

Hypocholesterolemic and attenuated oxidized-LDL of epinephrine-induced atherosclerosis rats using cardamom rhizome ethanolic extract: Study of functional-food components

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Abstract

This research was aimed to study the flavonoid level of cardamom-rhizome ethanolic-extract (CREE) and effect of the extract on the level of ox-LDL, total-cholesterol (total-c), and HDL-cholesterol (HDL-c) plasma of atherosclerosis *Sprague dawley* rats induced by epinephrine and egg-yolk. Twenty-eight female *Sprague dawley* white rats, aged 2-3 months, weighted 180-250 g, were adapted. Twenty six rats were injected by adrenalin and were fed with egg yolk for three weeks so that their total-c were > 45,5 mg/dl and HDL-c plasma were < 35 mg/dl. The atherosclerosis rats were divided into 4 groups: I, 7 rats were given extract of CREE; II, 7 rats were given simvastatin; III, 7 rats were given CREE+ simvastatin; and IV, 7 rats were given rat feed. In addition, 5 healthy rats were given rat feed. Intervention was conducted for 2 weeks. Blood was sampled 3 times, 1 ml each time. The sampling was done through *plexus Retroorbitalis* at baseline, 1 and 2 weeks post intervention. CREE significantly reduced ox-LDL level from 90.59 to 82.22 pg/ml and total-c from 57.89 to 38 mg/dl. In contrast, CREE increased HDL-c level from 25.66 to 32.9 mg/dl, which is lower than compared to treatment with statin, CREE-statin, or control. CREE was hypocholesterolemic, attenuated LDL-c oxidized, and repressed cardiovascular disease risk. In the future, the cardamom rhizome can be used as functional food component beneficial for health.

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Keywords

Ox-LDL

Cholesterol

Flavonoids

Cardamom rhizome

Atherosclerosis

Functional food

Introduction

Atherosclerosis according to Davies *et al.* (1999) is the hardening and narrowing of the arteries. Libby *et al.* (2011) defines atherosclerosis as an inflammatory process involving soluble mediators, macrophages and smooth muscle cells of blood vessels. Atherosclerosis is a high risk for cardiovascular disease (CVD), and is known to be the number one cause of death in the world (Soler and Ruiz, 2010). CVD mortality rate is quite high in the whole world. As many as 30% of deaths is due to coronary heart disease (CHD), 80% of which occur in low and middle income countries like Indonesia. This figure is expected to increase until 2030 (Wong, 2014). In Indonesia, according to Bahri (2004) higher incidence occurs in women (16.2%) than men (14%); however, Bintanah and Muryati (2010) states that the risk of CHD is equal between women and men.

Various factors such as hypertension, hyperlipidemia, diabetes, high level of LDL, oxidized-LDL (ox-LDL), and oxidative stress cause atherosclerosis (Arici and Walls, 2001). Hyperlipidemia includes high levels of total-

cholesterol (total-c), triglycerides, and LDL-cholesterol (LDL-c), and low HDL-cholesterol (HDL-c) level. Atherosclerosis is closely related to the metabolism of LDL-c, because that high level is important in atherogenesis. This is because LDL-c infiltrate the arterial wall, be accumulated in the artery wall intima, which is known prone to oxidation, thus forming ox-LDL. Increased in ox-LDL is associated with plaque vulnerability (Nishi *et al.*, 2002). Researches in animals and humans proved that ox-LDL level is more important predictor for atherosclerosis and CVD compared to total-c. However, native LDL is also a direct contributor to atherogenesis. Some researchers say that ox-LDL is closely related to CVD (Steinberg and Witztum, 2002; Toshima *et al.*, 2000), and both ox-LDL and native LDL rely on one's antioxidant status (Murr *et al.*, 2009; Mäkimattila *et al.*, 1999).

Oxidative stress plays an important role in the pathogenesis of atherosclerosis, especially in promoting the modification of ox-LDL (Peluso *et al.*, 2012). The ox-LDL contributes to the process of early atherogenesis in rats and humans (Rueckschloss *et al.*, 2003; Cathcart, 2004). Ox-LDL stimulates

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the inflammatory signal, and then stimulates macrophages to accumulate cholesterol and to form foam cells derived from fatty streaks. Such conditions characterizes early atherosclerotic lesions.

