Administration of Robusta Coffee (Coffea canephora) Decreased Erythrocyte Sedimentation Rate on Hyperlipidemia Rats

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ABSTRACT

Background: Hyperlipidemia is one of the factors that can trigger atherosclerosis which is characterized by abnormalities in lipid metabolism in the blood, especially increased Low Density Lipoprotein (LDL) levels above normal. The increase in level of LDL stimulates the production of several cytokines that can affect the increase in acute phase proteins, especially fibrinogen. The presence of fibrinogen functions in increasing the Erytrocyte Sedimentation Rate (ESR) through its positive charge to neutralize the surface of erythrocytes. Prevention efforts to reduce ESR use herbal plants that do not have side effects, namely robusta coffee. Anti-inflammatory and antioxidant activity in robusta coffee is thought to be effective in reducing ESR through inhibition of cytokine production in the inflammatory response.
Objective: This study aimed to determine differences in ESR values after administration of robusta coffee to hyperlipidemia rats.
Methods: Fifteen male Wistar rats were randomly divided into 3 research groups including the control group (K), the hyperlipidemia group (H), and the hyperlipidemia + coffee group (C). Induction of hyperlipidemia by feeding duck egg yolk and pork oil. The calculation of the ESR level uses the Westegreen method in mm/hour.
Results and Conclusions: Robusta coffee can affect the decrease in ESR values in hyperlipidemia rats.
KEYWORDS: Hyperlipidemia, Low Density Lipoprotein, Erytrocyte Sedimentation Rate, Robusta coffee.

INTRODUCTION

Hyperlipidemia is a pathological condition resulting from abnormalities in lipid metabolism, especially increased Low Density Lipoprotein (LDL) cholesterol levels in the blood which has a high prevalence rate as well as one of the highest causes of death in Indonesia. Based on epidemiological data, hyperlipidemia is a major risk factor for atherosclerosis, namely thickening of the lining of the blood vessel walls which can inhibit blood flow and stimulate blood clotting[1]. The prevalence of hyperlipidemia in Indonesia is increasing from year to year. In 2008 it was recorded at 35.1%. Then in 2013 it increased to 35.9%[2]. The higher the prevalence of hyperlipidemia, the higher the incidence of atherosclerosis which has an impact on death[3].

Hyperlipidemia is triggered by diet which is closely related to increased cholesterol due to high cholesterol and saturated fat intake[4]. This is why hyperlipidemia is always characterized by high levels of cholesterol which can cause fat deposition in the walls of blood vessels[1]. High cholesterol in the bloodstream will easily stick to the erythrocyte membrane so that it can change the morphology of the erythrocyte. Previous study revealed that the difference in erythrocytes in patients who have high cholesterol and normal patients is that the erythrocytes of patients who have high cholesterol will change shape to become flatter so that it can cause an increase in the surface area of the erythrocyte membrane[5]. Based on other research, an increase in the area of the erythrocyte membrane will easily cause erythrocyte aggregation[6]. The erythrocyte aggregation will trigger the formation of rouleaux so that it will make the erythrocytes heavier and cause them to fall faster. This will affect the increase in the sedimentation rate of blood that exceeds normal[7].

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Erythrocyte sedimentation rate (ESR) is the rate of settling of erythrocytes in a tube filled with anticoagulant blood within one hour[8]. ESR is a complete blood count in a simple non-specific routine hematological examination, but until now it is still commonly used as an indicator for assessing the activeness of a disease because it has several advantages. The advantages of LED are as a simple laboratory test, not classified as expensive, and can be done manually using the Westegren method which is used as a benchmark for the occurrence of infection in the body and monitoring the response to therapy[9]. ESR is found to be increased during acute inflammatory processes, acute and chronic infections, tissue damage (necrosis), collagen, rheumatoid disease, malignancy, and physiological stress conditions (eg pregnancy). ESR is found to decrease in other pathological conditions such as sickle cell disease, polychytemia, spherocytosis, congestive heart failure, and hypofibrinogenemia[10].

Coffee is a drink that has been consumed since ancient times and has now become one of the world's favorite drinks[11]. There are three species of coffee cultivated in Indonesia, namely arabica coffee (Coffea arabika), robusta coffee (Coffea canephora), and liberika coffee (Coffea liberica)[12]. Robusta coffee has the advantage of being superior to other types of coffee, namely the caffeine content in Robusta coffee beans is two times more than Arabica coffee beans, besides that the chlorogenic acid content in Robusta coffee beans is also more than Arabica coffee and Liberika coffee which is around 7- 14% [13,14]. The bioactive components contained in robusta coffee seeds such as caffeine, phenolic compounds, flavonoids, and chlorogenic acid are known to have functions asanti-inflammatory and antioxidant[15,16] which are thought to inhibit hyperlipidemia. The bioactive components of Robusta coffee willprevents the absorption of cholesterol in the intestine and inhibits the release of glucose into the bloodstream after eating. If this obstacle occurs, the pyruvate formation mechanism in the glycolysis process will also be reduced. The pyruvate oxidation process that converts pyruvate into acetyl-CoA also decreases, thereby reducing the formation of endogenous cholesterol made from acetyl-CoA[17]. Robusta coffee's bioactive compounds can also play a role in reducing blood cholesterol levels by increasing bile acid secretion and reducing blood viscosity so that fat deposition in blood vessels can be prevented [18].

