**In Silico Modelling, Regulation of Cell Viability and Anti Atherosclerotic Effect in Macrophage by Decaffeinated Coffee and Green Tea Extract**

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**ABSTRACT**

Background: The evidence of decaffeinated coffee and green tea extract (DCGTE) in amelioration of atherosclerosis through foam cell formation inhibition has not been established. This study tried to predict the potential role of coffee and tea in foam cell inhibition through in silico modelling and to investigate the effect of DGC TE on the viability and regulation of foam cell inhibition effect in macrophage cell. Methods: Prediction of physicochemical properties of secondary metabolite of coffee and tea was computed with Swiss ADME. Molecular docking was performed using PyRx Autodock Vina. Prediction of biological activities was done with PASS SERVER and analyzed the suitability with KEGG Pathway: lipid and atherosclerosis. Results: Molecular docking analysis revealed a strong affinity binding between all active compound of tea or coffee with CD36, but not with PPARγ. Conclusion: The DCGTE exhibit cell number in oxLDL-stimulated Raw264.7 compared to control (p=0.000). DGCTE (320/320 μg/ml) had a synergistic effect (Loewe score = 17.26417) and significantly reduced the foam cell number in oxLDL-stimulated Raw264.7 compared to control (p=0.000). Key words: Atherosclerosis, Coffee polyphenol, Foam cells, Molecular docking, Tea flavonoid.

**INTRODUCTION**

Atherosclerosis is the etiology of cardiovascular disease which is the leading cause of mortality worldwide ¹. To date, no specific therapies either pharmacologically or non-pharmacologically has been successfully prevent atherosclerosis. Statins and aspirin which are still become the available medication for atherosclerosis have several limitations. These medications only slightly lower the level of plaque. Moreover, the prolonged use of the drugs is widely reported for its adverse side effects like provoke toxicity to liver cells, induce rhabdomyolysis, increase the risk of cancer, and impair the cognitive function ²,³. Moreover, upper Gastrointestinal (GI) bleeding is the most common complication in patients consuming aspirin ⁴. However, only limited evidence showed that primary prevention either with statins or aspirin may be cost-effective and improve patient quality of life ⁵.

Based on these evidences, investigating the potency of natural product is urgently required for developing effective strategy to prevent the burden of the disease. Coffee is the most popular beverages in society nowadays which show safety in long term use. Yet, the presence of caffeine in coffee cause cardiovascular and non-cardiovascular side effect. Interestingly, a report from Lukitarsari et al (2020) showed that a combination of decaffeinated coffee combined with tea has a beneficial effect on improving the lipid profile, blood pressure, blood glucose, and liver PPARγ expression in the mice metabolic syndrome model ⁶. Remarkable studies demonstrated that the polyphenol found in coffee and tea has benefits in reducing cardiovascular disease risk by their action as hypolipidemic, antioxidant, and anti-inflammatory properties ⁷–¹¹.

However, the evidence of decaffeinated coffee and green tea combination in preventing early phase of atherosclerosis remains unclear. Foam cell formation is the pathological process that not only determines the initial phase but also the progression of advanced phase atherosclerosis ¹²–¹⁴. Two important molecules that dysregulate during foam cell formation are CD36 and PPARγ. Perturbation of CD36 is seen by the upregulation of this protein to continuously accelerate the oxLDL uptake. In addition, the lipid derivative from oxLDL inside the macrophage will diminish PPARγ function to induce ATP Binding Cassettes Transporter A1 (ABCA1) expression, reducing the cholesterol efflux.

Therefore, the recent study aimed to predict affinity binding of coffee and tea secondary metabolite as competitive inhibitor of oxLDL to bind with CD36 and PPARγ which never done before. This study tried to predict the anti-atherosclerosis role of bioactive constituents in coffee: caffeine, chlorogenic acid (CGA), diterpenes (Cafestol, Kahweol) and trigonelline ¹⁵, and in green tea: Epigallocatechin gallate (EGCG), Epigallocatechin (EGC), Epicatechin (EC), and Epicatechin Gallate(ECG)¹⁶. Secondly, in vitro study was performed to see the cytotoxicity of the decaffeinated coffee and green tea extract in Raw264.7 compared to individual extract. Another objective of this study was investigating...
the effect of DCGTE in foam cell formation. We propose that this
combination could provide a safe source for developing functional
foods for atherosclerosis-based disease prevention.

