In Potential Siliko Simulation of Clorogenic Acid in *Coffea canephora* to Transfering Macrophage Polarization of M1 in Tuberculosis Infection

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Abstract

The aim of this study to determine the potential of *Coffea canephora* by using chlorogenic acid (CGA) compound associated with macrophages polarization in tuberculosis condition. Exploration of compound by using the Knapsack database and Dr. Duke. Analysis of CGA by using a PASS server with the results ranged score 0-1 probability to be active score. Molecular binding docking receptor and ligand were used Autodock PyrXv9.5 and interactions between molecules using CLUSPRO 2.2. Potential activities of CGA compounds was 3,5-*dicaffeoylquinic acid*, *feruloylquinic acid*, and *p-coumaroylquinic acid* with Pa value close to 1, this indicates that the accuracy of prediction of antioxidant activity was quite high. The results of docking through the attachment pathway of several amino acids showed the potential for side methylation activity 'which is able to reduced DNMT1 activity thereby suppressing the anti-inflammatory process. Protein interactions based on affinity values were higher among DNMT1 and STAT1 bonds compared to STAT6. The results of the study concluded that the active compound in *Coffea canephora* was involved in the function of the immune system and it related to the inflammatory process. It is triggers the delivery of antimicrobial cytokine signals and increased antioxidant, it is very necessary in healing tuberculosis disease process.

Keywords: *Coffea canephora, Chlorogenic acid, Inflamation, DNMT1, STAT1, Tuberculosis*

Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb) that attacks the lungs and causing pulmonary TB and if it affecting other parts of the body is called extra pulmonary TB [(1).]

The prevalence of TB patients in Indonesia was 297 per 100,000 people, with a mortality rate of 69,000 people per year [(2)]. In 2013 an estimated 480,000 people worldwide progressed towards MDR-TB and an estimated 210,000 died due to this disease [(3)]. The number of new TB cases in Indonesia was 420,994 cases in 2017 with male are more dominance than women[(4)].

XI. Mtb is an intracellular pathogen that lives and develops in macrophages where the stages of immune response to bacterial infections are complex. Inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IL-1 play a major role in the defense of immunity against (Mtb) [(5)]. Innate immunity plays an important role as a host defense against Mtb that begins the process of introducing Mtb by innate immune cells against pathogen associated with molecular patterns (PAMP) through their pattern recognition receptor (PRR) [(6)].

Mtb hijacks host mechanisms defense by using manipulating host cellular pathways, innate immune responses, and cell death pathways to take an advantage [(7)]. Macrophages are very important cells in TB infection because their will involve in Mtb phagocytosis process and as an initiation of immunity by adaptive T cells. Elimination of Mtb by macrophages can also through the reactive oxygen forms and nitrogen species, phagosome acidification, and phagosome lysosomal fusion [(6)].

Involvement of macrophages (M) is a complex process with two types of macrophages, it are

macrophage1 (M1) and macrophage2 (M2) was described the main activities that contradict each other and innate immunity controls toward adaptive immunity and not vice versa [8]. The M1 and M2 communication lines in the polarization direction are based on the activities of STAT1 and STAT6. The main activities of NF-κB and STAT1 are promote polarization towards M1 producing pro-inflammatory function [9].

Coffee contains large of CGA, it is a group of quinic acid esters of phenyl propenic acid, especially caffeic acid and ferulic acid. 5-O-caffeoylquinic acid (5-CQA) is the largest of chlorogenic acid of 3-O-caffeoylquinic acid (3-CQA) and 4-O-caffeoylquinic acid (4-CQA) in coffee [10]. *Coffea canephora* species are coffee species that have the highest variability in the genus coffea [11]. Empirical data showed that the average levels of chlorogenic acid were lower in Arabica coffee extracts, while average levels of *coffea canephora* was higher [12].

Based on previous studies in vitro CGA induced LPS or IFNγ but inhibited gene response to IL-4 production by promoted STAT1 signaling and inhibited STAT 6 signaling [13]. In silico test described that CGA has a potential to interact with DNA methyltrasferase enzyme and several proteins associated with transducer sinyaling and transcription activators such as STAT1 and STAT6 and IFNGR1 genes that affected transcription of IFNγ cytokine proteins.

