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In Silico Insight the Prediction of Chlorogenic Acid in Coffee through Cyclooxygenase-2 (COX2) Interaction

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ABSTRACT

Inflammation was signs of pathological or abnormality in tissue to give an alert as a trouble signal to the system. Therapeutic using NSAIDs has some side effects. This research explored the potential role of chlorogenic acid as natural therapeutic compound to inhibit the inflammation target such as COX-2 by interaction model. The research method used in the study by molecular docking approach, which binds ligand and protein. Protein data provided by Protein Data Bank (ID: 6cox) while, chlorogenic acid obtain from PubChem (CID: 1794427). We docked COX-2 and chlorogenic acid using Hex 8.0.0. Visualization and analysis of the molecular interactions of chlorogenic acid and COX-2 conducted by the Discovery Studio Client 4.1 software. Chlorogenic acid has high permeability and is easily absorbed based on five Lipinski Rule. Interestingly, we found Fifteen amino acid was binding with chlorogenic acid that formed by hydrogen bond and van der Waals. The interaction between ligand-protein results in energy binding-327.59cal/mol. Chlorogenic acid has a potential role to inhibit inflammation pathway by inhibiting COX-2. We predicted chlorogenic acid has a potential as therapy anti-inflammatory to suppress COX-2 as mediator inflammation.

Keywords: amino acid; anti-inflammatory; chlorogenic acid; inflammation; in silico, COX-2

INTRODUCTION

Inflammation is a physiological response to abnormal conditions in the body. Inflammation can occur locally, systemically, acutely and chronically, causing severe pathological abnormalities (Abdulkhaleq *et al*., 2018; Phalitakul *et al*., 2011). When inflammation occurs, the body will respond by forming anti-inflammatory cytokines to produce symptoms of inflammation. Bare *et al*., (2018) in the previous study reported the protein profile of all tissues in inflammation (type 2 diabetes mellitus) group and the normal group was completely diverse as proper by Experion Pro260 analysis besides in SDS-PAGE analysis show same results.

Inflammation, which happened in human's body correlated with the mediator inflammation such as Cyclooxygenase-2 (COX-2). COX-2 has a function of initiation and maintenance during inflammation abnormal physiological conditions. These Cyclooxygenase-2 regulations by stimulating the production of prostacyclin (PGI2), also preventing platelet aggregation (Al-Saeed, 2011; Knights *et al*., 2010). In another study to developed COX-2

inhibitor as known as NSAID is aspirin, acetylsalicylic acid. Aspirin binds to the COX active site and has a higher affinity to Ser-530 (Smith & Murphy, 2016).

To therapeutic inflammation in human's body, most people use drugs such as use nonsteroidal anti-inflammatory drugs (NSAIDs). The drug treatment not only in fever and mild pain but also to act by reducing chronic inflammatory (Bäck *et al*., 2012). The function of NSAIDs inhibition of COX-2 (possibly by blocking PGI2 biosynthesis while not hindering TXA2 formation (Phalitakul *et al*., 2011).

Overconsumption of NSAIDs drugs has some sides effect such as gastrointestinal, cardiovascular risks (Al-Saeed, 2011), adrenal atrophy (Phalitakul *et al*., 2011), and induce the risk of atrial fibrillation (Bäck *et al*., 2012). Due to the side effects of the NSAIDs, researchers concern to find natural compounds for effective and reduce the effects.

Coffee one of the cultivation in Indonesia. Coffee has natural compounds, caffeic acid has functions inhibitor COX-2 (Bare *et al*., 2019a) and chlorogenic acid. Chlorogenic acid is one of the chemicals compounds in coffee beans (Moon *et* al., 2009; Watanabe *et al*., 2006). Shi *et al*., (2013) reported chlorogenic acid has potentially associated with various inflammatory response inhibition by in vitro study in case of reduces liver inflammation and fibrosis. Bare *et al*., (2019b) reported chlorogenic acid has a function to inhibit ACE. Using the chlorogenic acid expected to reduce toxicity and side effects to human body. In this paper, we analyze and investigate the potential of chlorogenic acid as anti-inflammatory agents by inhibiting COX-2 roles used in silico approach.

MATERIALS AND METHODS

Ligand and Protein Preparation. Protein Data Bank (PDB) from www.rscb.org was used to acquire a database to get 3D COX-2 with ID: 6cox (Kurumbail *et al*., 1996), while the chemical structure of chlorogenic acid (CID: 1794427) was acquired from the database of PubChem.com. Minimalizing energy chlorogenic acid by open babel in PyRx Virtual screening tool. Removing ligand from water molecules used Discovery Studio Client 4.1. Converting ligand from SDF format file to PDB file using PyRx Virtual screening tool software. Preparation COX-2 by removing ligand that binding with protein using Discovery Studio Client 4.1.