Statin drugs are generally used as a clinical treatment of atherosclerosis since it can reduce levels of LDL-c (Ray and Cannon, 2005). However, their long-term use can cause side effects on health such as damage to the liver and muscle toxicity (Maron *et al.*, 2003), myopathy, rhabdomyolysis, and acute renal failure (Kar and Chockalingam, 2013). Nowadays, attention is directed to natural products from plants, such as cardamom.

Cardamom is generally known from the seed that can be used as spices, cosmetics and pharmaceuticals. In vitro rhizome of cardamom has the lowest IC_{50} compared to cardamom leaves and stem, as well as standard ascorbic acid (Winarsi *et al.*, 2012). It is stated that cardamom leaves contain flavonoids around 130 mg/g extract. The flavonoids act as antioxidants (Manach *et al.*, 2005), antiatherogenic, and antiatherosclerotic (Kleemann *et al.*, 2011; Winarsi *et al.*, 2013), hypocholesterolemic (Zou *et al.*, 2005; Singh *et al.*, 2009; Winarsi *et al.*, 2013; Winarsi *et al.*, 2014), and cardioprotective (Stangl *et al.*, 2007; Abbass, 2011). Hypocholesterolemic effect is demonstrated by increased levels of HDL-c, a lipoprotein compound known to be antiatherogenic, thus it is believed able to prevent the development of atherosclerosis.

Flavonoids in cardamom leaves extract is also proven as antidiabetic, induces weight lost and hypocholesterolemic to alloxan-induced diabetic *Sprague Dawley* rats (Winarsi *et al.*, 2014). It is possible that cardamom rhizome ethanolic extract (CREE) contains higher flavonoids since it has lower IC_{50} than cardamom leaves. However, there has been no data that reveals the magnitude of the flavonoid in CREE and its inhibitory effect on the development of atherosclerosis. Therefore, this study aimed to determine the level of flavonoid in CREE and its effect on levels of ox-LDL, total-c, and HDL-c plasma of atherosclerosis rats induced by epinephrine and egg yolks.

Materials and Methods

Cardamom rhizomes were obtained from cardamom farmers in Sumbang village, Sumbang subdistrict, Banyumas Regency, Central Java, Indonesia. Cardamom rhizome extract was obtained by maceration of cardamom rhizome powder using 96% ethanol. Duck egg- yolk was used as cholesterol-rich diet. Rat oxidized low density

lipoprotein ELISA kit of Sunglong Biotech was used for determining the levels of ox-LDL. Total-c and HDL-c levels were determined by the CHOD-PAP method. Sprague Dawley rats were obtained from the Faculty of Animal Husbandry, University of Jenderal Soedirman, Purwokerto, Indonesia. Phapros epinephrine injection was used to induce atherosclerotic rats.

Preparation of cardamom rhizome ethanolic extract (CREE) (Winarsi, 2014 modified)

A number of Cardamom fresh rhizomes were washed, thinly sliced, dried and then grounded into cardamom rhizome flour (CRF). The CRF was then soaked in 96% ethanol for 3x24 hours, then filtered with 41 Whatman paper to obtain filtrate. The ethanol in the filtrate was evaporated using a rotary evaporator and then concentrated. As much as 2.3-2.8% concentrated filtrate was obtained and called cardamom-rhizome ethanolic-extract (CREE).

Determination of flavonoid content of CREE

The total flavonoid contents were measured by a colorimetric assay (Zou *et al.*, 2004; Rohman *et al.*, 2010). A 100.0 μ l aliquot of extracts in ethanol was added to a 10 ml volumetric flask containing 4 ml of distilled water. Initially, 0.3 ml of 5% sodium nitrite was added to the flask. After 5 min, 0.3 ml of 10% aluminium chloride was added and 6 min later, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the mixture was diluted with the addition of 2.4 ml distilled water and thoroughly mixed. Absorbance of the mixture, pink in color, was determined at 510 nm versus a blank containing all reagents except extract sample. Rutin was used as standard for the calibration curve. Total flavonoid content of the extracts and fractions were expressed as mg rutin equivalents (RE) per gram of sample (mg/g).