Method

Exploration of the content of the Chlorogenic acid compound Coffea canephora

The compound content of the coffee family is explores by using KnapSack database (http://knapsack3d.sakura.ne.jp/) and from the duke database (https://phytochem.nal. Usda.gov/p hytochem/search/list), which obtained from various published literatures.

Potential of Chlorogenic acid

To find out the potential of CGA, an analysis carried out using a PASS server (http://www.pharmaexpert.ru/passonline/index.php). The score obtained from the PASS server in the form of Pa (probability to be active) has a range of 0-1. The higher the score or more than 0.7 is interpreted the more accurate the prediction of its potential activity with the in vitro / in vivo test.

Sample acquisition and molecular docking analysis

3D samples of DMNT1 protein (PDB ID 4WXX), STAT1 (PDB ID 1YVL chain A), and STAT6 (PDB ID 5D39 chain A) were obtained from RCSB GDP database (https://www.rcsb.org). While the 3D structure of CGA (ID: 1794427) was obtained from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov).

To find out the binding affinity between CGA and DNMT1 protein, molecular docking analysis was carried out between receptors and ligands by using Autodock PyrX v9.5, while molecular interactions occurred between DNMT1 and STAT1; STAT6 and NFKB1 and STAT1; STAT6 were performed docking by using CLUSPRO 2.2 software. The approach taken form of blind docking without using an active side receptor. To determine the bond strength parameter is the highest score from the docking results in form of binding affinity scores. Docking should specifically by imitating the binding site DNMT1 inhibitor 5-Aza-2'-deoxycytidine as a control. The more negative docking result, mean that the stronger the bond occurred. Ligplot software was used analyzed the determination amino acids.

Findings

Potential of Chlorogenic acid as an anti-inflammatory

Based on the analysis, CGA and several major groups of CGA include 3, 5-dicaffeoylquinic acid, feruloylquinic acids, and p-coumaroylquinic acids which have a high potential for anti-inflammatory activity, with score more than 0.6. Pa value or close to 1 indicates the accuracy of the prediction of anti-inflammatory activity.

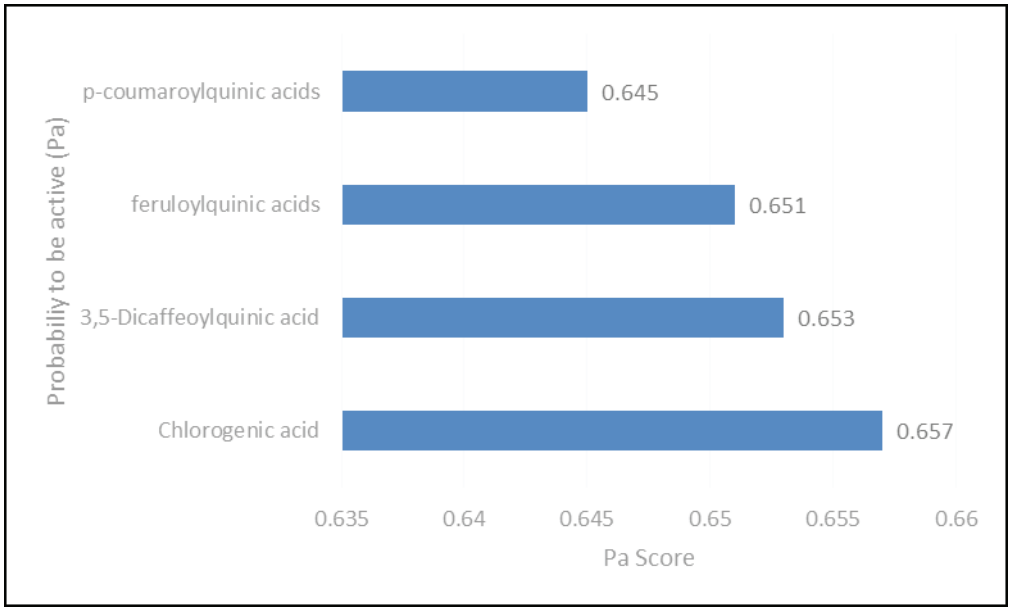


Figure 1. Potensial prediction of anti inflamatory of CGA.