MATERIAL AND METHODS

Pharmacokinetic properties and prediction function of
coffee and tea bioactive molecules

Various pharmacokinetic properties and Lipinski rule of 5 which
covers the drug-likeness properties of the active biomolecules were
predicted using Swiss ADME online software. Early Atherosclerosis
is determined by foam cell formation. the PASS Server http://www.
pharmexpert.ru/passonline/index.php investigated the biological
activities related to each active compound. The score listed in PASS
SERVER followed the criteria: If Pa > 0.7, the substance was very likely
to show activity in the experiment, but the probability of the substance
being an analogue of a known pharmaceutical agent is also high. If 0.5
< Pa < 0.7, the substance was likely to show activity in the experiment,
but the probability is less, and the substance was unlike any known
pharmaceutical agent. If Pa < 0.5, the substance is unlikely to show
activity in the experiment. Therefore, this study used cut off Pa > 0.5, 
the PASS SERVER data than matched with KEGG Pathway hsa 05417: 
lipid and atherosclerosis

Ligand preparation

The structure of 4 main active ingredients in coffee and tea were retrieved
from the Pubchem crystal database server in SDF format (https://
pubchem.ncbi.nlm.nih.gov). Then all the .sdf files were converted to
.pdb files using Pymol software. Four bioactive compounds of green
coffee named CGA, cafestol, kahweol, trigonelline (Compound ID :
1794427, 108052, 114778, 5570 ) were used in this study. EGCG,
ECC, EC, ECG (Compound CID: 65064, 72277, 72276, 10795) as
the main compound of green tea were also used in this study. The control
for CD36 receptor was AP525820. The ligand control for PPARγ was
Pioglitazone which was PPARγ agonist and used broadly as anti-
diabetic drug.

Receptor Preparation and Molecular docking

oxLDL are key factor that promote the foam cell atherosclerosis through
its binding with CD36 that is expressed in activated macrophage. Additionally, ox-LDL binding with PPARγ in macrophage could exert
the increase expression of CD36 thus accumulate the lipid inside
the macrophage and propagate the foam cell number. Hence, an
investigation to find alternative ligand for CD36 and PPARγ should
be performed.

The X-ray crystal structures of Human Platelet Glycoprotein 4 (CD36)
(PDB SLGD Chain A) and PPAR-γ (PDB 5Y2O) were retrieved from
the PDB database (http://www.rcsb.org). All proteins were modelled
with the Pymol program by removing all the water molecules and
sulphate ions present in the structure. Then the structure was optimized
by assigning the bond orders, bond angles, and topology. The formal
atomic charges were fixed for the amino acid residues. The optimized
structure was then energy minimized to remove the steric clashes
between the atoms.

The binding affinities of the ligand and receptors were determined by
docking CD36 and PPARγ with the bioactive molecule of coffee and
tea using PyRx AutoDock Vina. The active binding site information
was obtained through the webserver active site prediction http://www.
scbio-itd.res.in/dock/ActiveSite.jsp. This results in a higher level of
confidence in predicting one or more protein binding regions, as well
as a more accurate ranking of the likely binding sites. The position
of the active site region was used to determine the center of the grid
box for CD36 and PPARγ. The center of the grid box for CD36 and
PPAR γ was chosen based on the location of the active site region.. The
specific docking for CD36 and PPAR-γ with grid box were at Center
(X: -44.484540943, Y: -34.1146354603, Z: 22.1589056284), Domain (x
= 17.5598077147, y: 15.6841424984, z: 16.4724530197) and Center
(X: -48.584, Y: -1.611; Z: 77.668), Dimension (X: 10.278; Y:13.425;
Z:17.929), respectively. These suitable grid boxes volume was the site
where the ligands can easily be fitted and which covers the entire
active site pocket. Amongst them, the best-suited conformations with
the lowest root mean square deviation (RMSD) values were selected
to calculate the binding energetics. The obtained docked products
were visualized and analyzed the hydrogen bond interactions and
preparation of high-resolution images by Biovia Discovery Studio
software.

