Functional-drink rich in antioxidant cardamom-rhizome (*Amomum cardamomum* willd) suppresses inflammation and improves lipid profile

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Abstract. The aim of this research was to know the effect of functional drink rich in antioxidant cardamom rhizome (Fd-Carrhi) on level of IL-6, C-RP, and lipid profile of atherosclerotic. A total of 30 women with atherosclerosis, age 40-65 years old, hypertension, hypercholesterolemia, hypertriglyceridemia, lived in Purwokerto, Banyumas, Central Java, Indonesia, and were willing to sign informed consent, recruited as research subjects. They consumed simvastatin from doctors, divided by 3 groups of 10 people each. Group I, given Fd-Carrhi; II, placebo; and III, only simvastatin, for 2 months. As many as 100 ml of Fd-Carrhi or placebo were given every morning. Blood samples were taken 3 times, 1 ml, at baseline, 1 and 2 months after intervention. Blood plasma was determined levels of IL-6, C-RP, as well as total cholesterol (total-c), triglycerides (TG), LDL-c, and HDL-c. Result showed Fd-Carrhi versus placebo significantly decreased plasma level of IL-6, C-RP, total-c, and LDL-c, and otherwise increased HDL-c, but no differences were seen in TG. The findings clearly support Fd-Carrhi inhibit the development of atherosclerosis towards cardiovascular heart diseases (CHD) by suppressing IL-6 and CRP levels, and improving lipid profile.

Key words: *Cardamom rhizome,* functional drink, IL-6, CRP, lipid profile

1. Introduction

Inflammation is one of the factors that induce atherosclerosis. Clinical studies pointed that inflammation status is reflected from the high level of hs-CRP and IL-6 in plasma [1, 2]. It is stated that high level of cytokine contributes to the early stage of coronary artery disease [3]. hs-CRP is the result of hepatic inflammation whose performance is managed by cytokine IL-6, while messenger proinflammatory cytokines (IL-6) is secreted by macrophag and smooth muscle cells in atherosclerotic lesion. Therefore, hs-CRP and IL-6 can be used to predict the development of atherosclerosis toward cardiovascular disease (CVD) [4]. Kumar *et al*. [5] supported that CVD is more prevalent in women than men. High markers of inflammation is related to the increasing risk of atherosclerosis. Accordingly, Frostegård [6] ensured that antiiflammatory treatment is considered the new medication for CVD.

Hypercholesterolemia is also related to atherosclerosis. LDL-c transports cholesterol from liver into the tissues for body performance, while HDL-c transports cholesterol from the tissues to liver. However, when LDL-c level more than normal, it can accumulate in the artery and increase the risk of

plaque that clogs the artery, called atherosclerosis. In atherosclerotic lesion is found a more electronegative LDL-c than native LDL-c [7]. It is certain that the contributing factor of atherogenesis is not LDL native but oxidized LDL (LDL-ox), while the formation of LDL-ox is the effect of high level of LDL-c. Thus, it is crucial to minimize the level of either LDL-ox or LDL-c.

Hypercholesterolemia is not a disease but a metabolic disorder that can contribute to the occurrence of various degenerative diseases, especially cardiovascular disease. Several studies have reported that the emergence of degenerative diseases is due to accumulation of free radicals in the body [8,9]. One substance that is capable of controlling the work of free radicals are antioxidants. Numerous beverage products in markets are labelled rhizome-based and rich in antioxidant such as saffron-acid drinks, ginger ale, ginger drink, and many others but not supported by scientific evidence.

The cardamom rhizome, recently reported Winarsi et al. [10] contains flavonoid antioxidants of 324.51 mg / g, nearly 3 times the levels in cardamom leaves, and 15 times their levels in cardamom rods. Extracts of cardamom rhizomes given to atherosclerotic rats for 2 weeks, proven to lower total cholesterol, LDL-c, LDL-ox [10], Triglycerides, MDA, IL-6, and CRP [11], and otherwise increase HDL levels [10]. The cardamom rhizome has also been formulated into functional drinks by Winarsi and Hernayanti [12]. The drink is known to be rich in phenolic antioxidants (498.8 $\pm$ 0.01 ppm), greater than the ginger rhizome drink (447.93 ppm) found by Ibrahim et al. [13]. In addition to phenolic antioxidants, beverages based on the cardamom rhizome also contain vitamin C (36 mg/100 g), higher than sour turmeric drink (0.688 mg/100 g) [14]. The drink tastes sweet, semriwing, warms the body, and there is a preferred aftertaste [12].

