

Comparison of Several Indonesian Medicinal Plants Effects on LDL-C and IL-6 Levels in Wistar Rats After High Fat Feeding

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Abstract

High-fat diet habits lead to an increase in LDL-C levels that eventually influence the atherosclerotic plaque formation and progression, causing coronary heart disease. Atherosclerosis is a chronic inflammatory process that releases various pro-inflammatory cytokines, including IL-6. Indonesians often use medicinal plants to decrease cholesterol levels. This study aimed to compare the LDL-C and IL-6 levels after treatments of ethanol extracts from Java ginger (EEJG), turmeric (EET), garlic (EEG), and pomegranate flowers (EPPF) in a hypercholesterolemia animal model. This study was conducted at the Maranatha Biomedical Research Laboratory from June–December 2020. Male Wistar rats were divided into six groups (n=5 per group) and received high-fat feeding and 0.01% propylthiouracil. The following treatments were given for 28 days: oral carboxymethylcellulose 1% for negative control; 35 mg/200 g of oral EEJG, EET, EEG, EPPF for respective treatment groups (n=5 groups); and 0.36 mg/200 g of oral Rosuvastatin for positive control. It was demonstrated that the mean LDL-C levels were 65.75 mg/dL, 55.25 mg/dL, 56.75 mg/dL, and 59.60 mg/dL for EEJG, EET, EEG, EPPF groups, respectively, which were significantly different from that of the negative control (81.73 mg/dL). The IL-6 levels of the EEJG (27.55 pg./mL) and EEG (27.54 pg./mL) group were significantly different from the EPPF group (24.5 pg./mL) but not significantly different from the negative control (25.58 pg./mL), EET (25.60 pg./mL), and rosuvastatin (26.09 pg./mL) groups. The administration of ethanol extracts of Java ginger, turmeric, garlic, and pomegranate flower decreases the C-LDL levels; however, only the ethanol extract of pomegranate flowers administered for 28 days decreases the IL-6 levels of Wistar rat hypercholesterolemia model, albeit insignificantly.

Keywords: Garlic, Java ginger, high-fat feeding, IL-6, pomegranate flowers, turmeric

Introduction

High-fat diet habits cause an increase in LDL-C levels, which play a role in atherosclerotic plaque formation and progression, the cause of coronary heart disease. Atherosclerosis is a chronic inflammatory process that releases various pro-inflammatory cytokines, including IL-6. Interleukin 6 (IL-6) is one of the pro-inflammatory cytokines that mediate inflammatory reactions, which produce an acute response to tissue

injuries that disrupt homeostasis.¹ A meta-analysis study reported the range of IL-6 in the blood circulation of healthy human donors between 0 and 43.5 pg./mL, with an estimation of the normal level at 5.186 pg./mL.² Inflammatory reaction accompanied by increased levels of IL-6 may occur in some circumstances such as hyperlipidemia.¹ Hyperlipidemia causes an inflammatory reaction in the endothelial blood vessels triggered by oxidized cholesterol-LDL. It causes the formation of foam cells between the basal endothelial membrane and smooth muscle cells, triggering the release of pro-inflammatory cytokines, including IL-6. Increased cholesterol levels will also increase C reactive protein via increased IL-6.³

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High-fat feeding (HFF) given to animal subjects will lead to increased expression of the IL-6 gene.⁴ IL-6 is one of the mediators of inflammatory reactions, immune responses, and hematopoiesis.¹ As an inflammatory reaction, IL-6 might become a chosen parameter to reach a therapeutic target, any agent that reduces IL-6 levels or reduces the expression of the IL-6 gene has anti-inflammatory effects.⁴ The HFF can increase LDL-cholesterol, a condition that can increase IL-6 levels, meaning that hyperlipidemia causes chronic inflammatory reactions, and lipid-lowering drugs that can lower IL-6 levels also have anti-inflammatory effects.¹