Molecular docking of Chlorogenic acid and DNMT1

Based on molecular docking results, CGA had a stronger binding affinity compared to DNMT1 inhibitors, 5-Aza-2'-deoxycytidine, with -8.3 kcal / mol of score (Table 1). The adhering side of CGA and DNMT1 inhibitors were in the same place (Figure 2), namely in amino acid GLU 562, ASP 565, GLU 566, ASP 569, SER 570, PRO 574, GLY 593, GLN 594, VAL 658, GLN 687 , ARG 690 (Figure 3). This showed that CGA had same potential as DNMT1 inhibitors which is it can carry out methylation activity on the 5 'side of cytosis so it can reduce DNMT1 activity and suppress the inflammatory process in macrophages

Table 1 Binding affinity between DNMT1 and Chlorogenic acid

Receptor	**Ligan**	**Binding affinity (kcal/mol)**
DNMT1	5-Aza-2'-deoxycytidine (control)	-6.6
	Chlorogenic acid	-8.3

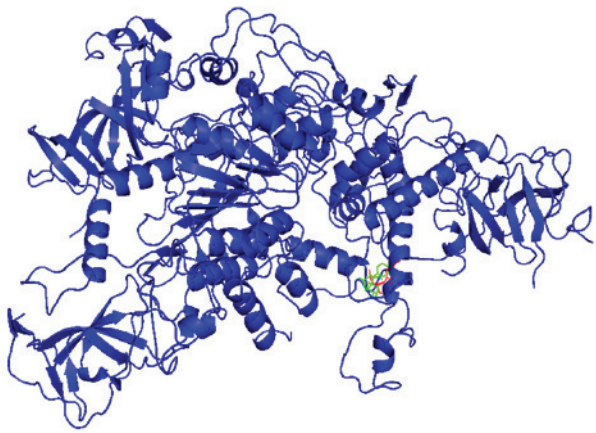


Figure 2. Docking complex between DNMT1 (ribbon; blue) and CGA (lines; green) also DNMT1 inhibitor (lines; red)

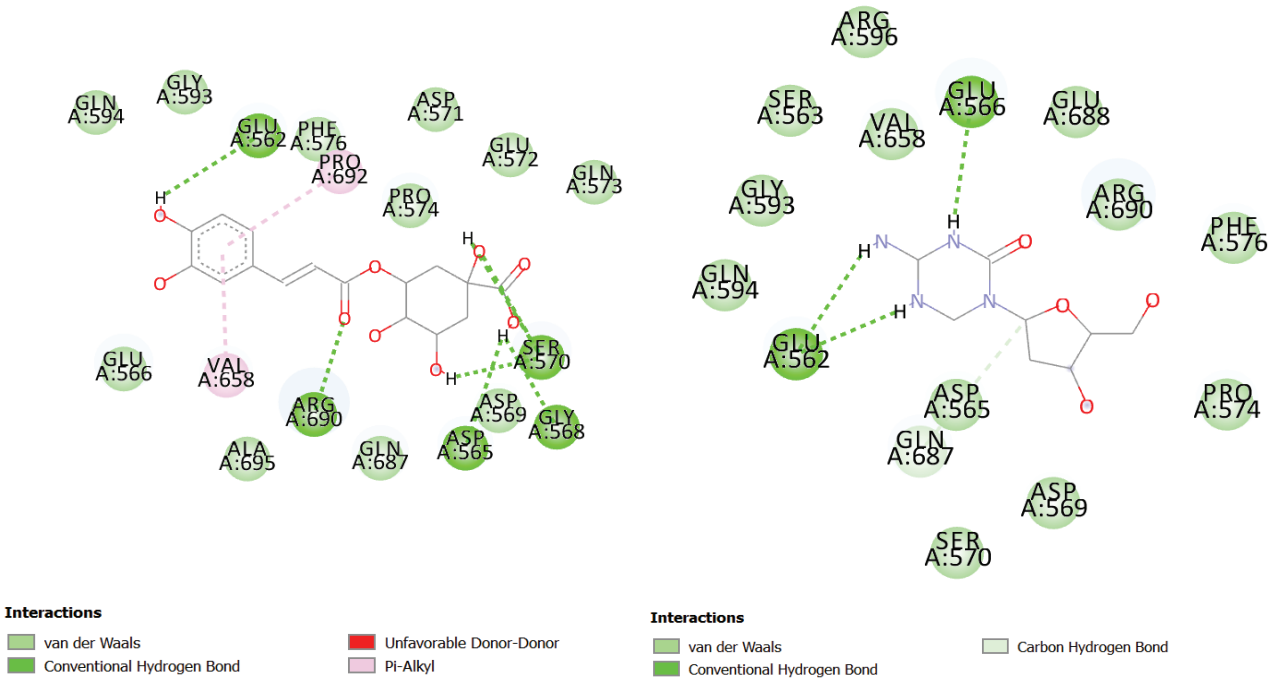
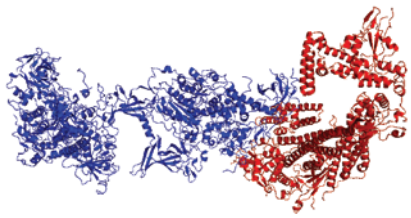
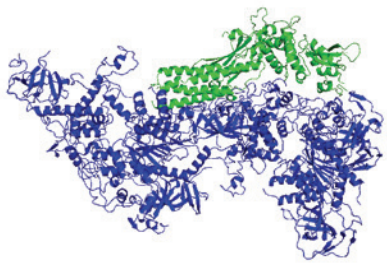