Preparation of atherosclerosis rats

Preparation of atherosclerosis rats were carried out in the laboratory of Clinical Pharmacology Department of Pharmacy, Faculty of Health Sciences, University of Jenderal Soedirman University, Purwokerto, Indonesia. Rat cages were made of stainless steel strimin wire sized 50 cm x 30 cm x 20 cm, and metally framed. There was no humidity measurement in the laboratory, but there was sunlight coming through a small glass window, and available fan was turned on at all times. During the study the rats were fed with AD II containing 51% carbohydrate, 15% crude protein, 3-7% crude fat, 6% crude fiber, 7% ash, and 12% water.

Twenty eight Sprague Dawley strain female rats, aged 2-3 months with the weight of 180-250 g were adapted for 7 days. They were given rat feed and drinking water ad libitum. Rats were fasted overnight and then injected with epinephrine intravenously at a dose of 0.006 mg/200 g BW (Fadhilah and Prasetyo, 2001). To support the occurrence of atherosclerosis, after injection of adrenalin, the rats were given egg yolks diet as much as 5 g/200g BW every day, for 4 weeks. The egg yolk diet was prepared by separating the yolk from the white egg, then the yolk was gently shaken to form an emulsion (Constantinides, 1994 modified). Rat was defined atherosclerosis if the total-c level was > 45.5 mg / dl and HDL-c plasma level was < 35 mg / dl (Suryohudoyo, 2000; Winarsi et al., 2013a).

Grouping of atherosclerosis rats and the intervention

Atherosclerosis rats were divided into 4 groups: I, 7 rats were given CREE ; II, 7 rats were given simvastatin (statins); III, 7 rats were given CREE plus simvastatin (CREE - statins); and IV, 7 rats were only given the rat feed as controls. In this study 5 additional healthy rats were fed by rat feed. Intervention was performed for 14 days or two weeks. CREE was given 100 mg/kg BW, dissolved in 1 ml of water, while statins was given 0.9 mg / kg BW (Corsini, 2000). The CREE, statin, CREE plus statins, or egg yolk was given using gastric sonde. Rat feed and water were provided ad libitum during the study.

Sampling of rat blood

Rat blood samples were taken three times through *Plexus Retroorbitalis* in the eye. The blood was taken 1 ml at baseline, 1 and 2 weeks after the intervention. Blood was centrifuged at 4,000 rpm for 10 minutes. Plasma obtained was tested for the ox – LDL, total-c and HDL-c levels.

Statistical analysis

Data were analyzed using Analysis of variance (ANOVA). Duncan multiple range test was used to further determine significant differences at the level of 5%.

Results

The flavonoid content of CREE was 324.51 mg/g. The effect of CREE showed significantly reduces ox - LDL level of atherosclerosis rats from 90.59 to 83.68 pg / ml ($P = 0.044$) one week after intervention. One week later, the level even further reduced to 82.22 pg / ml, lower compared to atherosclerotic rats only fed

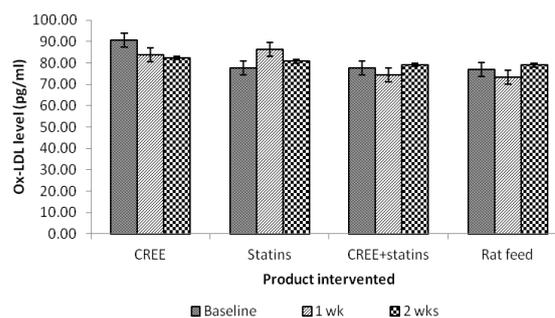


Figure 1. The effect of CREE on ox-LDL levels of atherosclerosis rats

Notes: CREE, atherosclerosis rats were given cardamom rhizome ethanol extract; Statins, atherosclerosis rats were given simvastatin; CREE+Statins, atherosclerosis rats were given cardamom rhizome ethanol extract plus simvastatin; rat feed, atherosclerosis rats were given rat feed; baseline, before intervention; 1 wk, 1 week intervention; 2 wks, 2 weeks intervention; $n = 7$; $P < 0.05$.

by rat feed ($P = 0.65$). Rats given the statin for 1 week showed significantly increase of ox-LDL level ($P = 0.03$), the increase was also observed one week later ($P = 0.343$). Combination of CREE and statins after 1 and 2 weeks did not alter the levels of ox - LDL ($P = 0.26$) and ($P = 0.65$) (Figure 1).

