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RESEARCH ARTICLE

The potential effect of the green coffee extract on reducing atherogenic index in hyperlipidemic rats

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Abstract

Introductions: Dyslipidemia is a risk factor for atherosclerosis and cardiovascular disease. The high prevalence of dyslipidemia triggers the development of green coffee supplement products, which are claimed as cholesterol-lowering and slimming agents. Nonetheless, research data on the effect of taking green coffee supplement products, especially regarding cardiovascular function, is limited. **Aims:** To determine the potential effect of green coffee extract (GCE) on improving atherogenic index of plasma (AIP) and cardiac histopathology in hyperlipidemic rats. **Methods:** 24 rats were induced by high-fat feed for 21 days. Then, the rats were treated with a GCE, dose of 200, 400, and 800 mg/kg bodyweight for 14 days. The next day, blood was collected from the rats to take measurements of their serum lipid profile and calculating their AIP. The heart organ was created by using histopathological preparations. **Results:** Administration of GCE in all doses significantly reduced the AIP and improved cardiac histopathology in the hyperlipidemic rats. **Conclusions:** GCE can be developed as a cardio-protector.

Introduction

Dyslipidemia is a significant fat component disorder, which includes an increase in total cholesterol and Low-Density Lipoprotein (LDL) levels (called hypercholesterolemia), an increase in triglyceride levels (called hypertriglyceridemia), and a decrease in High-Density Lipoprotein (HDL) levels, or a combination thereof (DiPiro *et al*., 2014). This abnormal condition needs special attention because it is a risk factor for atherosclerosis which will lead to cardiovascular disease that comes from narrowing of blood vessels due to fat accumulation (Nelson, 2013). This disease caused by excess fat is a fairly common health problem.

In 2008, about 39% of 25-year-old had increased cholesterol levels globally. This increase in cholesterol was estimated to be the cause of 18% of cerebrovascular disease cases and 56% of ischemic heart disease in the world. The death rate caused by dyslipidemia disease affects 4.4 million people (WHO, 2002). Based on the 2013 Riskesdas Report, for people aged below 15 years old, there was 35.9% abnormal total cholesterol, 22.9% low HDL, 60.3% optimal-borderline high LDL and 15.9% high-very high LDL, abnormal triglycerides with high borderline categories of 13.0% and high-very high categories of 11.9%. East Java is in the top six for obesity cases and second place after DI Yogyakarta with a cardiovascular disease prevalence of 0.2% (Ministry of Health, 2013).

Certain factors can trigger the high prevalence of dyslipidemia to cause serious diseases. The interaction of genetic factors and environmental factors can cause hyperlipidemia (PERKI, 2013). Unhealthy lifestyles prevalent in today's society can also trigger fat accumulation in the blood circulation; these include the penchant for consuming food from fast-food restaurants and other fat-rich foods, lack of activity and exercise, alcohol consumption, obesity and a family history of hyperlipidemia (LIPI, 2009). Generally, this

disease does not cause symptoms in sufferers, but it was found that some patients had symptoms of chest pains, anxiety, sweating, and shortness of breath (DiPiro *et al*., 2014).

Dyslipidemia is one of the risk factors associated with coronary heart disease. Low HDL levels, as well as high levels of triglycerides and LDL, correlate with an increased incidence of coronary heart disease. Several lipid ratio parameters, such as the atherogenic index of plasma (AIP), Castelli's Risk Index, and the atherogenic coefficient, can be used to predict the risk of cardiovascular disease (Bhardwaj *et al*., 2013). Controlling these various lipid ratios, hopefully, can help the prevention of cardiovascular disease events and the management of dyslipidemia therapy.

In addition to the primary therapy with the statin class and lifestyle modification (LIPI, 2009; Walker, & Whittlesea, 2012), recently there are several products in the form of cholesterol-lowering supplements, slimming or weight loss on the market to reduce fat in the body. One of them is green coffee extract supplements. Green coffee is coffee that has not undergone a roasting process like black coffee and is known to have a higher chlorogenic acid content (Farah, 2012). It is also supported by the fact that Jember city is one of the five most significant contributors to Robusta coffee in East Java (Ministry of Agriculture, 2016).