Indonesians often use medicinal plants, such as Java ginger, turmeric, garlic, and pomegranate flowers, to lower lipid levels for metabolic syndrome management.⁵ Java ginger (*Curcuma xanthorrhiza*) and turmeric (*Curcuma longa*) are parts of 15 species of *Curcuma* in Java.⁶ Turmeric has more curcuminoid (curcumin, bisdemethoxycurcumin, and desmethoxycurcumin) than Java ginger.⁵ In Vitro and in vivo studies have reported that curcumin showed a potent anti-inflammatory effect in hyperlipidemia.⁷ Among the genus of *Allium*, garlic (*Allium sativum*) is a medicinal plant that is widely used as an anti-hyperlipidemia and anti-inflammatory agent.⁸ Recent study reported that garlic could be suggested as a candidate for maintaining homeostasis by its immunomodulatory and immunotherapeutic activities, thus improving hyperlipidemia.⁹ Another medicinal plant with antioxidant compounds (cyanidin, pelargonidin, delphinidin, and ellagic acid) is pomegranate (*Punica granatum*).¹⁰ as a rule, is several times higher than the content of L-malic. The total acidity of pomegranate juice is high, on average 1.1 g of organic acids is present in 100 cm³ Pomegranate flower significantly reduced tissue lipid peroxidation, increased HDL-C, and reduced vascular inflammatory markers and cytokines.¹¹ This study aimed to compare the anti-inflammatory effects of Java ginger, turmeric, garlic, and pomegranate flowers ethanol extract in lowering IL-6 in an HFF-induced animal model.

Methods

This research was conducted at Maranatha Biomedical Research Laboratory from June 2020 to December 2020. This study is an experimental laboratory study, using animal research subjects of male rat Wistar, aged 6–8 weeks, weighing 180

g –200 g. This research has been approved by the Research Ethics Commission of the Faculty of Medicine at Maranatha Christian University with the number: 149/KEP/X/2020. The study subjects of male Wistar rats to be used adapted first to the laboratory atmosphere and then induced by high-fat feeding (HFF) for 14 days, and continued until 28 days later and treated with Indonesian medicinal plants (Java ginger, turmeric, garlic, and pomegranate flowers) for 28 days.¹²

Making ethanol extract is by mixing medicinal plants with 70% ethanol for 24 hours, then the solid material is separated, and a thick extract is made using a rotatory evaporator.

Animal research subjects, after induction with HFF for 14 days, were then divided into six groups (n=5) and given the following treatment: (1) negative control: given CMC 1% and HFF in rats per oral for 28 days; (2) Java ginger: given Java ginger (*Curcuma xanthorrhiza*) ethanol extract dose of 35mg/200 g rats per oral and HFF for 28 days; (3) turmeric: given turmeric (*Curcuma longa*) ethanol extract dose of 35mg/200g rats per oral and HFF for 28 days; (4) garlic: given garlic (*Allium sativum*) ethanol extract dose of 35 mg/200g rats per oral and HFF for 28 days; (5) pomegranate flowers: given a dose of pomegranate (*Punica granatum*) flower ethanol extract dose of 35 mg/200 g rats per oral and HFF for 28 days; (6) positive control: Rosuvastatin was given to rats orally and HFF for 28 days.¹³ At the end of the study, the rats were terminated, and the blood samples were stored in a -80°C refrigerator for further examination. The dose taken for all herbal ingredients was adapted from Huajing et al.¹⁴

The test principle for C-LDL examination was a Homogeneous enzymatic colorimetric assay using a spectrophotometer. The IL-6 examination is performed by ELISA Method using the ELISA Rat IL-6 kit (Elabscience, E-EL-R0015). Each group's data were analyzed using the Shapiro-Wilk Test, and all data were normally distributed. The data were analyzed using a one-way ANOVA, followed by the Tukey HSD test with alpha=0.05.

Results

The result of an examination of C-LDL Levels in Wistar rats was as follows: negative control (81.73+4.43 mg/dL), java ginger (65.75+2.59 mg/dL), turmeric (55.25+1.30 mg/dL), garlic (56.75+4.02 mg/dL), pomegranate flower (59.60+10.21 mg/dL), positive control/

