

## The potential role of caffeic acid in coffee as cyclooxygenase-2 (COX-2) inhibitor: *in silico* study

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### ABSTRACT

Caffeic acid was formed from hydrolyzation chlorogenic acid caused roasting coffee. Caffeic acid has anti-inflammatory properties by *in vitro* and *in vivo* analysis. Inflammation is the body will be activator COX-2 as mediator inflammation. This study purpose to prediction, investigate and analyze caffeic acid as potential theuraphic to inhibit COX-2 by *in silico* study. The method of this research using *in silico* compound interaction models. COX-2 Protein data was taken from Protein Data Bank, caffeic acid from PubChem. Protein-ligand interaction docking using HEX 8.0.0. Although visualization and analysis of the molecular interactions of caffeic acid and COX-2 conducted by the Discovery Studio software 4.1. Caffeic acid is a potential therapist because easily absorbed and has high permeability. The results show that interacted between COX-2 and caffeic acid. The interactions showed by seven amino acid residues, which bind with the caffeic acid with hydrogen bond type. Energy binding formed from ligand and protein -210.23cal/mol. Interaction caffeic acid and COX-2 has a positive impact which potential as inhibitor COX-2.

**Keywords:** *Anti-inflammatory; Caffeic Acid; Coffee; COX-2; In silico; Inflammation.*

### 1. INTRODUCTION

Coffee is one of cultivations in Indonesia. Caffeic acid is a major phenolic phytochemical in coffee. Coffee become one of the highly agricultural product has been consumed, because contains many chemicals compound such as chlorogenic acids, caffeic acid, kahweol, cafestol and so on compounds. One hand, Caffeic acid has the ability to suppress DNA methylation in cancer cells and inactivation of several ways participated in tumorigenic process such as apoptosis, stress and inflammatory response [1,2]. In another hand, Da Cunha et al., [3] reported caffeic acid has anti-inflammatory properties by *in vitro* and *in vivo* analysis with the anti-inflammatory actions of caffeic acid butyl ester.

Inflammation is the one effected of immune system response by infection and injury and occurs when abnormalities condition in human body [4,5]. When inflammation humans body will response by inducing inflammatory cytokines to be pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) [6-9]. Another enzyme which is responsible during inflammations is Cyclooxygenase-2 (COX-2) The role of COX-2 is to activate prostacyclin (PGI<sub>2</sub>) production in humans body [10-11]. In the

same time, the immune system will be produced anti-inflammatory cytokine to suppress inflammatory. Bare et al., [12] reported IL-10 as anti-inflammatory agent became mutation in case of inflammation. These conditions lead to reduce function IL-10 as an agent of anti-inflammatory. Other research showing that some of the protein bands have been losing in case of inflammatory disease [13]. Therefore, treatment in inflammation case uses drugs.

Compound of nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used medicines. NSAIDs are the drugs which use to suppress the COX-2 associated PGE<sub>2</sub> production [14].

The use of synthetic drugs as agent anti-inflammatory often cause several side effects so patients are suggested to take combinational oral medicine to maximize the healing chance and also to minimized the toxicity of the drugs [15-16].

The aims of this paper to investigate and analysis of potential chemical compound caffeic acid as an anti-inflammatory agent to inhibit COX-2 by *in silico* approach.

### 2. MATERIALS AND METHODS

#### 2.1. Ligand Preparation.

Structure 3D of caffeic acid (CID 689043) was taken from the database of PubChem.com. Ligand structure optimized energy by PyRx Virtual screening tool Open Babel tool. Ligand, which formed SDF file format converted to pdb format by in PyRx Virtual screening software.

#### 2.2. Protein Preparation.

Protein data bank (PDB) from <http://www.rcsb.org>. used to take the structure of COX-2 (ID= 6cox) [17]. COX-2 receptor downloaded in .pdb file. Removing water molecules, which incorporated with the receptor, used Discovery Studio Client 4.1.

#### 2.3. Molecular Docking.

Ligand and receptor were docked. Molecular docking ligand and protein established using the Hex 8.0.0 software. Visualization and analysis data from the molecular interactions of caffeic acid and COX-2 in the Discovery Studio software V 4.1. In this section, we

analyzed the free energy of binding ( $\Delta G$ ), amino acid residues and types of hydrogen bond. The higher ligand affinity towards the active site of the used receptor has more negative  $\Delta G$  [18].

### 3. RESULTS

Caffeic acid (3,4-Dihydroconnamic acid) is one of the coffee chemicals compounds that have potent antioxidants and impart several health benefits like protecting the body from the hazardous effects of free radicals. The presence of caffeic acid slows inflammation process in human body [19].

Caffeic acid has phytochemical properties which is easily absorbed and very high permeability (Table 1) because of the molecular weight of caffeic acid  $180.159 < 500$ , the log coefficient of octanol/air (log P)  $1.1956 < 5$ ; H-bond donor (HBD)  $3 < 5$ ; and H-receptor bond (HBA)  $3 < 10$  based on Lipinski et al., [20] can be continued in silico analysis.

