Trp372-iNOS (3.08 Å) strengthen of this fact (figure 3) with free energy binding of -9,79 kcal/mol (Table 3). The interaction between asiatic acid and iNOS also formed (with distance <5 Å) hydrogen bond between Ala262, Ser242, and Gly202 (figure 3).

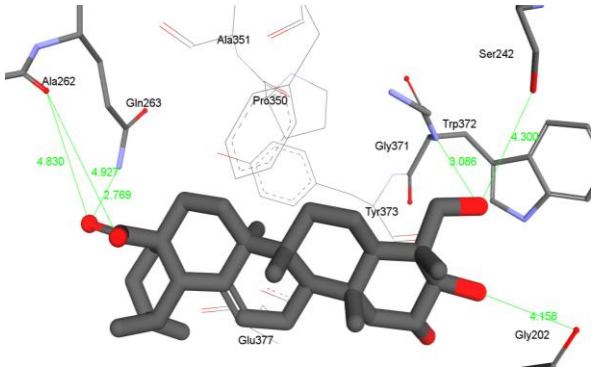

Fig. 3: Asiatic acid interaction with iNOS in the binding pocket

Table 3: Asiatic acid compound hydrogen bonds in iNOS binding pockets (*PDB:1NSI*)

Amino Acid Residue	Ligand Atoms	Binding Distance (Å)	ΔG (kcal/mol)
Gln 263	O of COOH	2,75	-9,79
Trp 372	O of OH	3,09	

In this study, Asiatic acid was docked into the active site of COX-2 also. The active site of COX-2 is devided into three important region, the first is a hydrophobic pocket there are Tyr 385, Trp 387, Phe 518, Ala 201, Tyr 248 and Leu 352; the second region being entrance of the acitive site lined by the hydrophilic residues Arg 120, Glu 524, Tyr 355, and the third is a side pocket lined by His90, Arg 513 and Val 523[19,20]. The interaction between AA and COX-2 occurred from the formation of hydrogen bond between oxygen atoms at hydroxyl group (C-2) of asiatic acid and N atoms (NH) from the hydrophilic residues of Arg 120 (donor) in the active site lined, and O atoms at carboxylic groups (acceptor) of AA and H atoms (OH) from amino acid residue of Tyr 385 (donor) (figure 5) in the hydrophobic pocket. In the side pocket of COX-2, hydrophobic interaction occured between olefin grup of AA and Val 523 (4.97 Å). The absence of ring aromatic in asiatic acid cause loss pi interaction with Arg120 and hydrogen bond interaction of AA at the side pocket of COX-2 may lead the AA could not compete into COX-2, with free energy of binding is -1.73 kcal/mol (Table 4).

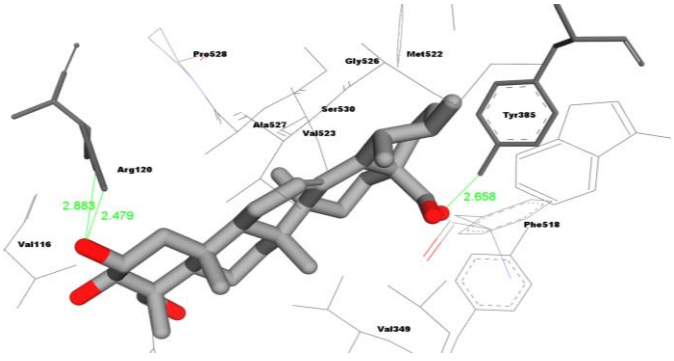

Fig. 5: Asiatic acid interaction with COX-2

Table 4: Asiatic acid compound hydrogen bonds in COX-2 binding pockets (*PDB:3NT1*)

Amino Acid Residue	Ligand Atoms	Binding Distance (Å)	ΔG (kcal/mol)
Arg 120	O of OH (C-2)	2,88	-1,73
Arg 120	O of OH (C-2)	2.86	
Tyr 385	O of COOH	2.65	

The interaction between asiatic acid and both receptors is also shown through hydrophobic bonds and Van der Waals interaction with some amino acids located inside the binding pockets (Figure 3,5). Based on the molecular docking simulation results, the active features of asiatic acid against iNOS and COX-2 are hydroxyl in the ring A and carboxylic groups. These groups act as hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) which are responsible for the biological activities not only against iNOS but also COX-2; moreover, their activities as inhibitors to iNOS are more selective that to COX-2.

CONCLUSION

Asiatic acid (AA) show affinity on both iNOS and COX-2, in which the affinity of AA on iNOS is higher than that of COX-2. In the case of iNOS, this affinity is due to hydrogen bonds between amino acid residue Gln 263 and Trp 372, while that of COX-2 between Arg 120 and Tyr 385. The important pharmacophore features are the hydroxyl (ring A) and carboxylic group acting as hydrogen bond acceptor (HBA), also olefin group as an one of hydrophobic function. The interaction of asiatic acid in inhibiting iNOS is bigger than COX-2. The anti-inflammatory activities of AA is suspected to inhibit iNOS enzymes.