Molecular Docking. Ligand chlorogenic acid was docked with protein COX-2 by in silico. Molecular docking was established using Hex 8.0.0 version software. Then ligand and protein that docked in HEX, we visualize and analyze the molecular interactions of chlorogenic acid and COX-2 by Discovery Studio client 4.1 software. The analyzation including amino acid resides, hydrogen bonds, van der Waals and energy binding which formed by the interactions.

RESULT AND DISCUSSION

The chlorogenic acid and COX-2 interactions were shown the binding of amino acid residues with chlorogenic acid. The types of chemistry bond, which formed between ligand, and amino acid residues are hydrogen bonds. Interactions between ligand and protein showed fifteen amino acid residues which correlating with the chlorogenic acid in B domain. The amino acid residues are HIS207, PHE210, LYS211, THR212, ASP213, HIS214, LYS215, ARG216, ARG222, ILE274, GLN289, GLU290, VAL291, ASN382, HIS386 The bond that occurs in amino acid residues LYS215, GLU290, ASN382 is hydrogen bond type conventional hydrogen bond while HIS207 is hydrophobic. Binding between ligand-protein had the energy binding -327.59cal/mol.

Farah *et al*. (2008) reported chlorogenic acid which consumed by the human has highly bioavailable in humans. Chlorogenic acid has molecular weight 354.311 <500, the value of the log coefficient of octanol/water (log P) -0.6459 > 5, H-receptor (HBA) under 8 and the donor H-bond (HBD) 6>5. Chlorogenic acid has been clear in humans' metabolism and kinetics, furthermore it has high permeability and is easily absorbed by the body based on Lipinski *et al*., (1997). In this research, we found hydrogen bond, binding chlorogenic

acid, and LYS215, GLU290, and ASN382. This interaction of those causes the disorientation of the substrate. In amino residue, HIS207 formed hydrophobic interactions, which affect the substrate and nucleophile orientation. The distance in hydrogen bond that formed in amino acid residue LYS215 smaller than amino acid residues GLU290 and ASN382 has an effect on the strength of the bond. The smaller distance of hydrogen to the acceptor leads, the bond will be stronger (Santoso *et al*., 2016).

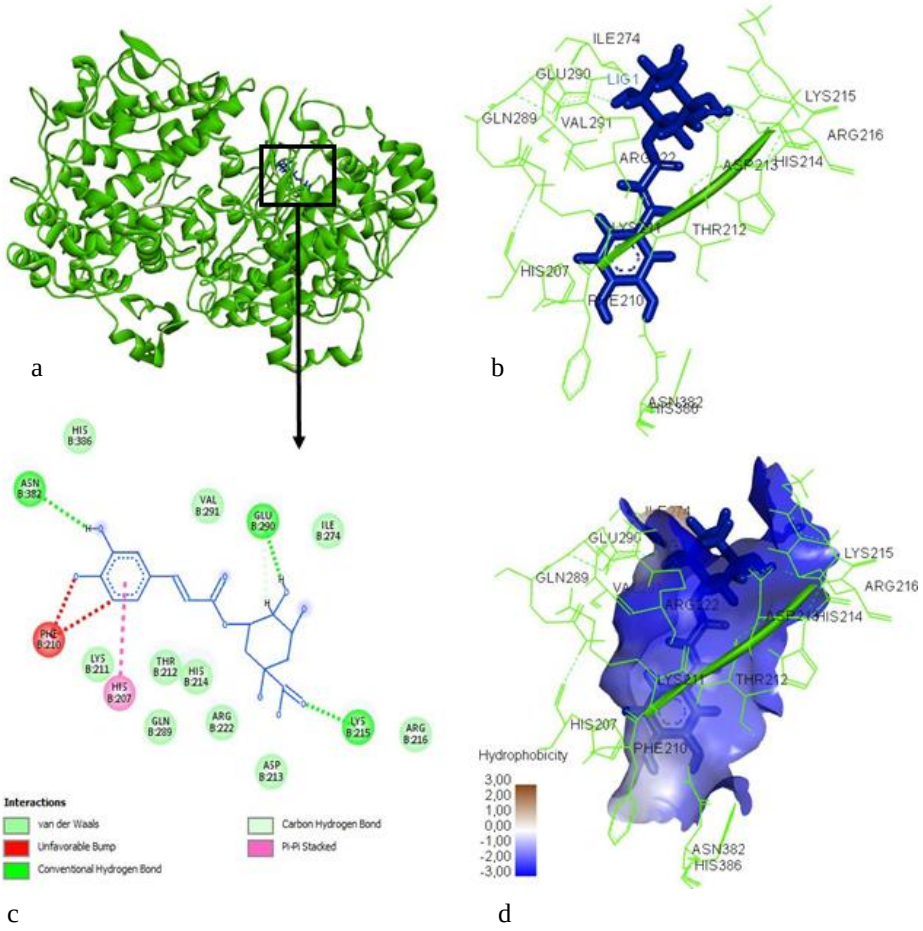

Figure 1. Molecular interaction between chlorogenic acid and COX-2: a. Ligand and protein interactions; b. 3D structure interaction. Interactions Van der Waals, unfavorable bump, conventional hydrogen bond, carbon-hydrogen bond, and Pi-Pi stacked; c. 2D structure interaction; d. Hydrophobicity complex