Several studies have shown that the active compounds in coffee have beneficial effects, such as antioxidant (Sato *et al*., 2011), hepatoprotective (Ji *et al*., 2013), and antidiabetic effects (Ong *et al*., 2013). Besides, the active compounds in coffee can prevent the storage of carbohydrates and lipids (Shimoda *et al*., 2006). Chlorogenic acid in green coffee can increase the oxidation of fatty acids (Li *et al*., 2009). Research conducted by Shimoda and colleagues (2006) and Choi and colleagues (2016) proved that green coffee bean extract could cause weight loss in mice. Regarding lipid profile parameters, giving green coffee ethanol extract can significantly reduce LDL levels, increase HDL, and significantly reduce total cholesterol levels in white rats when induced with a high-fat diet (Setyono *et al*., 2014). Other research stated that green coffee extract could lower cholesterol and lower total triglyceride levels (Choi *et al*., 2016). Meanwhile, the latest study showed that administration of green coffee extract for 14 days in hyperlipidemic rats could significantly improve lipid profiles, except HDL levels which remained unchanged. It also enhanced the rat aorta histopathology even though it did not match normal conditions (Christianty *et al*., 2020).

So far, however, there has been no research data on the activity of green coffee extracts related to lipid ratio parameters to predict cardiovascular events. Therefore, this study aimed to determine the potential of green coffee extract in preventing cardiovascular disease based on atherogenic index parameters and histopathological cardiac features of the hyperlipidemic rats.

Materials and methods

Materials

Green bean coffee as a sample was obtained from KSU Buah Ketakasi, Sidomulyo, Silo District, Jember City. The materials used in the study included ethanol, aqua dest, HCl_2N, normal saline, NH_4OH, simvastatin tablet 10 mg (PT. Hexpharm Jaya), CMC Na, used cooking oil, quail egg yolk, BR II feed (PT Japfa Comfeed), formaldehyde, ether, triglyceride and HDL reagents, hematoxylin-eosin staining, enthelan, xylol. The experimental animals used were male Wistar rats, healthy, weight 150-250 g in weight, and around six to eight weeks old.

Methods

Preparation of green coffee extract

The green coffee extract was prepared by the maceration method. Dried green coffee beans were ground to a Simplicia powder, weighed in a certain amount and macerated using 96% ethanol 7.5 times the weight of powder. The resulting filtrate was concentrated using a rotary evaporator and then dried using an oven at a temperature of 50°C until a constant weight was obtained. Furthermore, the green coffee extract suspension was made in CMC Na 1% at a dose of 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight.

In vivo activity assay

The whole procedure for the care and treatment of experimental animals had obtained the approval of the ethics committee of the Jember State Polytechnic with certificate number 615/PL17/LL/2018. A total of 25 rats were acclimatised first for seven days, then administered a high-fat diet, with a composition of quail egg yolk, using cooking oil and 0.01% propylthiouracil (PTU) orally for 21 days, except in the control group. Hyperlipidemic rats were then divided into several groups, namely the green coffee extract treatment group (dose 200 mg/kg, 400 mg/kg and 800 mg/kg body weight), and the positive control group was given simvastatin suspension 0.9 mg/kg BW and CMC Na 1% for negative control. All treatments were given orally for 14 days. On the 15^{th} day, the rats were killed, intracardiac blood was taken for measurement of lipid

profiles, and the heart organs were separated to make HE preparations.

Lipid profile measurements

The lipid profile measured was triglyceride and HDL levels. Triglyceride levels were measured using the enzymatic colourimetric method using glycerol-3-phosphate-oxidase (GPO-PAP). The test was started by mixing 10 µL of serum with 1000 µL of reagent and then incubated at 37°C for five minutes. The 1000 µL reagent mixture and 10 µL standard solution were treated the same, and then the absorbance was measured using a 546 nm wavelength Biolyzer-100TM photometer.