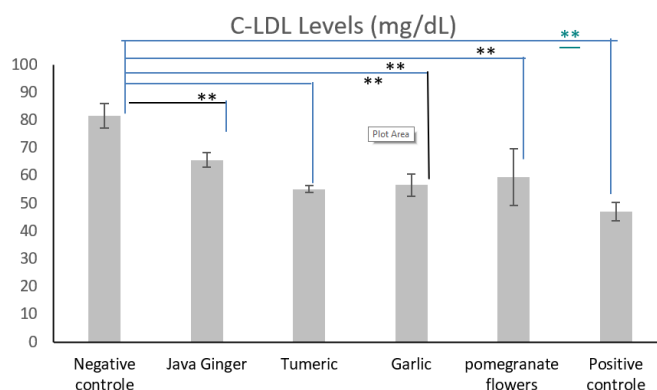


Figure 1 C-LDL Levels of Each Group

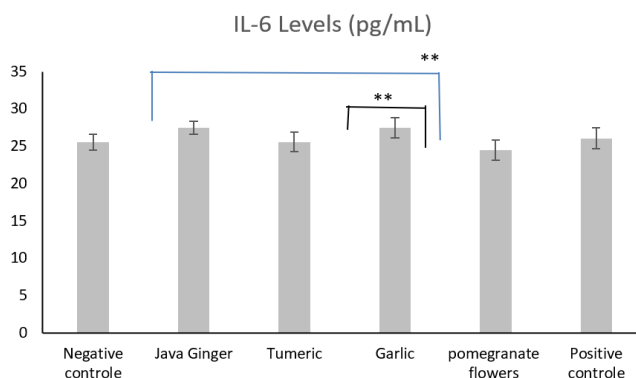


Figure 2 IL-6 Levels on 28 Days of Treatment

rosuvastatin (47.20 ± 3.27 mg/dL). Examination of IL-6 levels in Wistar rats was conducted on the 28th day of treatment. The results were as follows: negative control (25.58 ± 1.07 pg./mL), Java ginger (27.55 ± 0.88 pg./mL), turmeric (25.60 ± 1.30 pg./mL), garlic (27.54 ± 1.34 pg./mL), pomegranate flower (24.56 ± 1.35 pg./mL), and positive control/rosuvastatin (26.09 ± 1.40 pg./mL). The result was shown in Figures 1 and 2.

Discussion

High-fat diet triggers hyperlipidemia, and hyperlipidemia causes endothelial dysfunction and increases pro-inflammatory cytokines such as IL-6.³ Increased levels of IL-6 can have an impact on several organs, such as the onset of hepatocellular carcinoma, coronary heart disease, and high blood pressure.¹ IL-6 is also associated with the aging process, including

atherosclerosis-related hypercholesterolemia.¹⁵ Pro-inflammatory cytokines, including IL-6, play a role in the pathogenesis of aging-related diseases, such as atherosclerosis, type 2 diabetes, Alzheimer's, dementia, cancer, and arthritis.¹⁶ Pro-inflammatory cytokines, including IL-6, are a low molecular weight regulatory protein secreted by various cell types.¹ Inflammation is a normal body reaction as a response to injury or infection for eliminating inflammatory microorganisms and can lead to cell death.¹⁷

Inflammation is generally divided into two types: acute and chronic. Acute inflammation has a short duration and a rapid onset. Acute inflammatory reactions occur in the recruitment of neutrophils and the discharge of blood plasma into the tissues. The main signs of inflammatory reactions are redness, swelling, pain, and warmth. Chronic inflammation has manifestations of persistent macrophages and lymphocytes with a long duration.¹⁸ tumor, calor, and dolor, scientific investigations have revealed

chemical components, cells, and pathways involved in the process of inflammation. The body's initial defense in response to infection, trauma, or inflammation is through the acute-phase response (APR Acute inflammatory cytokines include IL-1, IL-6, IL-8, IL-11, TNF alpha (tumor necrosis alpha), and IL-16, IL-17, G-CSF (granulocyte-macrophage colony-stimulating factor). Chronic inflammatory cytokines are divided into two groups, groups that regulate hormones such as IL-4, IL-5, IL-6, IL-7, and IL-13, and groups that regulate cellular responses such as IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, interferons, and TNF α and β . Some cytokines play a role in both acute and chronic inflammation. The cytokines are IL-1, IL-6, IL-11, IL-17 and TNF- α . Pro-inflammatory cytokines play a role in the pathogenesis of cardiovascular disease and cancer.¹⁹

HFF-induced inflammation occurs in the central nervous system and peripheral tissues such as the liver, skeletal muscle, and intestine.^{20,21} chronic overnutrition, such as consumption of a high-fat diet (HFD These two mechanisms lead to pro-inflammatory cytokines production such as IL-6, IL-1 β , and tumor necrosis factor/TNF α) in the gut that is distributed to circulation, activating low-grade systemic inflammation.^{20,22} Chronic overnutrition, such as consumption of a high-fat diet (HFD Besides that, increased levels of free fatty acid caused by HFF directly affect intestinal cells.²⁰ chronic overnutrition, such as consumption of a high-fat diet (HFD These two mechanisms lead to pro-inflammatory cytokines production such as IL-6, IL-1 β , and tumor necrosis factor/TNF α) in the gut that are distributed to circulation, activating systemic low-grade inflammation.^{20,22} chronic overnutrition, such as consumption of a high-fat diet (HFD Therefore, an anti-inflammatory agent is needed to counteract the effect of HFF on pro-inflammatory cytokine production.

