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In Silico Study of Apigenin as COX-2 Inhibitor and *In vivo* Studies of Apigenin contained in Methanolic Extract of of *Atactodea striata* for decreasing Malondialdehyde Levels On Inflammatory Bowel Disease Rats

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Abstract: Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease in the gastrointestinal tract, especially the small intestine and the colon. One cause of IBD is a side effect of the use of non steroidal anti-inflammatory drugs (NSAIDs), such as Indomethacin. Indomethacin is not selectively inhibit both COX-1 and COX-2. COX-2 isused to reduce toxicity in the gastrointestinal tract. This study consisted of 2 phases, were study of molecular docking trough *in silico* studies using COX-2 with Apigenin compound as a ligand contained in methanolic extract of of *Atactodea striata*. Also *in vivo studies* of methanolic extract of *Atactodea striata* theraphy in IBD rats to reduce MDA level. *In vivo* studies used male rats (*Rattus norvegicus*), Wistar strain, divided into 4 groups: negative control (healthy rats), positive control (IBD rats) and group IBD with theraphy rats dose of 100 mg/kg BW and 400 mg/kgBW.The Results of i*n silico* studies showed molecular interactions by molecular docking between ligand (Apigenin) and COX-2 (pdb code: 3LN1) resulting the level of bond interaction and visualization of interaction level. It had value of Ki (inhibition constant) of 16.88µM and the value of bond energy to be -6.51 kcal/mol. Statistical analysis indicated that methanolic extract of of *Atactodea striata* therapy showed significant differences ($p < 0.05$) in reducing of MDA levels on IBD rats.
Keywords: Apigenin, COX-2, molecular docking, MDA, IBD rats.

Introduction

Inflammatory bowel disease (IBD) is a multi-factorial disorder. IBD can be caused by genetic factors, environmental and immune response. One cause of IBD is a side effect of the use of nonsteroidal anti-inflammatory drugs (NSAIDs) that can inhibit cyclooxygenase 2 (COX-2). The COX-2 synthesizes an endogenous prostaglandin (PG) as a protective factor for inflammation. The inhibition of prostaglandin synthesis can cause the increasing of intestinal permeability and lead to an easy access of pathogenic bacteria in the intestines surface[1,2].The induction of indomethacin is proven to be able to activate macrophages which can cause the release of inflammatory cytokines and can produce Reactive Oxigen Species(ROS), radical $O2^{\bullet-}$, OH• and H_2O_2 derived from the activation of neutrophils and macrophages (phagocytosis process) because of an infection. The oxidation process of metabolites indomethacin (iminokuinon) and the leakage of electrons from the specific side of mitochondrial electron transport chain of enterocytes cell. Administering of indomethacin orally once with a dose of 15mg/kg within a period of 24h can cause mucosal damage (villi) in

the small intestine [3,4].Methanolic extract of *Atactodea striata* contains flavon compounds [5].One of flavones contains in the methanol extract of *Atactodea striata* is Apigenin. In the previous research was mentioned that Apigenin compounds may function as an anti-inflammatory [6]. But until now, the strength of the inhabitation of COX-2 by Apigenin role has not been widely studied through computational methods. The Apigenin as a COX-2 inhibitor can be determined by *in silico* studies through molecular docking. The Inhibition value of the COX-2 were determined based on the bonding strength interaction between ligand and receptors on COX-2.

This research were conducted through *insilico* studies of interactions between COX-2 with apigenin of *Atactodea striata* as inhibitors of COX-2 and an *in vivo studies of* anti-inflammatory effect in the digestive tract of IBD rats.

Material and Methods

Molecules Model Analysis

The preparation of ligands and receptors.Ligands were Apigenin compounds contained in the methanolic extract of *Atactodea striata* and COX-2 receptor. The structure of the ligand and receptor was obtained from NCBI. The ligands and receptors preparation used "pdbqt" using *Autodock* application. Then running grid was performed and Autodockrun between ligands and receptors. To determine the bond strength interaction between ligand and receptor performed using the Autodockprogram and the bond structure can be displayed by using the application of Accelerys Discovery.

The Treatment of Experimental Animals

The rats (*Rattus norvegicus*), Wistar strain, male, age of 2 months, weigh of 200 grams. Itwere divided into 4 groups: first group (healthy rats, negative control), second group (positive control rats, IBD rats) induced by indomethacin with a dose of 15 mg/kg BW, once time orally. The third and fourth groups were therapy groups with dose of 100 mg/kg BW and dose of 400 mg/kg BW, for 14 days, respectively. The administration both indomethacin and extract were orally.