Extraction of coffee and tea

Robusta green coffee beans from the Dampit district of Malang
Regency, Indonesia, were used in this study as the raw material. The
sample utilized was powder green coffee beans that had been roasted
for 3 minutes at 180° C. For the tea extract processing, 3 green tea
leaves were taken from the tallest section of trees in the Ciwidey
district of Bandung.

The coffee and green tea were extracted using infusion technique. The
Fischer and Liang et al. in 2007 method was used to decaffeinate
the coffee and green tea, respectively. The light-roasted ground coffee
beans were boiled for 10 minutes at 90°C in mineral water, then filtered
through fine filter paper. The filtrate was decaffeinated for 8 hours at
60°C 27 using activated charcoal. After the dried tea leaves were boiled
for 5 minutes at 50°C in mineral water then filtered using Whatman
paper No 1, the filtrate was infused in 90°C water for 30 minutes.

Cell culture

RAW 264.7 macrophages, a mouse macrophage cell line obtained from
ATCC passage 13-15, were cultured in Dulbecco’s modified Eagle’s
medium (DMEM high glucose), supplemented with 10% Fetal Bovine
Serum (FBS, Gibco) and 1% Penicillln/Streptomycin (P/S, Gibco) in a
5% CO2 incubator at 37°C in 60 mm dish culture.

Viability test and synergistic prediction

Briefly, RAW 264.7 was seeded at 50.000 cells /well in 96 well plates
and incubated at 37°C in 5% CO2 incubator for 24 hours. The next day,
the medium was replaced with tea and coffee extract at 0, 10, 20, 40, 80,
160, 320, 640, 1280, and 2560 μg /ml and incubated for 24 hours.
WST-1 based cell proliferation and cytotoxicity assay kit obtained from
Roche Diagnostics GmbH (Mannheim, Germany) Cat. No. 05015944001
was added to each well at the end of the treatment, and the plates were
incubated for 1 h in the dark. Finally, absorbance was measured at 450 nm

The synergy finder was used to see the synergistic potency of the coffee
and tea combination. The summary synergy scores can be interpreted
as follow: (1) Less than -10: the interaction between two drugs is
likely to be antagonistic; (2) From -10 to 10: the interaction between
two drugs is likely to be additive; (3) Larger than 10: the interaction
between two drugs is likely to be synergistic.

Construction of Foam cell model and staining

The RAW 264.7 cells were plated in 24-well plates placed with coverslip
with an initial cell density of 15.000 cells/well and cultured for 24 hours.
After this period, cells were treated with M-CSF 50 ng/ml. The next day
after treated with coffee and tea combination extract, a foam cell model
was established by incubation of cells with oxLDL 50 μg/ml for 24 h.
Before staining, the working solution of ORO was made from Oil Red O
stock solution. The Stock solution was made by dissolving 0.5 g Oil
Red O powder (Sigma) in 80 ml isopropanol (100%) in water bath at 56 °C overnight. The container was sealed to prevent evaporation of isopropanol. The final volume of the stock was adjusted to 100 ml and mixed under gentle stirring (IKA Working Group, Germany). Before staining, the stock solution was pre-warmed to 60 °C and filtered with number 1 filter paper (Whatman, UK). A working solution was prepared by diluting the stock solution 3:2 with deionized water, allowing to stand for 10 min at room temperature and then filtered (0.22 μm, Millipore).  

The medium was aspirated and cells were rinsed twice with 0.01 M PBS. The cells were fixed in 10% paraformaldehyde for 10 min. Rinsing The cells were rinsed in PBS once (1 min) then rinsed in 60% isopropanol for 30 minutes to facilitate the staining of neutral lipids. The cells were stained with filtered Oil Red O working solution at 37°C for 30 min in darkness. Aspirated the ORO staining, then washed with PBS for 3 times, 3 min each. Mounting on microscope Positive-staining (red) cells were macrophage-derived foam cells, which were observed via light microscope (Olympus) and then photographed using Image Pro-Plus 6.0 software (Media Cybernetics).

Statistical analysis

In silico data were presented using descriptive statistic. Statistical analyses of RAW 264.7 viability and foam cell number after administration of DCGTE were determined with SPSS 24.0 software by Anova one way test. Differences with P values ≤ 0.05 were considered statistically significant.