One week after the intervention of CREE, the total-c level of atherosclerosis rats decreased from 57.89 to 38 mg/dl ($P = 0.00018$). One week later, the level was further decreased ($P = 0.00045$). In this study, a significant decrease in total-c level also occurs in atherosclerotic rats that were given statins at the first week ($P = 5,28E -05$), and 1 week later ($P = 0.475$). In this case there were similarities between the cholesterol-lowering effects of statin and combination of CREE - statin. Treatment during the first week can significantly lower the total-c level of atherosclerotic rats, that was from 57.43 to 34.57 mg / dL ($P = 0.0011$). One week later, the level was also declining but not significant ($P = 0.254$). The total-c level of atherosclerotic rats fed only by rat feed did not decrease ($P = 0.325$) (Figure 2). In this case a single CREE, single statins, and combination of both can lower the total-c levels, however, the biggest reduction occurs on CREE treatment. In one week after CREE intervention, HDL-c level of atherosclerotic rat was remained, but one week later, the level was significantly increased from 25.66 to 32.9 mg/dl ($P = 0.038$) compared to statin, combination of CREE - statin, and control (Figure 3).

Discussions

Flavonoids in CREE

Flavonoids level in CREE was determined using the rutin standard method (Rohman et al., 2010).

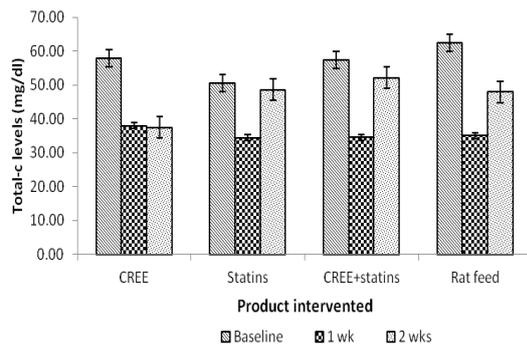


Figure 2. The effect of CREE on Total-cholesterol levels of atherosclerosis rats

Notes: CREE, atherosclerosis rats were given cardamom rhizome ethanol extract; Statins, atherosclerosis rats were given simvastatins; CREE+Statins, atherosclerosis rats were given cardamom rhizome ethanol extract plus simvastatins; rat feed, atherosclerosis rats were given rat feeds; baseline, before intervention; 1 wk, 1 week intervention; 2 wks, 2 weeks intervention; n = 7; P<0.05.

The CREE flavonoid content was almost three times of that in cardamom leaf ethanol extract (CLEE) (Winarsi *et al.*, 2012). This finding was consistent with the CREE IC₅₀ value that was lower than IC₅₀ value of CLEE, meaning that the antioxidant potential of CREE was higher than that of CLEE. Winarsi *et al.* (2013a) reported that the CLEE lowered atherogenic index, as well as reduced oxidative stress, as indicated by the decreased plasma MDA and increased SOD activity (Winarsi *et al.*, 2013b). CLEE was also potential for body weight lost, as antidiabetic and hypocholesterolemic to alloxan-induced diabetic Sprague Dawley rats (Winarsi *et al.*, 2014). Aside from its function as antioxidant, flavonoid, including CREE flavonoids, was also reported potential to protect lipids from oxidation and was cardioprotective.

The effect of CREE on ox-LDL level of atherosclerosis rats

Susceptibility of atherosclerotic rats LDL-c to oxidation was higher than that of healthy rat. Initially atherosclerosis rats had high level of ox-LDL (90.59 pg/ml), whereas healthy rats was 80.75 pg/ml. Some researchers had previously mentioned that the LDL-c molecule is easily modified by glycation (Lopes – Virella *et al.*, 2011), oxidative stress (Vogiatzi *et al.*, 2009), lipid and fatty acid compositions, as well as the size of LDL-c particles (Tribble *et al.*, 2001; Williams *et al.*, 2003). Increased ox-LDL in plasma proves the high level of susceptibility of LDL to oxidation in atherosclerosis patient. The high level of ox-LDL can also be produced from native LDL mediated by the inorganic oxidants such as H₂O₂. According to Sangle *et al.* (2008) ox-LDL promotes thrombosis mediator. In addition, blood vessel cells