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REFERENCES

1. Jeong BS, Asiatic Acid Derivatives for New Wound Healing Agent, *Archives of Pharmacal Research,* 2006; 29, No.7 : 556-562.
2. S.S Huang, C.S Chiu, H.J Chen, W.C Hou, M.J Sheu, Y.C Lin, P.H Shie, G.J Huang, Antinociceptive Activities and the Mechanisms of Anti-Inflammatory of Asiatic Acid in Mice, *Evidence-Based Complementary and Alternative Medicine*, Volume 2011; Article ID 895857, 10 pages.
3. K.J Yun, Y.J Kim, J.B Kim, K.W Lee, S.Y Jeong, H.J Park, H.J Jung et al., Inhibition of LPS-induced NO and PGE2 production by asiatic acid via NF-κB inactivation in RAW 264.7 macrophages: Possible involvement of the IKK and MAPK pathways, *Int. Immunopharmacol*, 2008; 8 : 431–441.
4. Yadav VR, Prasad S, Sung B, Kannappan R, Aggarwal BB, Review Targeting Inflammatory Pathways by Triterpenoids for Prevention and Treatment of Cancer, *Toxin*, 2011; 2: 2428-2466.
5. Suh N, Honda T, Finlay HJ, Novel Triterpenoids Suppress Inducible Nitric Oxide Synthase (iNOS) and Inducible Cyclooxygenase (COX-2) in Mouse Macrophages, *Cancer Res*, 1998; 58: 717-723.
6. Calixto JB, Otuki MF, Santos ARS, Anti-Inflammatory Compounds of Plant Origin. Part I. Acion on Arachidonic Acid Pathway, Nitric Oxide and Nuclear Faktor kB (NF-kB), *Planta medica*, 2003; 69: 973-983.
7. Yang EJ, Yim EY., Song G, Kim Gi-Ok, Hyun CG, Inhibition of nitric oxide production in lipopolysaccharide-activated RAW 267.4 macrophages by Jeju plant extracts, *Interdisc Toxicol*, 2009; 2(4) : 245-249.
8. Blobaum AL. and Lawrence JM, Structural and Functional Basis of Cyclooxygenase Inhibition, *J. Med. Chem*, 2007; 50(7), 1425-1441.
9. Smith RM., DeWitt DI., Garavito RM., Cyclooxgenases : structural, cellular and molecular biology, *Ann Rev Biochem*, 2000; 69: 145-182.
10. Liang YC, Huang YT, Tsai SH, Shiau SYL., Chen CF., Lin JK., Supression of Inducible cyclooxgenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages, *Carcinogenesis*, 1999; 20: 1945-1952.
11. Rohith KA, Sri RT, Shravan KG, 3D QSAR and Docking Studies Of Flavonoid Derivatives on p56lck Protein Tyrosine Kinase Using PLS, Int J Pharm Pharm Sci, 2011; 3(4), 44-52.
12. Goodsell DS. & Olson AJ, Automated Docking of Substrates to Proteins by Simulated Annealing, *Proteins: Str. Func. Genet.* 1990(**8**):195-202.
13. Kroemer RT, Molecular modelling probes: docking and scoring. Biochemical Society Transactions, 2003; 31: 980–984.
14. Schaeffer L., *The Practice of Medicinal Chemistry*, Thrid edition, 2008 (26), Elsevier.Ltd. p: 464-471
15. Li H, Raman C.S, Glaser C.B, Blasko E, Young T.A, Parkinson J.F, Whitlow M, Poulos T.L., Crystal structures of zinc-free and -bound heme domain of human inducible nitric-oxide synthase. Implications for dimer stability and comparison with endothelial nitric-oxide synthase, *J Biol Chem*. 1999 Jul 23;274(30):21276-84.
16. Duggan KC, Walters MJ, Musse J, Harp JM et al., Molecular Basis for Cyclooxygenase Inhibition by the Non-steroidal Anti-inflammatory Drug Naproxen, *J. Biol Chem*. 2010; 285(45): 34950–34959.
17. Morris, G.M and M. Lim-Wilby, *Molecular Docking, Methods in Molecular Modelling in Molecular Biology, In Molecular Modelling of Protein*, Kukol, A. (Ed), Human Press, Totowa, New Jersey, pp : 365-382.
18. D`Mello P, Gadhwal MK, Joshi U, Shetgiri P, Modelling of COX-2 Inhibitory Activity of Flavonoids, Int J Pharm Pharm Sci, 2011; 3 (4): 33-40.
19. Llorens O, Perez J, Palomer A and Mauleon D: Differential binding mode of Diverse cyclooxygenase inhibitors, Journal of Molecular Graphics and Modelling, 2002; 20:359–371.
20. Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC: Structural Basis for Selective Inhibition of cyclooxygenase 2 by antiinflammatory agents, *Nature*, 1996;384:644.
21. Francis, S.M., A Mittal, M. Sharma and P.V. Bharatam, Design of Benzene-1,2-diamines as selective inducible nitric oxide synthase inhibitors: A combined de novo design and docking analysis, J. Mol. Model., 14: 215-224.