RESEARCH METHODS

This research has fulfilled the eligibility requirements by the Ethics Commission of the Faculty of Dentistry, Gadjah Mada University with letter number 001062/KKEP/FKG_UGM/EC/2022.
Fiveteen experimental animals were divided into 3 groups, the group that was not given any treatment/control group (K). the group fed hyperlipidemic diet (H) and the group fed hyperlipidemic+coffee (C). ESR measurement using the Westegreen method. The principle of this method is that venous blood and anticoagulant are put into a tube and the settling speed of the erythrocytes is recorded in mm/hour.

This study consisted of 3 research groups, namely the control group which was given a standard feed, the hyperlipidemia group which was given a high cholesterol diet, and the coffee group which was fed a high fat diet and added 3.6 ml/day of brewed Robusta coffee. High fat diet is made by mixing duck egg yolk and lard in a ratio of 3:2 which is given as much as 5 ml/day [13]. Brewing coffee is made by dissolving 3 grams of coffee powder in 200 ml of boiling water, then stirring and filtering it [14], then 3.6 ml/day of the rats. After 56 days of treatment, the rats were fasted for 12 hours. 3 ml of rat blood was collected via the infraorbital vein for ESR value using the Westegreen method in mm/hour.

The data obtained was then tested using the normality test with Shapiro Wilk and the homogeneity test with the Levene-test, then analyzed by one way annova test and continued with the Least Significant Difference (LSD) test to find out the differences in each group.

RESULT

The results of the ESR examination in experimental animals showed that the highest value was found in the sample group that was treated with hyperlipidemic feed (group H) with an average value of 2.5 mm/hour, followed by the sample group that was treated with hyperlipidemic feed and robusta coffee (group C) with an average value of 1 mm/hour, and the control group (group K) with an average value of 0.6 mm/hour as the group that has the lowest ESR value (Figure 1).
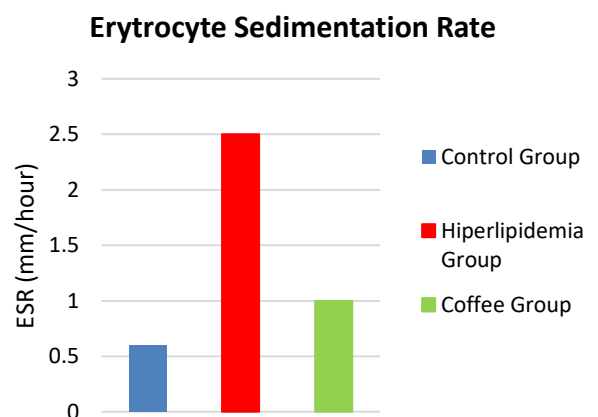

Figure 1. Average ESR level

The results of the normality test that had been carried out showed that all treatment groups were normally distributed (p>0.05). Then a homogeneity test was carried out using the Levene test and a value (p> 0.05) was obtained which indicated that the data was homogeneous. Then the analysis was continued with the One Way ANOVA test to obtain a significant data value (p <0.05). This shows that there are

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differences in all study groups. Furthermore, the data were analyzed with the LSD test to determine differences between each research group. The results of the LSD test showed that there was a significant difference significant (p<0.05) between groups H with C and K, there is no significant difference (p>0.05) between groups K and C group. The results of the LSD test can be seen in Table 2.

Table 2. LSD Test Results

Group		Sig.
Control Group	Hiperlipidemia Group	0,278
	Coffee Group	0,002*
Hiperlipidemia Group	Control Group	0,002*
	Coffee Group	0,002*
Coffee Group	Control Group	0,002*
	Hiperlipidemia Group	0,278

*: significant difference (p<0,05)

DISCUSSION

Hyperlipidemia is a condition in which there is an increase in LDL cholesterol levels in the blood that exceed normal limits, causing HDL which acts as good cholesterol to be unable to transport fat deposits in the body to the liver to be excreted with bile through the feces[19]. Consumption of cholesterol containing excess saturated fat tends to increase the lipid profile in the blood with the risk of accumulation or deposition of cholesterol on the walls of blood vessels[20]. Cholesterol deposition on the walls of the inner blood vessels will further impede the flow of blood and oxygen[21].