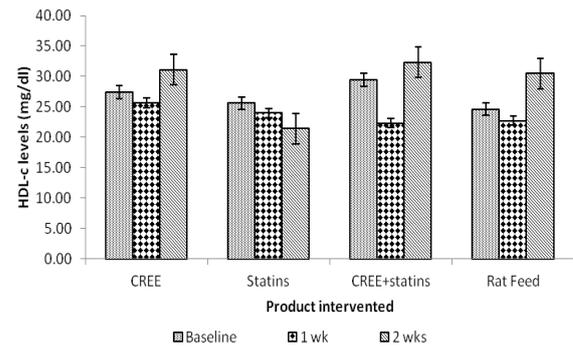


Figure 3. The effect of CREE on HDL-cholesterol levels of atherosclerosis rats

Notes: CREE, atherosclerosis rats were given cardamom rhizome ethanol extract; Statins, atherosclerosis rats were given simvastatins; CREE+Statins, atherosclerosis rats were given cardamom rhizome ethanol extract plus simvastatins; rat feed, atherosclerosis rats were given rat feed; baseline, before intervention; 1 wk, 1 week intervention; 2 wks, 2 weeks intervention; n = 7; P<0.05.

in atherosclerotic lesions undergo apoptosis with high ox-LDL. This was due to the cytotoxic properties of ox-LDL, which continues induce apoptosis. Ox-LDL induce apoptosis of endothelial cells through increased production of malondialdehyde (Yang *et al.*, 2014). Thus, clarifying that high ox-LDL was associated with low antioxidant status in atherosclerosis rat. Therefore, it was appropriate to carry intervention using antioxidant-rich of CREE to atherosclerosis rats. In this study, rats were given CREE 100 mg / kg BW, which was equivalent to 32.45 mg of flavonoids per kg body weight per day. This corresponds to flavonoid doses routinely given to rats by Kamalakkannan and Prince (2006) as much as 25-100 mg / kg BW of rat.

In this case, rats given the statin significantly increase ox-LDL level. While combination of CREE and statins after 1 or 2 weeks did not effect the levels of ox-LDL. That condition reflects the occurrence of ox-LDL turn over in the development of new lesions that not only occur in coronary arteries, but also in systemic arteries thus causing atherosclerosis. It is clear that decreased levels of ox-LDL caused by CREE alone. High level of ox-LDL is the beginning of atherogenesis. Therefore decrease level of ox-LDL by CREE can be an indication that this product can be regarded as antiatherogenic.

According to Winarsi *et al.* (2012) the flavonoid level in CREE was nearly three times that level in cardamom leaf extract. Epidemiological study indicates that high consumption of flavonoid is inversely related to the risk of CVD (Arts and Hollman, 2005). This phenomenon may be related to the flavonoid antioxidant potential in reducing various types of radicals in the environment (Keaney, 2000). Flavonoids are also able to inhibit apoptosis

induced by ox-LDL in endothelial cells through lipid peroxidation scavenger, as well as in the formation of reactive oxygen species (ROS) (Choi *et al.*, 2008).

Flavonoids can promote endogenous antioxidants as radical scavengers with a variety of mechanisms. When flavonoids were oxidized by radicals, the radical compounds become more stable and less reactive, meaning flavonoids stabilize ROS. Certain flavonoids can directly reduce superoxide radicals, while other types of flavonoids can reduce reactive oxygen radicals called peroxynitrite (Choi *et al.*, 2002). The ability of routine flavonoids to act as radical scavengers was associated with flavonoids inhibitory activity against xanthine oxidase enzyme (van Hoorn *et al.*, 2002). Through these mechanisms, flavonoids can inhibit the formation of ox - LDL, which means that flavonoids can act as lipid preventive (Winarsi, 2014).

LDL-c is the main lipoprotein carrying cholesterol in the blood involved in the occurrence of coronary heart disease. Oxidation of LDL-c plays an important role in the pathogenesis of atherosclerosis (Steinberg and Witztum, 2002). In this case, ox - LDL can be captured by macrophages through the scavenger receptor, which subsequently led to the formation of foam cells. The accumulation of foam cells in the subendothelial space of blood vessels is the earliest evidence of atherosclerotic plaque growth known as fatty streak (Manach *et al.*, 2005; Kleemann *et al.*, 2011; Peluso *et al.*, 2012).