RESULTS

The physicochemical characteristic, prediction of pharmacokinetics, and mechanism of coffee and tea secondary metabolites

Designing new drugs must consider the assessment of absorption, distribution, metabolism, and excretion (ADME) which could be performed using computer models that constitute a valid alternative to experiments. Six physicochemical as properties are taken into account that gives a global description of the structure that could predispose the Bioavailability of the molecule and also highlight the propensity of the molecule to interfere with biological assays when research is conducted. The results on predictive data for pharmacokinetics, bioavailability, drug-likeness of established 8 Phyto-ligands were depicted in Table 1. The analysis of drug-likeness using the Lipinski Rule concluded that EGCG did not fulfill the criteria of a drug and also have low bioavailability score. 

Early atherosclerosis is marked by upregulation of inflammatory transcription factor and its product, dysregulation of cholesterol metabolism inside macrophage due to high LDL penetration, and modification in subendothelial space. Therefore, the prediction of the potential biological process of coffee and tea to regulate the important process of atherosclerosis contributes to important data for further research as depicted also in Table 3. It was concluded that all active constituent of tea had a strong potency to inhibit atherogenesis through several pathways, but not with coffee. The cafestol and kahweol did not exert antiatherogenic properties based on PASS SERVER analysis.

Molecular Docking Simulation

Molecular docking analysis of 4 coffee (CGA, Cafestol, Kahweol, Trigonellin) and tea compounds (EGCG, EGC, ECG, EC) with target proteins (CD36, PPARy) were presented in Table 3.

The results obtained were validated based on the binding affinities score and hydrogen bond interaction. EGCG, the largest content of tea active compound, became the strong candidate to bind with CD36 (Binding affinity = -9.0>-8.5). Interestingly, the bioactive molecule interaction with PPARy showed reverse predictive potency compared with the binding with CD36. The highest potency of binding with PPARy was CGA, the most active constituent of coffee. The kahweol, cafestol, EC, and ECG, which have binding affinity value close to control, had a potency to further investigation as an anti-atherosclerotic agent. The binding affinity of trigonelline to CD36 and PPARy was the lowest among others, thus we assumed that the potency as an oxLDL inhibitor for the receptors was low. The detail interaction of binding was presented in Figure 2.

The effect of decaffeinated coffee or green tea extract on RAW264.7 viability

The WST-1 method was used to determine cell viability after being treated with coffee or green tea extracts for 24 h. This test was used to determine the ratio of decaffeinated coffee extract (DCE) and decaffeinated green tea extract (DGTE) that would be used for combination and to identify the dose that tended to reduce the viability of the cells below 100% . The comparison of mean and standard deviation of Raw viability value from Table 4 between DCE and DGTE group was similar in each dose, thus for the combination we used ratio 1:1. Figure 3 demonstrated the significant difference of percentage viability in cells treated with DCE and DGTE (0-1280 μg/ml) compared with control (p<0.05). The cell administered with the extract showed higher viability than control.

The synergistic effect of decaffeinated coffee and green tea extract on RAW264.7 viability

The serial dose 0-640/640 μg/ml of DCGTE was exposed to Raw264.7 for 24 hours and compared with the administration of DCE or DGTE (0-640 μg/ml). The data showed the viability of DCGTE was higher than DCE or DGTE as shown in Table 5.

To validated the suggestion of synergistic effect found in coffee and tea combination, we performed a serial viability test with a variety of doses and analysed the result in synergy finder. The total score of coffee and tea combination using the Loewe method was 11.96, thus it was concluded that the combination was synergistic. The percentage viability of Raw264.7 after administered with coffee-tea combination and Loewe score were depicted in Figure 4.

Effect of decaffeinated coffee and green tea extract on foam cell formation

The effect of the DCGTE on foam cell formation was assessed and compared to control + (p=0.000). Hence, the DCGTE 320/320μg/ml reduced foam cell number in oxLDL-exposed Raw264.7.
Table 1: Pharmacokinetics and biological process analysis related to coffee and tea main active ingredients.