The antioxidant compounds are capable of suppressing inflammation, protecting endothelial cell membranes by modulating lipid profiles [15], and prevent the development of atherosclerosis [10]. But, no data revealed the potential of Fd-Carrhi in suppressing levels of IL-6 and C-RP, and improving lipid profile. The aim of this study was to investigate the effects of Fd-Carrhi on levels of IL-6, C-RP, total cholesterol, triglycerides, LDL-c, and HDL-c in plasma of women with atherosclerosis.

2. Materials and methods

This randomized double-blind placebo-controlled trial was a continuation of the research Winarsi and Hernayanti [12] conducted through several stages. The research was approved by the ethics committee of the Medical Faculty of Diponegoro University and Central General Hospital Dr. Kariadi, Semarang, Indonesia.

2.1 Production functional drink rich in antioxidant rhizome cardamom (Fd-Carrhi)

The cardamom rhizome was obtained from cardamom farmers in the village of Sumbang, Banyumas, Central Java, Indonesia. After washing, cardamom rhizomes were thinly sliced then dried in cabinet dryer at 57^{o}C, so the water content was 4-6%, called cardamom chip rhizome (CCR). The drinks was composed of a variety of CCR coupled with spices such as wooden cup, cinnamon, cloves, star anise, ginger, lemongrass, and lime leaves, as well as low calorie sweetener and a little salt [12].

2.2 Selection, classification, and intervention of research subjects

Research subjects were 30 atherosclerotic women, aged 40-65 years and suffered from hypertension (>140/90 mmHg) [16], hypercholesterolemic (>200 mg/dl), and obesity (BMI > 25 kg/m2) [17]. Subjects lived in Purwokerto, Banyumas Regency, Central Java, Indonesia and were willing to sign the informed consent. Subjects were randomly assigned into three groups of 10 and took simvastatin from the doctor. Group I was given Fd-Carrhi, II placebo (=Fd-Carrhi without CCR), and III only simvastatin. Intervention was performed in two months straight. Fd-Carrhi and placebo were given 100 mL/day one time a day, and delivered to the subject's house every morning between 06.00-08.00 to directly taken.

2.3 Blood sampling and test

Blood sample was taken three times 3 ml each on the baseline, then 1 and 2 month after intervention. Blood sample was taken using venoject with 10% EDTA. Blood was sentrifuged at 3,000 rpm for 10

minutes, separated the plasma [18] then evaluated the IL-6, C-RP, total cholesterol, trigliseride, LDL-c, and HDL-c.

2.4 Data Analysis

The result was expressed as mean ± SE. The obtained data were subject to ANOVA followed by Duncan test in case of significance with 5% error.

3. Result and Discussion

3.1 Effect Fd-Carrhi on IL-6 levels

In this research, the CCR-scented was semriwing, less savory aroma, and chelate. Preparation of Fd-Carrhi refers to Winarsi and Hernayanti [12]. Level of IL-6 was determined using Human IL-6 Elisa Kit, SunRed-Bio. On the baseline, the three groups were homogenous (P=0.84), but after 2-month intervention IL-6 level decreased from 82.67 to 54.69 ng/L (P=0.02) in Fd-Carrhi group. Opposite result occurred in placebo and statin group (P=0.53 and P=0.13) (Figure 1).

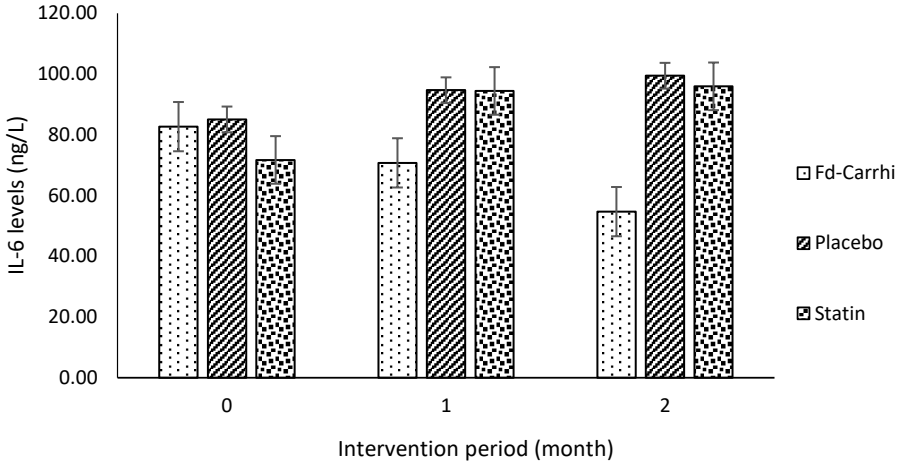

Figure 1. Effect of Fd-Carrhi on IL-6 levels in blood plasma atherosclerotic women.