**Table 1.** The physicochemical properties of caffeic acid compounds used the pkCSM online tool. MW = Molecular Weight; LogP = logarithm of octanol/water partition coefficient; Torsion= a bond between atoms that can rotate; HBA = Hydrogen Bond Acceptors; HBD = Hydrogen Bond Donors; PSA = Polar Surface Activity.

Chemical Compound	MW	LogP	Torsion	HBA	HBD	PSA (A2)	Lipinski Legal Requirements
Caffeic Acid	180.159	1.1956	2	3	3	74.381	Yes

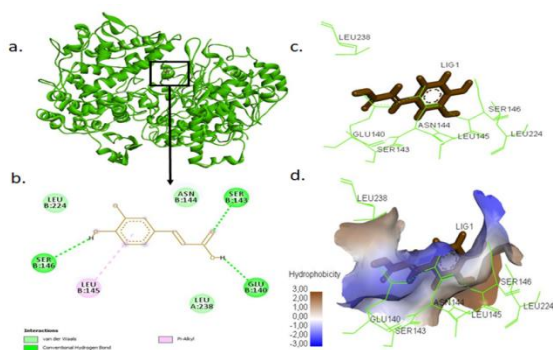
Interaction between ligand and receptor result in seven amino acid residues that bind with caffeic acid. They are LEU224, SER146, LEU145, GLU140, ASN144, SER143 (Domain A) and LEU 238 (Domain B). Interestingly, we found Pi-Alkyl in LUE145 (Figure 1). Hydrogen bonds we found in amino acid residues SER146, GLU140, and SER143, besides that Van Der Waals interaction in Amino Acid Residues LEU224, LEU238 AND ASN144. Hydrogen bonds have variety distance interactions around 2.2467-5.37782. The small distance formed by the hydrogen bond and amino acid residue from COX-2 in amino acid residue GLU140 will make the hydrogen bond stronger (table 2). Other function the bond caused interaction between caffeic acid and COX-2 protein bond is more stable. Xu D, Tsai, & Nussinov (1997) analyzed the smaller distance of hydrogen to the acceptor lead the hydrogen bond will be stronger [21]. Interaction ligand and protein had the energy binding of -210.23cal/mol (Table 2).

In our previous study, showed that effect of therapeutic nutrition against inflammation has a negative effect on COX-2 activity [22]. COX-2 blocking has a serious effect on tissue improvement. In another side, COX-2 inhibitor also has the potential function to become therapeutic amelioration from damaged tissue. COX-2 has function in biosynthesis of prostaglandins. The gene activated when the inflammation process occurs in human body. COX-2 will increase inflammation [23].

Interaction between cafeeic acid and COX-2 mediated by blocking JNK, p38 and ERK phosphorylation. These interaction indicated that ligan (caffeic acid) has a potential role effective to suppress the UVB-activated signaling pathway. Caffeic acid one of the polyphenols that have small molecules with structures same with other pharmacological kinase inhibitors, and these can bind with specific kinases to reduce their activities [24].

Compare to other research, caffeic acid affects inhibited COX-2 conducted some metabolic effect. COX-2 pathway metabolites also directly linked to the development of tissue damage such as neuron tissue. Freshwater et al., [25] reported increased COX-2 expression and activity, as measured by PGE2 content, in the spinal cords of diabetic rats compared to normal rats. Increasing COX-2 expression was associated with enhanced pain behavior and hyperglycemia. In this research model, selective COX-2 inhibitor and resulted that COX-2 inhibitor can reduce markers of hyperglycemia within affected in a neuropathy.

When inhibit COX-2 will stop conversion of prostaglandin G2 to prostaglandin H2 and decreased in superoxide production and, subsequent lipid peroxidation, therefore reduce the inflammation in human's body.



**Figure 1.** Molecular interaction between Caffeic Acid and COX-2. a. Ligand and Protein interactions, b. 2D structure interaction, c. 3D structure interaction, d. Hydrophobicity complex.

**Table 2.** Interaction Caffeic Acid and Cyclooxygenase-2 (COX-2).

Complexes	Energy (cal/mol)	Name	Distance	Category	Types	from chemistry	to chemistry
Caffeic Acid-COX-2	-210.23	B:SER143:HG - :LIG1:O	2.60103	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
		:LIG1:H - B:SER146:OG	2.69016	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
		:LIG1:H - B:GLU140:OE1	2.2467	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
		:LIG1 - B:LEU145	5.37782	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl

#### 4. CONCLUSIONS

Caffeic acid has easily absorbed and has high permeability interaction with protein based on Lipinski Rules. The interactions between COX-2 and caffeic acid by hydrogen bond type. Seven amino acid residues which interacted with the caffeic acid. This interaction with energy binding -210.23cal/mol. Based on the

result, Caffeic acid has therapy potential to inhibit mediator inflammation COX-2 and will be blocked peroxidase dependent that will the conversion of prostaglandin G2 to prostaglandin H2 and caused decreased in superoxide production and, subsequent lipid peroxidation.

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