<table>
<thead>
<tr>
<th>Physical and chemical properties</th>
<th>Tea active constituents</th>
<th>Coffee active constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consensus Log $P_{ow}$</td>
<td>1.01</td>
<td>-0.38</td>
</tr>
<tr>
<td>Log S (ESOL) mg/ml solubility (mol/l); class</td>
<td>0.42</td>
<td>3.24</td>
</tr>
<tr>
<td>3.56; .27e-01; 2.08;2.57e+00; 2.76e-04; soluble</td>
<td>1.23</td>
<td>3.18</td>
</tr>
<tr>
<td>8.39e-03; soluble 2.00e-04; soluble 5.98e-03; soluble</td>
<td>0.85</td>
<td>3.18</td>
</tr>
<tr>
<td>2.76e-04; soluble</td>
<td>-3.70; 8.85e-02; 2.22; 1.74e+00; 8.50e+00; 2.40e-02; soluble</td>
<td>-3.96; 3.44e-02; -4.03; 2.96e-02; -1.39; 5.59e+00; 4.08e-02; very soluble</td>
</tr>
<tr>
<td>GI absorption</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>BBB permeant</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>P-gp substrate</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CYP1A2 inhibitor</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CYP2C19 inhibitor</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CYP2C9 inhibitor</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CYP2D6 inhibitor</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CYP3A4 inhibitor</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Lipinski</td>
<td>No; 2 violations: N or O&gt;10, NH or OH&gt;5</td>
<td>Yes; 1 violation: NH or OH&gt;5</td>
</tr>
<tr>
<td>Bioavailability Score</td>
<td>0.17</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Figure 2: 3D and 2D binding interaction view of top 3 coffee and tea active ingredients with CD36 and PPARγ.
3D visualization: red cylindrical spiral; Blue and grey strand indicated secondary structure of protein α-helices and β-sheet, respectively. 2D visualization: Green, light green, purple, orange circles represented conventional hydrogen bonds, carbon hydrogen bonds, alkyls, anions, respectively.
Figure 3: The effect of DCE and DGTE on viability of Raw 264.7 cells. Cells in 96 well plate (5x10^4 cells/well) were incubated with : (A) Complete medium; (B) DCE and (C) DGTE dose 320/320 μg/ml for 24 hours. (D) The histogram depicted viability percentages of cell after treated with the extract. Each value is expressed as mean ± SD in three different experiments performed in triplicate.* indicated p<0.05 relative to control (dose 0 μg/ml).

Figure 4: The synergistic Loewe score of DCGTE. (A) The 2D plot of synergy distribution; and (B) The 3D synergy landscape plot. The overall Loewe score was 11.962, therefore the combination showed synergistic effect. The optimal dose based on Synergy Finder was at 320/320 μg/ml (score=17.26417).
Figure 5: Foam cell percentage in oxLDL-stimulated Raw264.7. (a) The foam cell formation was evaluated under light microscope 200x magnification. (K) Control groups: Raw 264.7 + M-CSF 50 ng/ml; (K+): Raw 264.7+M-CSF 50 ng/ml + oxLDL 50 μg/ml, and (P1): Raw 264.7+M-CSF 50 ng/ml + DCGTE 320/320 μg/ml + oxLDL 50 μg/ml. The foam cell is the cell coloured red in the cytoplasm after ORO staining. The data were taken after triplicate experiment. Histogram showed the % of foam cell in triplicate. * p<0.05 relative to control (K), and # p<0.05 relative to K+.

Table 2: Predicted biological activities of coffee and tea active compound.

<table>
<thead>
<tr>
<th>Biological process</th>
<th>Tea active constituents</th>
<th>Coffee active constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ECG</td>
<td>EGC</td>
</tr>
<tr>
<td>Lipid peroxidase inhibitor</td>
<td>0.946</td>
<td>0.92</td>
</tr>
<tr>
<td>Hypolipidemic agent</td>
<td>0.70</td>
<td>0.71</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>0.62</td>
<td>0.61</td>
</tr>
<tr>
<td>Transcription factor NF kappa B inhibitor</td>
<td>0.57</td>
<td>0.71</td>
</tr>
<tr>
<td>TNF inhibitor</td>
<td>0.57</td>
<td>0.71</td>
</tr>
<tr>
<td>Ubiquinol-cytochrome-c reductase inhibitor</td>
<td>0.59</td>
<td>0.71</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>0.82</td>
<td>0.87</td>
</tr>
<tr>
<td>ApoA1 enhancer</td>
<td>0.85</td>
<td>0.85</td>
</tr>
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</table>

Table 3: Affinity binding of coffee and tea active compounds for CD36 and PPARγ ligand.