Notes: Fd-Carrhi, group consuming statin + functional drink based on cardamom rhizome; Placebo, group consuming statin + placebo; Statin, group consuming statin only; n=30; P<0.05.

The decrease of IL-6 level in Fd-Carrhi group may be due to the phenpolic antioxidant in the drink. Hopkins *et al*. [19] stated that supplementing antioxidant can lower IL-6 level in blood. Phenolics are heterogenous compound derived from plant secondary metabolism. According to Bravo [20] phenolic compounds can be classified inti flavonoid and non-flavonoid. Epidemiologic data showed that high phenol diets reduced the occurrence of chronic diseases such as diabetes, cardiovascular disease, alzheimer, parkinson, and inflammation [21]. Phenolic compounds are assumed to responsible of beneficial effects on the diseases. Chronic acute inflammation contributes to the development of chronic disease such as atherosclerosis. Intervention that can modify inflammation in chronic diseases is assumed to be potential for prevention [22].

Several phenolic compounds show anti-inflammation properties. Although the antiinflamation mechanism is not thoroughly known, correlation between high intake of phenol-enriched diet and inflammation downregulation response [23]. Nieman *et al*. [24] supported that as anti inflammation, phenolic compound lowered TNF-α level through inhibiting Nuclear Factor kappa-B (NF-kB). NF-kB controls gene expression related to cytokine and chemokine proinflammation TNF-α, IL-1β and IL-6. As NF-kB is inhibited, IL-6 expression and the content decrease. Phenolic compounds work the same way as non-steroid anti-inflammatory drugs (NSAID), such as inhibiting pro inflammation mediator besides COX by limiting the activity or expression of the genes. Moreover, some phenolic compounds

increase or decrease transcription factor such as NF-kB on the tract of inflammation and antioxidant [25,26].

The structure of phenolic compounds significantly affects anti inflammation mechanism. Lättig *et al*. [27] reported that unsaturation C ring stabilized intermediate radicals through resonance. Besides, the double bond in C2 and C3 induced coplanarity between ring A and C and stimulated flavonoid interaction with the active enzyme. Eventually, lignan of phenolic compounds participates in the formation of covalent bond between flavonoid and macromolecule [28]. Meanwhile, Chuang and McIntosh [29] stated that phenolic compounds showed antiinflamation activity by inhibiting the synthesis of pro inflammation mediator, modifying eicosanoid synthesis, inhibiting activated immune cells and NOS (nitric oxide synthase) and xyclooxygenase-2 by the inhibitory effect of NF-κβ.

Some flavonoid diets are shown to modulate inflammation mediator like IL-6, such as flavonol (a type of flavonoid) that affects the concentration of blood plasma IL-6 [30]. Epidemiology data also proved the decrease in chronic disease incidence in people taking phenolic-enriched diet [31]. Every type of phenolic in extracts has different effect of pro inflammation mediator. For example, one type can inhibit pro inflammation mediator while the other inhibits the expression. Therefore, Ambriz-Pérez *et al*. [32] stated that phenolic compounds, either mono or combined, can serve as the alternative therapy for inflammation because of the ability to perform under diverse mechanisms, while medicine has specific work on the body. Fd-Carrhi is viable as an alternative natural diet for inflammation therapy to atherosclerotic patients.

3.2 Effect of Fd-Carrhi on CRP levels

C-RP level was determined using Human C-RP Elisa Kit, SunRed-Bio. Three groups were homogenous on the baseline (P=0.88); however, it decreased after 2-month intervention from 2.92 to 1.34 mg/L (P=0.047) in Fd-Carrhi group, while opposite result occurred in placebo and statin group (P=0.67 and P=0.93) (Figure 2).

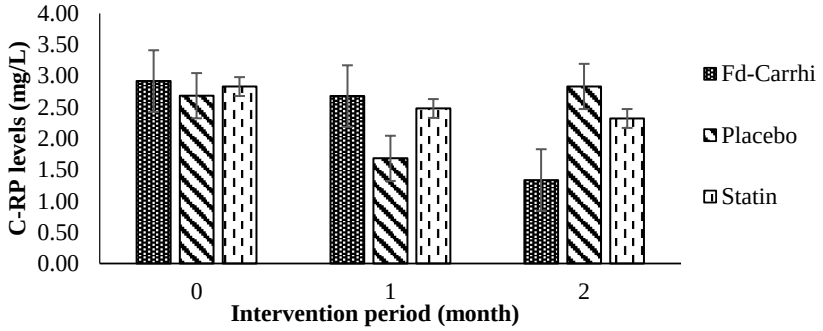

Figure 2. Effect of Fd-Carrhi on C-RP levels in blood plasma atherosclerotic women.
Notes: Fd-Carrhi, group consuming statin + functional drink based on cardamom rhizome; Placebo, group consuming statin + placebo; Statin, group consuming statin only; n=30; P<0.05.