Based on the results of the study, the hyperlipid group had the highest ESR valuewhen compared to the control group and the coffee group, while in the control group the lowest ESR value was obtained because it was only given standard normocholesterol feed so that the ESR value obtained is a physiological process of the body[22]. The hyperlipid group that had the highest ESR value was thought to be due to the influence of hyperlipid feeding in the form of lard and duck egg yolk which succeeded in inducing a vascular inflammatory response due to the accumulation of LDL cholesterol that exceeded normal limits. This is in accordance with the theory in other research, that hyperlipid feeds that can significantly increase LDL levels are lard at a dose of 3 grams/200 grams BW/day and duck egg yolk at a dose of 2 grams/200 grams. body weight/day[23]. Egg yolks and lard oil are sources of cholesterol and saturated fat intake which can increase plasma cholesterol levels[24]. According to previous study, lard oil has the highest cholesterol content compared to other animal oils while duck egg yolk has a high enough cholesterol content reaching 35%, so that both are thought to be able to induce an increase in blood LDL which

in turn will trigger inflammation in the vascular wall. through a pathological condition of hyperlipidemia[25]. The presence of hyperlipid feed can result in increased absorption of cholesterol in the intestine. Increasing cholesterol absorption in the intestine will increase LDL cholesterol synthesis in the liver so that LDL cholesterol in rat blood exceeds normal limits[26].

LDL cholesterol levels that exceed normal limits will interfere with endothelial cell function through the formation of oxygen free radicals that deactivate nitric oxide, which is the most important endothelial relaxing factor. Fat chemical changes triggered by free radicals generated in macrophages or endothelial cells in the arterial wall will produce oxidized LDL, which will be engulfed by macrophages through scavenger receptors that are different from LDL receptors in general[4]. In the inflammatory process there is also an increase in monocytes and macrophages adhering to the endothelium through specific endothelial adhesive molecules such as P-Selectin, intercellular adhesive molecules, and vascular cell adhesive molecules Vascular Cell Adhesion Molecules-1 (VCAM-1) that form on the surface of the cells. endothelial cells. Macrophages that are present during the inflammatory process will then secrete cytokines, namely IL-6, which in turn will stimulate hepatocyte cells in the liver to produce acute phase proteins, such as fibrinogen[22]. The increase in acute phase proteins, especially fibrinogen, will result in a decrease in the negative zeta charge potential on the surface of erythrocyte cells, so that the aggregation of erythrocyte cells can easily occur and the process of formation of erythrocyte rouleaux occurs more quickly. The speed of rouleaux formation will make the erythrocytes heavier and cause them to drop faster[7]. The series of processes above are believed to be the cause of the increase in ESR values that exceed normal limits in the hyperlipidemia rat group.

Test results of *One Way Annova* significance value of p=0.003 (p<0.05) was obtained which indicated that there was a significant difference in all sample groups. This is presumably due to the influence of hyperlipidemia diet and administration of robusta coffee which are in accordance with the procedures both in terms of dosage and determination of the length of treatment time. The dose of steeped Robusta coffee is in accordance with research conducted by previous study, which is 3.6 ml/day[27].

In the LSD test results there was no significant difference between the control group and the coffee group with a p=0.278 (p>0, 05). This is thought to be evidence of the existence of robusta coffee bioactive compounds which have a role as both anti-inflammatory and antioxidant in inhibiting the inflammatory response due to cholesterol accumulation that exceeds normal limits, so that the LED conditions that occur in the coffee group have physiological conditions that are close to normal as in the control group rats. The existence of anti-inflammatory activity in Robusta coffee is proven by the theory that the presence of phenolic

compounds in Robusta coffee is known to reduce the production of histamine, bradykinin, and lecotrienes so that in the end it can also reduce the increase in capillary permeability during the inflammatory phase. Flavonoid compounds in robusta coffee also act as anti-inflammatories by reducing protein levels of Tumor Necrosis Factor-α (TNF-α), IL-6, Macrophage Inflammatory Protein 1-α (MIP1-α) and P-selectin[29]. By inhibiting inflammatory mediators, especially IL-6, fibrinogen synthesis in the liver decreases. The decrease in fibrinogen will inhibit the aggregation of erythrocyte cells and the process of rouleaux formation so that it will reduce the ESR value[4]. The existence of antioxidant activity in robusta coffee is proven by the theory which states that robusta coffee phenolic compounds have a protective effect against oxygen free radicals by inhibiting lipid peroxidation. The presence of flavonoid compounds in Robusta coffee can also suppress the LDL oxidation process through the mechanism of free radical scavengers by releasing hydrogen atoms from the hydroxyl groups so that these free radicals become stable and stop oxidizing. The process of inhibiting LDL oxidation will stimulate nitric oxide to vasodilate blood vessels and reduce the level of LDL oxidation, thereby reducing the accumulation of cholesterol in blood vessels[30]. Reducing the accumulation of cholesterol in the blood vessels will reduce the inflammatory effect and prevent increase ESR values.

CONCLUSIONS
Based on the research, it can be concluded that Robusta coffee decreased erythrocyte sedimentation rate on hiperlipidemia rats.

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