<table>
<thead>
<tr>
<th>Chemical compound</th>
<th>Binding affinities (kcal/mol)</th>
<th>Number of H-bond</th>
<th>Interacting residues</th>
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</thead>
<tbody>
<tr>
<td>CD36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP 5258</td>
<td>-8.5</td>
<td>3</td>
<td>ASN118, PRO203, SER269</td>
</tr>
<tr>
<td>EGC</td>
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<td>4</td>
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<td>EGC</td>
<td>-7.8</td>
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<td>ARG96</td>
</tr>
<tr>
<td>ECG</td>
<td>-8.4</td>
<td>3</td>
<td>LYS334, ASP209, TYR238</td>
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<td>EC</td>
<td>-8.2</td>
<td>1</td>
<td>ARG96</td>
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<tr>
<td>CGA</td>
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<tr>
<td>Cafestol</td>
<td>-8.2</td>
<td>1</td>
<td>ALA208</td>
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<td>Kahweol</td>
<td>-8.4</td>
<td>1</td>
<td>ASN118</td>
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<tr>
<td>Trigonelline</td>
<td>-5.3</td>
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<td>SER268, SER269, ASP270, LYS385</td>
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<tr>
<td>PPARγ</td>
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<tr>
<td>Pioglitazone</td>
<td>-8.8</td>
<td>3</td>
<td>HIS323, SER289, TYR473</td>
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<td>-6.9</td>
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<td>CYS285, HIS449, SER289</td>
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<td>Cafestol</td>
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<td>Trigonelline</td>
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<td>TYR327, HIS449, SER289</td>
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DISCUSSION

Exploring natural product as candidate agent for prevention of atherosclerosis is crucial effort for reducing the cardiovascular disease prevalence. Many studies reported the effect of coffee consumption in reducing cardiovascular risk, thus become one functional food candidate in the prevention of the disease. Yet, several reports did not successfully reveal the beneficial effect of coffee in lowering cardiovascular disease. Therefore, our current study tried to alleviate the coffee health effect by several strategies: (1) performing light roasting to coffee bean, (2) decaffeination, and (3) combining coffee with tea based on preliminary study. The total CGA content of green coffee beans (34.43 - 41.64 mg / g) is much higher than that of light and medium roasted coffee (2.05 - 7.07 mg / g) because CGA undergoes isomerization and decomposes with heat. Decaffeination process minimize the caffeine concentration which cause cardiovascular or non-cardiovascular side effects by consuming coffee 34,35.

Our findings showed that either DCE, DGTE, or DCGTE did not induced cytotoxicity on macrophage cell. These results were supported by other in vitro studies. Five studies were examining the viability test after the cell exposed to coffee extract and three studies with green tea leaves extract. The average dose of green coffee extract that still viable to macrophage cell 90% was 500 µg/ml, while for green tea was 125 µg/ml 36,37. The synergistic combination of active ingredients in coffee and tea has been reported by Mao et al 2017. The study showed that combination of CGA and EGCG has higher antioxidant activity compared to the tea leaves extract. The study has several limitations since we did not investigate the molecular mechanism how the could lower foam cell. The mechanistic action of these extract might be explained by the in silico result. Based on molecular docking result, the tea preferably inhibits oxLDL uptake by competitive binding to CD36, meanwhile the coffee strongly suggested act as ligand for PPARy. A cluster of differentiation 36 (CD36) is a transmembrane glycoprotein family, a member of class B scavenger receptor. This receptor plays an important role in atherosclerosis development due to high expression in macrophage and facilitating the uptake of ox-LDL into macrophages 23. Fatty Acid, another lipid derivate such as HODE in an oxLDL bind with high affinity to CD36. Many studies demonstrated that amino acids 160-168 of CD36 represent the core of the binding site for ox-LDL and that the electrostatic interaction between evolutionary 39. The PPARγ will activate the ABCA1 and mediated the efflux of cholesterol from the macrophage. Thus, our in silico findings gave information for developing coffee and tea as an antitherosclerosis agent based on the molecular docking result and molecular function prediction. The prediction biological process supports the in vitro results. All coffee and tea active ingredients have potency either as: lipid peroxidase inhibitor, hypolipemic, anti-hypocholesterolaemia, anti-inflammatory, TNFα inhibitor, ubiquitin inhibitor, and ApoA1 enhancer agent that has been matched with KEGG Pathway: lipid and atherosclerosis. Our study has several limitations since we did not investigate the molecular mechanism how the could lower foam cell.