HDL levels were measured by the precipitation method and determined enzymatically. A sample of 200 µL was mixed with 500µL of HDL reagent, left to stand for 10 minutes at 15-25^{o} C, then centrifuged ten minutes at 4000g. The supernatant was removed from the residue within one hour. Next, pipette 100µL supernatant and mix with 1000 µL cholesterol reagent, incubated at 37^{o}C for five minutes. For the control, use 100 µL of aqua bidest with 1000 µL of cholesterol reagent, treated the same as the sample. The absorbance of the sample was measured within 60 minutes using a 546 nm wavelength Biolyzer-100TM photometer.

Atherogenic index determination

The atherogenic index was calculated based on the logarithm of the ratio between triglyceride levels and HDL levels in molar units (mmol/L), with the following formula (Frohlich & Dobiasova, 2003): Atherogenic index of plasma (AIP) = Log (TG/HDL)

Statistical analysis

The lipid profile data and AIP were expressed as means ± standard deviation ($\bar{x}$ ± SD). Between-group comparisons were performed using one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) procedure for multiple range tests. A value of $p < 0.05$ was considered significant.

Results

In this study, the induction of high-fat feed used a combination of quail egg yolk and used cooking oil (7:3) which was administered once a day, and 0.01% PTU suspension in distilled water given ad libitum. The induction was carried out for three weeks, and the results of the increase in lipid profile were obtained.

Based on the results shown in Table I, it is found that the triglyceride levels in the treatment group (both with green coffee extract and simvastatin) are lower than the negative control group. The results of statistical analysis using one-way ANOVA showed that there were significant differences ($p = 0.023$) between groups. The post hoc test with LSD showed that administration with green coffee extract had triglyceride levels that were significantly different from the CMC Na 1% group ($p < 0.05$) but not separate from the simvastatin group ($p > 0.05$). Between groups, the dose of the green coffee extract also did not show a significant difference. It means that treatment with green coffee extract (starting at a dose of 200mg/kg BW) can reduce triglyceride levels in hyperlipidemic rats, and it is equivalent to 0.9mg/kg BW of simvastatin. However, this was not the case for HDL levels, which between groups did not show a significant difference ($p = 0.379$).

The lipid profile (especially triglyceride and HDL level) data was then used to determine the AIP. Table I shows that the treatment with green coffee extracts for 14 days can reduce the AIP. According to one-way ANOVA analysis, as written in Table I, the AIP between groups has a significant difference ($p = 0.007$). The post hoc test with LSD showed that the green coffee extract group at various doses was significantly different from the CMC Na 1% group but not significantly different when compared to the simvastatin control group. Among the three doses of green coffee extract, the atherogenic index value was not significantly different. It means that green coffee extract (starting at a dose of 200 mg/kg BW) can reduce the AIP in hyperlipidemic rats, equivalent ability with 0.9 mg/kg BW simvastatin. However, based on the atherogenic index risk category classification, namely low (< 0.11), moderate (0.11 - 0.21), and high (> 0.21) (Dobiasova *et al.*, 2004), it was found that all groups had a low-risk category.

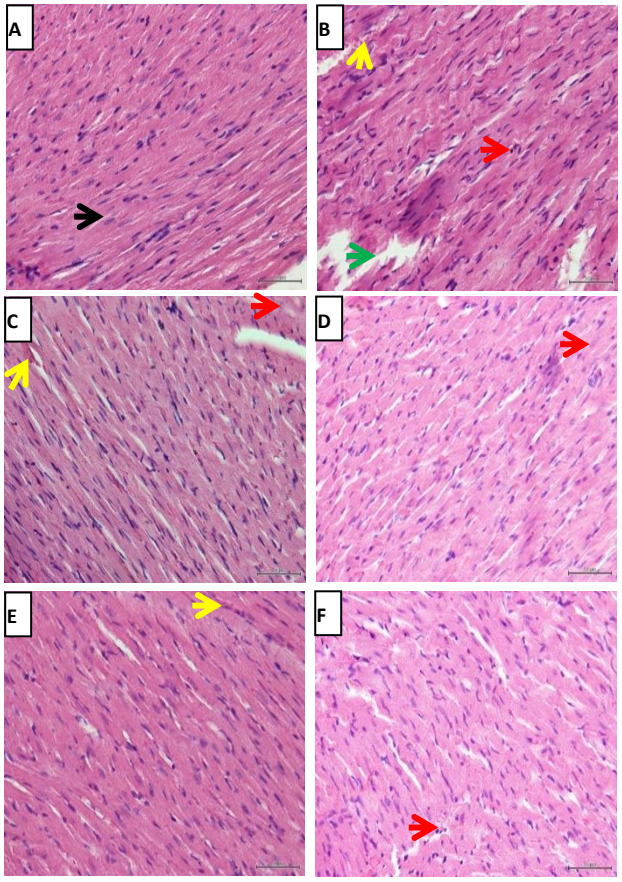
Table I: Profile of triglyceride and HDL level, and atherogenic index of plasma (AIP)