The measurement of Malondialdehyde Levels

The jejenum sample was collected: one gram of Jejenum was cut into small pieces dan crushed on cold mortar which placed on ice cube. Then 1 mL physiological NaCl wasadded. Furthermore, homogenates were centrifuged at 8000 rpm, 4^{o}C, for 20 min, and supernatant were collected.

Supernatant was used for analysis of MDA levels: 100 µL supernatant was added by 550 µL distilled water, 100 µL of TCA, 250 µL of 1NHCl and 100 µL of Na-Thio then mixed and homogenized using vortex, then it was centrifuged at 500 rpm, 4^{0}C, for 15 min. Then the supernatant were collected into new microtube and incubated at a 100 °C temperature for 10 min. Samples were measured at λmax 532 nm. After the data obtained, the absorbance data were plotted on a standard curve equation.

Results and Discussions

The mechanism of flavonoid theraphy for IBD is through inhibitation of the COX-2 which lead no conversions of of *arachidonic* acid to prostaglandins, reduce intestinal bleeding, and protect the intestinal mucosa, but does not interfere the function of Prostaglandin (PG)[7]. Apigenin is a class of flavon compounds that can be used as an anti-inflammatory in by inhibiting the activity of COX-2 [6].

The results of the analysis of molecular interactions using the docking method between ligand (apigenin) and COX-2 (pdb code: 3LN1). The interaction of both ligand (apigenin) and COX-2 resulting the bond interaction. The visualization of Apigenin inhibition confirmed by the value of Ki (inhibition constant) as 16.88 µM and the value of bond energy of -6.51 kcal/mol. The results of bond energy and inhibition constants indicated that Apigenin compound was potential as a natural COX-2 inhibitor. Validation results through molecular re-docking Colecoxib natural ligand to COX-2 (code pdb3LN1) produced docking energy of -11.52

kcal/mol, inhibition constants of 3:57μM, and Root Mean Square Deviation (RMSD) of 0.267 Å. The RMSD value was < 2, which means that the position of the ligand copy similar to the native ligand. So, this molecular docking method is valid to be used to predict the COX-2 inhibitor.

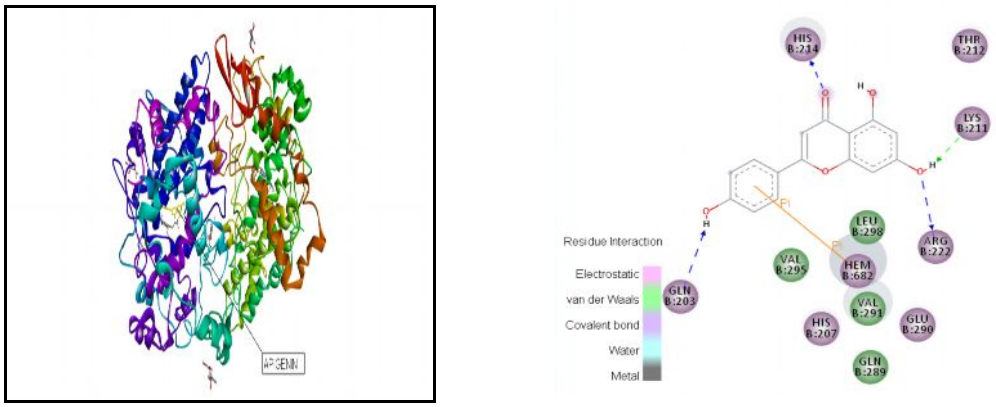

Figure 1.Molecular Model Interactions of COX-2 with Apigenin Compound

Results of *in silico* studies on inhabitation of COX-2 by Apigenin shown by the presence of Van der waals bonding between amino acids of COX-2 eiValine (Val B: 295); Leusine (Leu B: 298); Glutamine (Gln B: 289) with Apigenine. There was also the covalent bonding of the amino acids of COX-2 ie :Histidine (His B: 214); Threonine (Thr B: 212); Lysine (Lys B: 211); Arginine (Arg B: 222); Glutamine (Glu B: 290); and Hemo Protein (Hem B: 682); Histidine (His B: 207); Glutamin (Gln B: 203) with Apigenina flavonoid compound (Table 1). This result demonstrated the interaction between Apigenin (as a ligand) with COX-2.It was evident that Apigenin can be developed as an anti-inflammatory candidate specific COX-2 inhibitor. Apigenin as a specific inhibitor COX-2 without adverse effect on interstinal inflammation in the presence of prostaglandin produced by intestine when compared by Colecoxib. The Colecoxib have too great interaction in inhibiting COX-2. Apigenin and COX-2 interaction showed in Fig.1.