The chlorogenic acid has an affinity with the activator side of COX-2 in amino acid residues, which bind with a hydrogen bond. The inflammation pathway was role by COX-2 such as PI3K, NF-κβ, and Akt was blocked when chlorogenic acid deactivated COX-2 by interaction on (active side domain of COX-2) (Rachmad *et al*., 2018).

Table 1. Interaction chlorogenic acid and COX-2 protein

Complexes	Energy (cal/mol)	Name	Distance	Category	Types	from chemistry	to chemistry
Chlorogenic acid-COX-2	-327.59	B:LYS215:HN - :LIG1:O	1.78522	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
		:LIG1:H - B:GLU290:OE1	2.48003	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor

	:LIG1:H - B:ASN382:OD1	2.573	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
	:LIG1:H - B:GLU290:OE1	1.99185	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
	B:HIS207 - :LIG1	4.53111	Hydrophobic	Pi-Pi Stacked	Pi-Orbitals	Pi-Orbitals
	B:PHE210:O - :LIG1:C	2.25178	Unfavorable	Unfavorable Bump	Steric	Steric
	B:PHE210:CB - :LIG1:O	2.25097	Unfavorable	Unfavorable Bump	Steric	Steric

COX-2 is a central enzyme in the biosynthesis of prostaglandins. These activated by inflammatory inducements, such as cytokines and lipopolysaccharide. In the cell metabolic pathway, COX-2 was induced by prostaglandin production implicated to increase inflammation, matrix tissue remodeling, fibrosis progress and expansion of tumor genesis (Shi *et al*., 2013).

Recently study shown that Ser-530 was homolog in COX-1 and COX-2 indicated that NSAID was non-selected inhibitors for COX-2 (Smith & Murphy, 2016). Interestingly, our study found another active site that binds chlorogenic acid. Chlorogenic acid has activity to induce inactivation of COX-2 by the interactions in amino acid residues LYS215, GLU290, ASN382 and HIS207.

Compare to affinity character between chlorogenic acid and COX-2 in this research indicated that interaction was blocking at COX-2 in different active site location compare to other ligand complexes COX-2 NSAID (Perez *et al*., 2019). Different binding sides in COX-2 have potential role for inhibiting COX-2 function in different effects compare to chemical drugs.

Interaction leads effectively to reduce chronic inflammation that indicated to pathophysiological damage. This mechanism has been extensively shown in preclinical and epidemiological studies that support the targeting of the COX-2 pathway for the prevention and treatment of malignancy. In another study, the blocked activity of COX-2 and upregulating of PGE2 synthase have prevalent roles to inhibit and reducing cancer factors progression. Inactivation COX-2 can down-regulated these effects through several signaling pathways such as stimulation of vascular endothelial growth factor (VEGF) leading to increased cell proliferation, metastatic endothelial and angiogenesis (Xu *et al*., 2014). Another effect can lead to upregulating the protooncogenes, BCL-2, and the epidermal growth factor receptor (EGFR), were mediated by the initiation of the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K)/AKT pathway, respectively (Buchanan *et al*., 2003). In some cases, increased transcriptional activity of the anti-apoptotic mediator nuclear factor-κB (NF-κB) (Poligone & Baldwin, 2001).

Inactivated COX-2 protein expression in target tissue, Pop-Busui *et al*. (2008) showed that inactivation gen of COX-2 in diabetic mice with COX-2 specific inhibition in diabetic rats prevented nerve conduction deficits. In this case, diabetes encouraged production-specific biomarkers by oxidative stress and inflammation. Systematical effect with potential to progression of PG and correlated with imbalance in the peripheral nerves. In another result also shown that COX-2 gene inactivation protects against diabetes-induced damage in some targeted tissue (Rachmad *et al*., 2018).

CONCLUSION

Based on interaction model, Chlorogenic acid has potential role as therapeutic agent anti-inflammation by inhibiting cyclooxygenase-2. The interactions between ligand and protein formed fifteen amino acid residues, which interacted with chlorogenic acid. Besides that, we found type of interactions such as hydrogen bonds, van der Waals, hydrophobic with the energy binding-327.59cal/mol. Inhibiting

COX-2 might correlated with oxidative and derivate of prostaglandins compound but need further study to prove the efficacy of chlorogenic acid.

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