C-RP is acute-phase protein whose level increases during chronic inflammation [33]. Epidemiology study stated that CRP is both marker and agent of atherosclerosis development [34]. CRP is also the clinical marker of the heightened risk of cardiovascular [35]. In vitro and in vivo research showed that CRP served as pro atherogenic factor and promoted atherothrombosis [36], promoted activation and dysfunction of endhotel cell [37], affected migration and proliferation of vascular smooth muscle [38], induced changes in biology matrix [39], and promoted coagulation [40].

In early study, CRP level of atherosclerotic patients was 2.72$\pm$2.23 mg/L. however, the level significantly decreased in Fd-Carrhi group compared to the placebo and statin groups, assumedly due

to phenolic compounds in Fd-Carrhi. Similar findings by Fitó *et al*. [41] stated that IL-6 and C-RP decreased after 28 coronary heart disease (CHD) patients consumed 161 mg/kg BW phenol rich olive oil. Phenolic protective effect against heart disease is derived from the ability to reduce thrombocyte aggregation [42], to improve vasorelaxant [43], to reduce lipid peroxidation [44], and to suppress procoagulant [45]. Ambriz-Pérez *et al*. [32] has traced the potential of grape phenolic that provides protection against atherosclerosis by suppressing CRP expression, the protein compounds that significantly performs in atherosclerotic pathogenesis [36]. Decreasing CRP level also occurred in the plasma of lactating mothers after consuming soy bean milk rich in isoflavone [18].
CRP serves in atherosclerosis pathogenesis because the decreased level of CRP is effective to inhibit atherosclerosis. A number of studies have proven that CRP predict the risk of CVD such as myocard infarction, coronary artery disease (CAD), stroke, peripheral artery disease, even sudden death [46]. CRP is the additional marker for 10-year risk score in predicting future CVD in healthy American women [47]. Based on the findings above, Fd-carrhi brings the atherosclerotic patients to the middle risk of CVD

3.3 Effect of Fd-Carrhi on lipid profile
Total cholesterol was determined using CHOD-PAP method (Cholesterol Oxidase-Peroxidase Aminoantipyrine Phenol). The initial total cholesterol was not different across the groups (P=0.60), but after 2-month of intervention the level decreased in Fd-Carrhi group from 291 to 186.3 mg/dl (P=3.71E-07), and in placebo group from 294.3 to 209.7 mg/dl (P=7.84E-05). Despite significant decrease in total cholesterol in placebo, the level was still more than normal. Cholesterol level in statin group remained (P=0.72) (Figure 3).

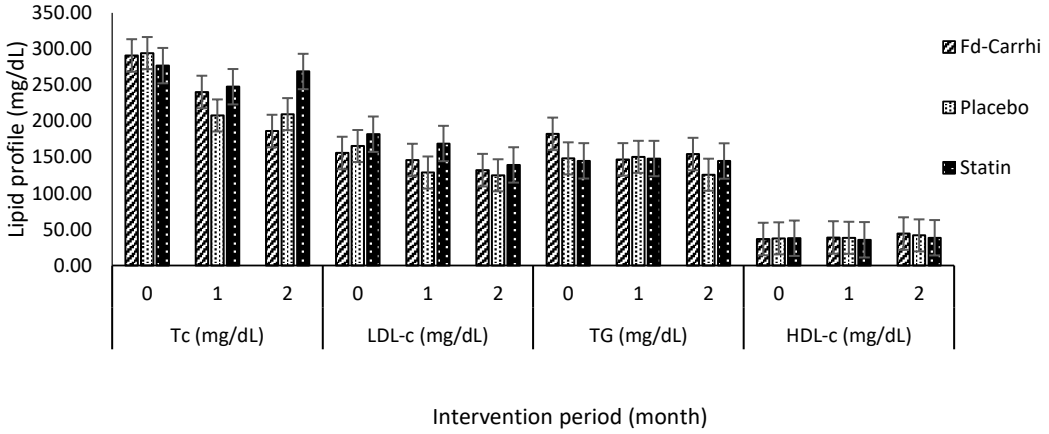

Figure 3. Effect of Fd-Carrhi on lipid profile in blood plasma atherosclerotic women.
Notes: Fd-Carrhi, group consuming statin + functional drink based on cardamom rhizome; Placebo, group consuming statin + placebo; Statin, group consuming statin only; Tc, total cholesterol; LDL-c, low density lipoprotein cholesterol; TG, triglyceride; HDL-c, high density lipoprotein cholesterol; n=30; P<0.05.