Groups	Mean level (mmol/L) ± SD		AIP	Interpretation (Based on risk category)
	Triglyceride	HDL		
Normal	0.61±0.32^{a}	0.91±0.20	-0.21±0.17^{a}	Low
CMC Na 1%	1.18±0.33^{b}	1.05±0.32	0.10±0.14^{b}	Low
Simvastatin	0.67±0.21^{a}	1.43±0.48	-0.33±0.19^{a}	Low
GCE 200 mg/kg bw	0.79±0.12^{a}	1.04±0.39	-0.15±0.19^{a}	Low
GCE 400 mg/kg bw	0.51±0.13^{a}	1.34±0.22	-0.42±0.06^{a}	Low
GCE 800 mg/kg bw	0.78±0.29^{a}	1.06±0.26	-0.18±0.21^{a}	Low

Different superscript letters indicate that there are significant differences between groups ($p < 0.05$); Anova test followed by LSD; GCE = Green coffee extract

The administration with green coffee extract and simvastatin could improve the cardiac histopathological feature of hyperlipidemic rats on

microscope observation. In normal rats, myocyte cells (longitudinal/elongated muscle fibres) were neatly arranged, parallel, and normal cell nucleus (→) (Fig. 1A). In the high-fat diet-induced rat, there were structural changes/degeneration in myocytes (→), vascular dilatation and congestion (→), and necrosis (→) (Figure 1B, 1C, 1D, 1E, 1F). The necrosis shown included pyknosis (cell nucleus shrinkage), karyorrhexis (destroyed cell nuclei), and karyolysis (cell nuclei disappeared). There was an improvement in the histopathological picture of cardiac myocytes in all treatment groups. Although some cells experienced necrosis and congestion, there was no degeneration of myocytes in the treatment group.



A. Normal rat heart muscle cells (myocytes), B. negative control (CMC Na 1%), C. positive control (simvastatin 0.9 mg/kg BW), D. GCE 200 mg/kg BW, E. GCE 400 mg/kg BW, F. GCE 800 mg/kg BW, → normal myocytes, → myocyte degeneration, → cell necrosis, → vascular dilatation and congestion.

Figure 1: Histopathology of rat heart, a cross-section with hematoxylin-eosin staining, magnification 400x

Discussion

The induction of high-fat feed used a combination of quail egg yolk and used cooking oil (7:3) which was administered once a day, and 0.01% PTU suspension in distilled water given ad libitum for three weeks successfully made hyperlipidemic model in rats. Quail egg yolk has the highest cholesterol content compared to other egg yolks, namely 2139.17 mg/100 g of eggs (Dwiloka, 2003; Aziz *et al.*, 2012). Used cooking oil contains saturated fatty acids and can increase the number of cells experiencing necrosis in rat hearts (Nurfadilah *et al.*, 2013). The addition of PTU is intended to damage the thyroid gland. Hypothyroidism can cause a decrease in the synthesis and expression of LDL receptors in the liver. That makes LDL circulate a lot in the plasma and drives hypercholesterolemia (Kapourchali *et al.*, 2014).