Table 1. Molecular *Docking*Apigenin, Vicenin-2 and Celecoxib with COX-2 (pdb code: 3LN1).

Compound	Free Energy (KCal/mol)	Ki (μM)	The position of the interaction of the ligand (Apigenin) and COX-2	
			Covalent bond	van der Waals
Apigenin	-6,51	16,88	HIS B:214; THR B:212; LYS B:211; ARG B:222; GLU B:290; HEM B:682; HIS B:207; GLN B:203	LEU B:298; VAL B:295; VAL B:291; GLN B:289
Celecoxib	-11,52	3,57	ARG A:106; TYR A:341; ARG A:499; SER A:339; HIS A:75; LEU A:338; GLN A:178; ILE A:503; PHE A:504	VAL A:102; LEU A:245; LEU A:517; VAL A:335; ALA A:513; ALA A:502; GLY A:512; TRP A:373; VAL A:509; SER A:516; LEU A:370; TYR A:371; PHE A:367

Apigenin can be used as an anti-inflammatory as a COX-2 Inhibitor. Apigenin acts as an anti-free radical by stabilizing free radicals and inhibiting the activity of cyclooxygenase enzymes that mediate the onset of pain [8]. Flavonoids are polyphenols which play a role as an anti-inflammatory and antioxidant [9]. Antioxidants are compounds that have a molecular structure that can provide electrons to the free radical molecules and break the chain reaction of free radicals. The methanolic extract of *Atactodea striata* contain flavonoids (Apigenin). The Apigenin as a free radical scavenger can reduce levels of free radicals (ROS) due to the effects of indomethacin based on the MDA level (Table 2).

Table 2. MDA Profile of Jejunum Rats Exposed by Indomethacin after Methanolic Extract of *Atactodea striata* Theraphy

Groups	Average levels of MDA (µg/ml)	Increase levels of MDA (%)	Decrease levels of MDA (%)	
Negative Control	$0,614 \pm 0,107^{a}$	-	-	
IBD	$3,677 \pm 0,422^{d}$	498,86	-	
Therapy with 100 mg/kgBW	$2,885 \pm 0,406^{c}$	-	21,54	
Therapy with 400 mg/kgBW	$1,169 \pm 0,109^{b}$	-	68,21	

The methanolic extract of *Atactodea striata* significantly ($P<0.05$) lowered MDA levels. The best therapuetic dose of *Atactodea striata* extract was dose of 400 mg/BW, although still significantly ($P>0.05$) to the negative control group. In the normal metabolism, the body produces high energy particles in a small amount known as free radicals. Free radicals in the body's defense system, through the action of the dual function monooxygenes, by oxidatives enzymes such as xanthine oxidase, and a mediator autooxidation with heavy metals. Result of MDA levels analysis in this study indicated that the evels of MDA in IBD rats was significantly different ($p<0.05$) compared with negative control group.

In the IBD rats, there was an increasing of MDA levels caused by indometacin (a claas of NSAID drugs) which can cause lipid peroxidation resulting MDA as a final product.High levels of MDA in IBD rats caused by lipid peroxidation process, which directly indicate by the production of free radicals. High expression of free radicals on Jejenum IBD rats, due to the response of indometacin exposure through oxidative stress. This study also showed that the apigenin contained in methanol extract of *Atactodea striata* acts as an antioxidant, which can neutralize free radicals in the body. The higher content of free radicals in tissue is directly proportional to the high oxidative stress that can lead to increased lipid peroxidation as MDA that uses as marker of damage cells due to free radical. Whereas,in the group treated with dose of 100 mg/kgBWand 400 mg/kgBW decreased MDA level tobe 2.885 µg/mL and 1.169µg/mL, respectively.The principe of MDA measurement is the reaction of one molecule MDA with two molecules of Thiobarbituric acid(TBA) to form a MDA-TBA complex, then meausred spectrophotometrically on 532 nm[10].The result means that the apigenin contained in the methanolic extract of *Atactodea striata* act as an antioxidant that stabilizes free radical. The MDA levels measured in this study proved to be able of inhibiting the COX-2 activity that has been describe above in computing studies.

Conclusion

Inconlusion, Apigenin compounds contained in *Atactodea striata* methanolic extract proved as COX-2 inhibitor that resulting binding energy of -6.51 kcal/mol and inhibition constant of 16.88 µM.Therapy of *Atactodea striata* methanolic extract reduced levels of Jejenum malondialdehida (MDA) on IBD rats.

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