CONCLUSION

Based on in silico and in vitro data, it was concluded that coffee and tea might have potential role in foam cell atherosclerosis. The DCGTE showed no cytotoxicity to the Raw264.7 cells and could inhibit foam cell formation. Further in vitro and in vivo research using atherosclerosis mice model need to be performed to see the cellular and molecular mechanism of combination of decaffeinated coffee and green tea on the atherosclerosis initiation and progression. The toxicity and safety aspects of products are also required to explore before commercialization. Apart from all, our findings give evidence that decaffeinated coffee and green tea could be a potential functional food candidate to replace the use of statin or aspirin as a previous drug to inhibit atherosclerosis.

ETHICS STATEMENTS

This is a computational and in vitro study, which doesn't include animal or human experiments.

### Table 4: Viability of Raw 264.7 after treated with DCE and DGTE.

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>% Viability groups DCE</th>
<th>p</th>
<th>% Viability groups DGTE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>167.72 ± 3.58</td>
<td></td>
<td>176.97 ± 5.13</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>163.54 ± 19.11</td>
<td></td>
<td>154.22 ± 4.04</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>159.66 ± 21.11</td>
<td></td>
<td>152.03 ± 7.51</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>156.39 ± 1.769</td>
<td></td>
<td>147.29 ± 11.76</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>154.42 ± 33.53</td>
<td>0.000**</td>
<td>137.07 ± 2.39</td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>152.42 ± 10.66</td>
<td></td>
<td>124.07 ± 9.87</td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>147.65 ± 37.36</td>
<td></td>
<td>116.17 ± 11.53</td>
<td></td>
</tr>
<tr>
<td>1280</td>
<td>110.21 ± 6.86</td>
<td></td>
<td>110.42 ± 10.66</td>
<td></td>
</tr>
<tr>
<td>2560</td>
<td>89.74 ± 5.68</td>
<td></td>
<td>61.13 ± 7.65</td>
<td></td>
</tr>
</tbody>
</table>

*The data were presented as mean and standard deviation in each group.* p<0.05 relative to control; **p<0.001 relative to control

### Table 5: The effect of DCE, DGTE, and DCGTE on macrophage Raw264.7 viability.

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>% Viability of DCE</th>
<th>p</th>
<th>% Viability of DGTE</th>
<th>p</th>
<th>Dose (µg/ml)</th>
<th>% Viability of DCGTE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>161.79 ± 4.54</td>
<td></td>
<td>164.25 ± 14.61</td>
<td></td>
<td>10/10</td>
<td>190.03 ± 14.93</td>
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<tr>
<td>20</td>
<td>158.85 ± 10.62</td>
<td></td>
<td>163.88 ± 10.72</td>
<td></td>
<td>20/20</td>
<td>175.49 ± 25.88</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>138.38 ± 5.80</td>
<td></td>
<td>139.25 ± 6.58</td>
<td></td>
<td>40/40</td>
<td>159.77 ± 16.72</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>135.16 ± 6.92</td>
<td>0.000**</td>
<td>137.89 ± 5.66</td>
<td>0.000**</td>
<td>80/80</td>
<td>154.75 ± 4.84</td>
<td>0.009**</td>
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<tr>
<td>160</td>
<td>132.58 ± 4.99</td>
<td></td>
<td>119.06 ± 3.82</td>
<td></td>
<td>160/160</td>
<td>149.79 ± 4.01</td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>127.77 ± 3.99</td>
<td></td>
<td>112.21 ± 8.89</td>
<td></td>
<td>320/320</td>
<td>155.94 ± 8.12</td>
<td></td>
</tr>
<tr>
<td>640</td>
<td>121.28 ± 5.72</td>
<td></td>
<td>111.12 ± 3.32</td>
<td></td>
<td>640/640</td>
<td>133.92 ± 34.00</td>
<td></td>
</tr>
</tbody>
</table>

*The data were expressed as mean and standard deviation in each group.* p<0.05 relative to control; **p<0.001 relative to control
**DECLARATION OF COMPETING INTEREST**

The authors declare no conflicts of interest.

**REFERENCES**


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