Figure 3. Amino acid Interaction between DNMT1 - 5-Aza-2'-deoxycytidine (let) and DNMT1- Chlorogenic acid (right)

The interaction of macrophage polarization proteins DNMT1 and STAT1, STAT6

The results showed that DNMT1 had a tendency to bind STAT1 compared to STAT6, this indicated by the results of a higher affinity value -1118.6. The same thing is observe in NFKB interactions that tended to bind STAT1 with an affinity value -941.1 (Table 2).

Table 2 Interaction of DNMT1 protein, STAT1 and STAT6

Receptor	Ligand	Binding Affinity (E-Total)
DNMT1 (blue; cartoon)	STAT1 (red; cartoon)	-1118.6 
	STAT6 (green; cartoon)	-1045.5 

Pathway Analysis of Chlorogenic acid, DNMT1, STAT1 and STAT6

Based on pathway analysis, the interaction between CGA and DNMT1 were not seen directly, this indicated by

a gray line. DNMT1 connected with STAT1 and STAT6 through binding with the HDAC1 protein shown by a blue line (Figure 4).

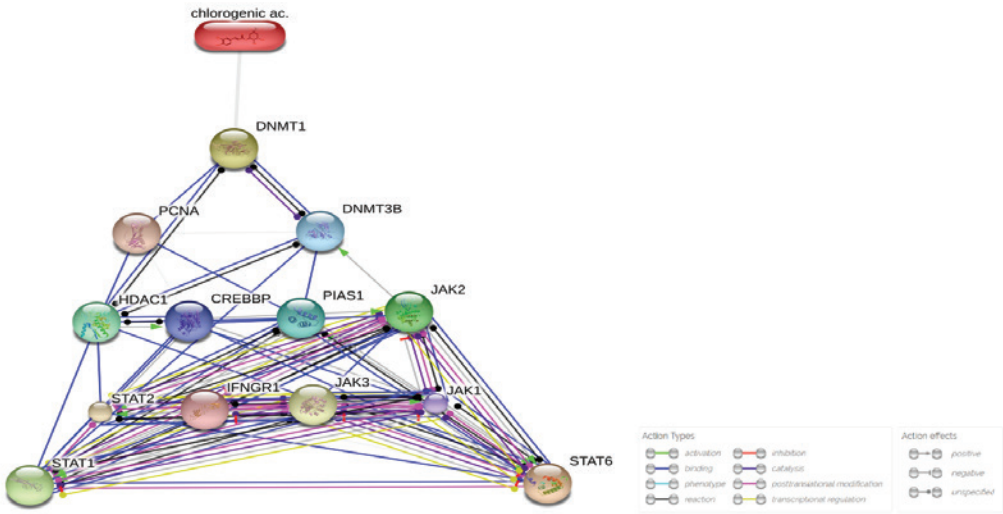


Figure 4. Pathway prediction form between Chlorogenic acid and DNMT1

Discussion

Molecular docking results based on binding affinity showed that CGA had a stronger bond than 5-Aza-2'-deoxycytidine (DNMT1 inhibitor) with a score of -8.3 kcal / mol against several of the same amino acid. CGA compounds as they are known are not a classic form of treatment, but these compounds have the potential alternative pharmaceutical benefits that can exert a physiological good effect [14]. CGA phenolic compounds have a low molecular weight which has anti-tumor effect [13].

DNMT1 is DNA methyltransferase 1 and it plays a role in maintaining the composition of the methylation formulation during DNA replication [15]. The most important epigenetic activity is the ability to modulate the defense of the host immune system against microbial pathogens and it carried out through the process of DNA methylation, histone modification and non-RNA coding activities [16]. Cellular functions such as inflammatory gene expression, DNA repair and cell proliferation were regulated changes in acetylation of histone and non-histone proteins, as seen in cancer, this is associates with changes process in histone acetylation patterns [17].

Indirect NF-κB activation by 5-Aza-2'-deoxycytidine occured in upstream phosphorylation inhibition [18]. Responsive NF-κB influence cellular processes such as apoptosis, cell survival, and often directly plays a role in the control of pathogenic infections [19]. Signaling of stressed cells results in the formation of reactive oxygen intermediates (ROI) which is contribute to the activation of NF-κB [20]. Pattern recognition molecules released by physically stress of cells or metabolically will provide innate adaptive responses to threat the pathogens [21]. NF-κB is a gene