This study found the inhibition of atherosclerosis by flavonoids in CREE. It is possible that flavonoids work by enhancing endothelial function or inhibit ox-LDL. Antioxidant flavonoids in CREE were able to prevent the occurrence of atherosclerotic lesions. Flavonoids are included in polyphenol group. In *in vitro* condition, flavonoids in CREE were powerful antioxidants that act as radical scavengers in the form of oxygen radical including hydroxyl. There were several mechanisms by which flavonoids act as antioxidants, among these are by protecting LDL-c from oxidation (Vaya *et al.*, 2003) through its ability to chelate metal (Kessler *et al.*, 2003), inhibit free radicals, as well as to recycle other antioxidants. Likely, CREE flavonoids protect LDL-c from oxidation reaction, so that the lipoprotein molecule was capable of transporting cholesterol optimally from the liver to tissues which was reflected by the decrease in total blood cholesterol level.

The effect of CREE on total cholesterol of atherosclerosis rats

One risk factor for atherosclerosis is hyperlipidemia. Since the rats were defined as

atherosclerosis, the total-c level was 57.10 mg/dl. Hyperlipidemia circumstance can lead to accumulation of total-c and cholesterol ester of lipoproteins containing Apo B - 100 in connective tissue of the coronary arteries walls. Such condition, supported by high free radicals resulting from auto - oxidation of blood lipids, promotes the occurrence of endothelial lesions (Moldovan and Moldovan, 2004). Endothelial lesions stimulate monocytes to migrate into the subendothelial layer of the coronary arteries, and induces the secretion of endothelial growth factor. At this subendothelial layer monocytes differentiate to become macrophages which then phagocyte cholesterol in that layer so that foam cells were formed. Macrophages also will damage the endothelial, thus trigger the emergence of platelet aggregation. Further result of these growth factor secretion and aggregation platelet stimulate endothelial proliferation resulting in thickening of the blood vessel which subsequently closing the lumen and give rise to atherosclerosis. However, one week after the intervention of CREE, the atherosclerosis rat total-c level decreased, similarly one week later, the level further decreased. The decrease was possibly due to the effect of flavonoids CREE. Some of the following mechanisms support reduction in cholesterol levels by flavonoids.

In vitro and *in vivo* studies showed that flavonoids can inhibit the activity of HMG - CoA reductase, thereby suppress cholesterol synthesis which finally lowered total-c levels in the blood (Prahastuti *et al.*, 2011). According to Nekohashi *et al.* (2014) flavonoids reduce total-c levels by inhibiting the absorption of cholesterol, then acts as a cofactor of cholesterol esterase enzyme (Olivera *et al.*, 2007). As inhibitor of cholesterol absorption in the intestine, flavonoid inhibits the formation of micelles so that absorption was inhibited. Flavonoid inhibits cholesterol absorption in the intestine by forming a complex bond (flavonoid - cholesterol) that does not dissolve, and then binds with bile acid to form micelles, and also increased the binding of cholesterol by the fiber.

A significant decrease in cholesterol level also occurs in atherosclerotic rats that were given statins at the first week and the following week. In this case there were similarities between the cholesterol-lowering effects of statin and combination of CREE - statin. Treatment during the first week can significantly lower the total-c level of atherosclerotic rats. One week later, the level was also declining but not significant. But, the total-c level of atherosclerotic rats fed only rat feed did not decrease. In this case a single CREE, single statin, and combination of both

can lower the total-c levels, however, the biggest reduction occurred on CREE treatment.

Flavonoid antioxidant potential was undoubted. Flavonoid compound abundant in CREE lowers the total-c levels through a variety of mechanisms. Flavonoid binds bile acids in the digestive tract, and interferes with enterohepatic circulation, resulting in an increase of steroid excretion in fecal. Decreased level of bile acid in the digestive tract causes increased production of bile acid derived from cholesterol. However, because the enterohepatic circulation was inhibited by flavonoid, the cholesterol absorbed through the gastrointestinal tract was inhibited, and comes out with feces. As a result, there was a decrease of cholesterol synthesis in the liver which then brings about increase of LDL-c receptor, then increases LDL-catabolism, and increases the activity of HMG-CoA reductase. From this it appears that the effects of flavonoids depends on the ability of liver cells to improve the function of the LDL-c receptor for binding circulating LDL-c.