Initial triglyceride level (TG) was homogenous across the groups (P=0.29). After 2-month intervention, the level decreased but not significantly in all groups, and still within normal range (30-200 mg/dl). LDL-c level on the baseline was not different across the groups (P=0.23). After 2-month intervention, the level significantly decreased in Fd-Carrhi, placebo and statin group, that is P=0.0009; P=0.029705; and P= 0.006279, respectively (Figure 3).
High total cholesterol (T-c) and LDL-c level is the risk factor of atherosclerosis and the primary cause of CVD, but high level of high density lipoprotein cholesterol (HDL-c) is considered the protector and anti inflammation [48]. Subjects in the present research suffered from hypercholesterolemia (287.4 mg/dl), with high LDL-c (167.76 mg/dl), and low HDL-c (37.37 mg/dl). This condition is prone to CVD. However, in Fd-Carrhi group, the T-c level and LDL-c decreased but HDL-c level increased,

assumedly due to the phenolic compounds in Fd-Carrhi that decreased T-c. This result supported Winarsi *et al*. [10] that flavonoid (one type of phenolic) of cardamom rhizome decreased T-c level of atherosclerotic rats induced with epinephrine. Phenolic compounds belong to flavonoid that can inhibit the activity of HMG-CoA reductase enzyme [49], thereby decreasing cholesterol synthesis and thus cholesterol content. Flavonoids inhibit cholesterol absorption by limiting the formation of micelles. The inhibited cholesterol absorption in intestines is due to insoluble flavonoid-cholesterol complex, then bound with bile acid and excreted in feces. Cholestyramine, sequestrant of bile acid distracts the circulation of enterohepatic bile acid by releasing it and preventing the reabsorption in the intestine. Consequently, bile acid pool is reduced. The more cholesterol converted into bile acid (to maintain steady level in blood circulation), the lower plasma cholesterol level [50]. Cicerale *et al*. [51] stated that in the body of 200 healthy men consuming high phenolic diets, T-c/HDL-c decreased. The increasing HDL-c is noted linear to the increasing phenolic [52]. Olive oil phenolic intake has improved HDL-c circulation around 5.1-6.7% in human [53]. Gimeno *et al*. [54] also reported LDL-c decrease after one week consumption of phenol-enriched olive oil.

Upon FD-Carrhi consumption, HDL-c level increased from 36.69 to 44.26 mg/dl (P=0.014). Similarly, placebo and statin groups experienced HDL-c increased but not significant (P=0.09) and (P=0.71), respectively (Figure 3).

The increasing HDL-c level also occurred in Fd-Carrhi subjects during 2 months which may due to phenolic content. Mechanism of HDL-c level increased by phenolic FD-carrhi was unclear, but Lamon-Fava *et al*. [55] confirms that flavonoid increases the production of apolipoprotein A1 (Apo-A1) and regulation of its expression through signaling pathways of mitogen activated protein kinase. Apolipoprotein-A1 is a compound which contributes to the formation of prebeta-HDL-c, which will then be converted to alpha-HDL-c, then mature through the process of esterification of free cholesterol to cholesterol ester by lecithin-cholesterol acyl transferase enzyme. HDLc is an antiatherogenic, antioxidant, anti inflammatory [56]. Increased concentrations of HDL-c can reduce the progression of atherosclerotic lesions.

In this study there was no change in TG levels in the group taking Fd-Carrhi, placebo, or statins, but the levels were within the normal range (167.76 mg / dl). According to Sudhop *et al*. [57] such levels belong to high normal limit criteria (borderline), therefore it needs to be controlled with Fd-Carrhi.

4. Conclusion

Fd-Carrhi can reduce of IL-6 by 33.85%, C-RP 54.24%, T-c 35.98%, and LDL-c 15.22%, while increasing HDL-c 20.63% and maintaining triglycerides level. Therefore, Fd-Carrhi can inhibit atherosclerosis toward CHD by suppresses inflammation and improves lipid profile.

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