target for transcription factors and the factors involved in the progression and development of inflammation [22]. NF-κB is the main regulator of innate immune response and it plays an important role in the activity of inflammatory response process in pathogens. The aim of the innate immune system is to regulate and adjust the inflammatory response to pathogens so that a balance of pathogenic destruction results and limits hyper inflammation are potential to endanger the host [23].

Mtb infection can cleare by innate immune system before the initiation of the adaptive immune response [24]. The first step of activating an innate immune response to Mtb infection is the introduction of pathogens [25]. Mtb adapts by replicating in macrophage cells and subverting cell function. This situation is able to inhibit phagosome maturation, avoid the autophagial process, or weaken the production of proinflammatory cytokines

[26]. Mtb infection induces polarization of macrophages from monocytes. Moreover, Mtb also has the potential to modulate macrophage polarization. In the first stages is tubercle granuloma formation, polarization of macrophages is M1 [27].

Transcription factors such as NF-kB are located in the cytoplasm, before further activation undergoes a transfer to the cell nucleus and as a response to influencing the signals to activate the target gene transcription, whereas the STAT protein must phosphorilation before being bound to DNA [28]. Mtb inhibited the transcription of inflammatory NF-kB and it is causes a decreasing of lysosomes enzyme into phagosomes so that there was a decreation in the ability to kill bacteria [29]. Previous studies of macrophage polarization in tuberculosis suggested that non-classical IFN-γ responses on macrophages were come from monocytes in tuberculosis patient [30]. Signal transduction and activator transcription (STAT) play an important role in cytokine production [31]. The activation of STAT1 and STAT6 are two important signals in polarization of macrophages, and it significantly pushed and suppressed in macrophage cells by influenced the CGA [13].

Conclusion

In this research can conclude that in Silico CGA is able to influence the transfering towards polarization of M1 macrophages to modulates are more strong on innate immune system in tuberculosis infection. Experimental laboratory studies are need to prove the results.

Ethical Clearance-obtained from the ethics commission of Medicine Faculty, Brawijaya University.

Conflict of Interest-no conflict of interests regarding the publication

Source of Funding-the Directorate of Research and Community Service, Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education (Kemenristekdikti).

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