Indonesian people like to use medicinal plants as a complementary therapy for many diseases and to boost their immune response.^{5,23} Among them, Java ginger, turmeric, garlic, and pomegranate are known for their various health benefits, often used in Indonesian food recipes, and available in abundance. Those medicinal plants are known to have anti-hyperlipidemia and anti-inflammatory effects because of their compounds that exhibit potent antioxidant properties.^{7,8,24} Java ginger and turmeric have curcumin that could decrease free fatty acids in plasma, inhibits pro-inflammatory cytokines (IL-6, IL-8, TNF- α , nuclear factor-Kappa B/NF-kB),

lipid peroxidation, and protein carbonyl.⁵

This study compared the effect of various medicinal plants on lowering pro-inflammatory cytokine IL-6 after 28 days of induction of HFF. Before treatment, the rats were induced with HFF for 14 days, whose administration passed on during treatment for up to 28 days. The treatment given in this study is Java ginger, turmeric, garlic, and pomegranate flowers for another 14 days, along with the HFF This study found a very significant difference in the pomegranate group compared to the Java ginger and garlic groups ($p=0.009$). In contrast, we did not find any significant differences in other groups. Among 15 genus of *Curcuma* found in Indonesia, turmeric (*Curcuma longa*) has the highest compound of curcumin;⁵ thus, the effect of Java ginger might not be as strong as turmeric in reducing IL-6 levels. The result of the study is somehow different from a study conducted by Jain et al that found a decrease of IL-6 in diabetic rats after curcumin supplementation.²⁵ However, the study was performed for seven weeks, while this study was conducted for four weeks. Garlic compounds such as alliin and *S-allyl cysteine* (SAC) were proven to have anti-inflammatory properties.²⁶ Recent studies showed that aged garlic extract has more health benefits than fresh garlic extract,²⁷ methylglyoxal or ribose this might explain why the IL-6 level in the garlic group is higher than in other groups. Triterpenoids oleanolic acid, and ursolic acid are bioactive compounds in pomegranate flowers.¹⁰ as a rule, is several times higher than the content of L-malic. The total acidity of pomegranate juice is high, on average 1.1 g of organic acids is present in 100 cm. The reduction of IL-6 levels found in pomegranate group is in line with other studies using the pomegranate flower as an anti-inflammatory agent after HFF induction.^{11,28}

This study found no significant difference in other groups, including in positive control, turmeric, and rosuvastatin groups; the short duration of HFF might cause this, and treatment has been given; also, time-modulation of IL-6 might have taken part in this study. The limitation of this study is that we did not measure IL-6 levels at some time points to investigate its modulation and did not measure other inflammatory cytokines that might also take part in low-grade systemic inflammation caused by HFF.

This study concluded that administering Java ginger, turmeric, garlic, and pomegranate flower extracts ethanol decreases C-LDL Levels compared to the negative control. The administration of extract ethanol pomegranate

flowers lowers IL-6 levels in Wistar rats induced with 28 days of a high-fat diet compared to the administration of extract ethanol Java ginger and garlic. Further studies that can be done include research linking total cholesterol, C-LDL, C-HDL, and triglyceride levels with IL-6 levels.

The induction with high-fat feeding to Wistar rats for 14 days has only succeeded in creating an experimental animal model of hypercholesterolemia or a model of dyslipidemia. Atherosclerosis is a chronic inflammatory process in which the inflammatory response is prolonged, so the increase in LDL-C levels within 14 days has not caused significant vascular endothelial injury and the accumulation of atherosclerotic plaques significantly for created animal atherosclerosis model, so the increase of IL-6 levels is not significant. This research needs to be continued by using experimental animal atherosclerosis models and a normal control group.

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