The green coffee extract could reduce the triglyceride level, but not with an HDL level. These results are in line with previous research, where green coffee extract could decrease all lipid profiles in high-fat feed induced rats, except HDL (Christianty *et al.*, 2020). Another study showed that the ethanol extract of green coffee beans at doses of 200 and 400 mg/kg BW could significantly reduce triglyceride levels (Shimoda *et al*., 2006). A slightly different result was found in the study conducted by Setyono and colleagues (2014), where giving green coffee ethanol extract at a dose of 400 mg/kg BW was more efficient in increasing HDL significantly in white rats induced by a high-fat diet. Still, there was no difference in lowering triglycerides (Setyono *et al*., 2014). This difference is influenced by its habitat and the processing of coffee. The difference in altitude where it grows will also affect the levels of active compounds in a plant, as well as the processing.

The increase in triglyceride levels is influenced by the rise in energy intake from the high-fat feed given. It can increase the activity of lipogenesis so that more free fatty acids are formed. The mobilisation of free fatty acids to the liver will also increase, and then it will be esterified with glycerol to form triglycerides. Meanwhile, HDL levels are more influenced by triglyceride metabolism due to lipoprotein lipase activity (Rodwell *et al*., 2018).

The green coffee extract could also lower the index atherogenic. Based on lipid profile, only the triglyceride level was affected due to differences in treatment, whereas HDL was not. Of course, it will affect the AIP, which is the result of the ratio logarithm of triglyceride and HDL levels. The higher the triglyceride level, the greater the atherogenic index value. Otherwise, the higher the HDL level, the smaller the AIP value. The AIP describes the distribution of lipids and lipoproteins in the body, which correlates significantly with the presence of risk factors for atherosclerosis, such as gender, age, dyslipidemia, and diabetes, as well as coronary angiography (Frohlich & Dobiasova, 2003). Also, the atherogenic index can be used as a parameter for routine daily monitoring, especially in patients with

other risk factors for cardiovascular disease (Niroumand *et al*., 2015). Compared to different parameters, the AIP has the most excellent sensitivity (84%) for predicting atherogenicity and the incidence of cardiovascular disease (Khazaál, 2013) and has the largest correlation coefficient compared to other lipid ratio parameters to the incidence of coronary artery disease (Bhardwaj, 2013).

From the results above, all of the groups are at low risk of developing a cardiovascular event. This was potentially due to the introduction of high-fat feed for only 21 days. The low atherogenic index in this study suggests that the risk of developing atherosclerosis and cardiovascular disease is still lacking.

The previous study showed that green coffee was known to improve inflammation and abnormalities in the heart, liver, and diastolic stiffness without increasing glucose sensitivity or lipid profiles in rats with metabolic syndrome (Bhandarkar *et al*., 2019a). Chlorogenic acid, as the main component of green coffee, can improve left ventricular diastolic stiffness by reducing collagen deposition and inflammatory cell infiltration in the left ventricle in high-fat feed rats (Bhandarkar *et al*., 2019b) and reducing interstitial collagen accumulation of the heart in an isoproterenol-induced myocardial infarction model in rats (Akila *et al*., 2017).

The AIP and cardiac histopathological feature improvement were presumed due to the main active compound in green coffee, namely chlorogenic acid. This compound is known to have a primary hypocholesterolemic effect and secondary effects such as atheroscleroprotective, cardioprotective, and hepatoprotective. The mechanism was presumed to increase the use of fatty acids in the liver through upregulation of peroxisome proliferation-activated receptor α (PPAR α) mRNA (Wan *et al*., 2012). Also, chlorogenic acid can activate AMPK (Ong *et al*., 2013), which causes metabolic responses, such as inhibiting fatty acid synthesis (through inhibition of fatty acid synthase) and increasing fatty acid oxidation (by increasing carnitine palmitoyltransferase (CPT) activity), inhibits lipolysis (through inhibition hormone-sensitive lipase (HSL)), and triglyceride synthesis (Meng *et al*., 2013).

Conclusion

This study concluded that green coffee extract has the potential to be developed as a protective agent against cardiovascular function through a series of advanced preclinical and clinical studies.

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