Mechanism of total-c reduction by flavonoid also occurs by increasing the activity of lipase lipoprotein, so that catabolism of triglyceride-rich lipoproteins such as VLDL and IDL increases. HDL-c level increased indirectly due to decreased level of triglyceride, VLDL or due to increased production of apo AI and apo AII. LDL-c lowering effect may be associated with increased VLDL and IDL in the liver, resulting in a decrease in the production of LDL-c (Kwan *et al.*, 2007).

Statin drugs reduce lipid level by blocking the HMG-CoA reductase, an enzyme in the liver that was involved in the early stages of cholesterol synthesis. Statin not only inhibits the synthesis of cholesterol, but also many important intermediate metabolites, including mevalonate pyrophosphate, isopentenyl pyrophosphate, geranyl-geranyl pyrophosphate and farnesyl pyrophosphate. Inhibition of this compound caused statin provides a real effect on blood cholesterol reduction. Studies in humans and animals showed that statin is pleiotropic, for example provides positive effect on the cardiovascular occur independently of the decline in total-c level (Bonetti *et al.*, 2003; Davignon, 2004).

The effect of CREE on HDL-c of atherosclerosis rat

HDL-c is a lipid molecule rich in protein, serves to bring the remnants of free fatty acids and LDL-c from peripheral tissues to the liver, as well as protecting blood vessels from damage caused by ox-LDL. HDL-c performs protection by removing cholesterol that sticks to the walls of blood vessels and inhibit the formation of new plaque (atheroma).

In this study, HDL-c level of atherosclerosis rat was 26.77 mg/dl, lower than that of the normal rat (38 mg/dl) (Winarsi *et al.*, 2016). HDL-c is antioxidant, which can prevent oxidation of LDL-c (Nofer *et al.*, 2002). Inhibition of ox-LDL by HDL-c is usually associated with high antioxidant content in the lipoprotein. Apo-AI antioxidant and some enzymes such as glutathione peroxidase prevent oxidation of LDL-c or lowering its bioactive product. This is proven at the beginning of atherosclerosis wherein the level of ox-LDL was high because many of LDL-c molecules were oxidized. Moreover, HDL-c level should be increased to maintain the integrity of the vascular endothelial, so that the blood vessels become stronger and not easily damaged.

Two week after CREE intervention, HDL-c level of atherosclerotic rat was significantly increased. This proves that the CREE contains bioactive compound that can increase HDL-c synthesis. HDL-c metabolism is rather complicated, but it should be understood that the HDL-c level is influenced by several factors, such as increased production of AI apolipoprotein; enhancement of ATP-binding cassette transporter A1 / G1 activity; or inhibitory activity of cholesterol ester transport protein (Iizuka *et al.*, 2012).

Mechanism of HDL-c level increased by CREE flavonoid was unclear, but Lamon-Fava *et al.* (2004) 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 pre-beta HDL-c, which will then be converted to become alpha-HDL-c, then mature through the process of esterification of free cholesterol to cholesterol ester by lecithin-cholesterol acyl transferase enzyme.

Increased concentrations of HDL - c and decreased of ox - LDL can reduce the progression of atherosclerotic lesions. In this study, rats given a daily of CREE as much as 32.45 mg of flavonoids per kg body weight for 14 days showed increasing HDL-c, and lowering total-c, and ox - LDL plasma levels in atherosclerotic rat. These findings prove that flavonoid-rich of CREE can reduce the risk of CVD in atherosclerosis rats.

A number of epidemiological studies stating that a 1% increase in HDL-c lowers the risk of CVD as much as 1-3% (Birjmohun *et al.*, 2005; Bhatt *et al.*, 2010). In this study, the flavonoids in CREE increase the HDL-c level by 28.2%. When converted, the risk of CVD decreased by 85%. It can be said that atherosclerosis rats epinephrine-induced that were given CREE were less likely to evolve towards CVD.

Conclusion, cardamom rhizome ethanolic extract

containing flavonoids 324.51 mg / g, which lowers levels of total-c, increases HDL-c, and attenuates ox-LDL in atherosclerosis rats epinephrine-induced and egg-yolks. In addition to inhibition of oxidation reactions, the CREE flavonoids improve lipid profile. These findings prove that the CREE can prevent the development of atherosclerosis towards CVD. In the future, the CREE rich in flavonoids can be used as functional food components beneficial for health. Therefore, it is necessary to conduct further research to improve lipid profile and suppress level of ox-LDL in women with atherosclerosis.

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