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The effects of Robusta coffee on blood fibrinogen level rat induced high fat diet

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Abstract
Hyperlipidemia is one of the risk factors of stroke and atherosclerosis. Hyperlipidemia is a condition of increasing fat levels in the blood, especially for Low density Lipoprotein (LDL). Increased LDL can cause the increasing of oxidation level in the blood, from LDL to become oxidative LDL which is one of the risk factors for chronic inflammation. Fibrinogen levels is one of major inflammatory marker. Fibrinogen levels in blood plasma increase if there was inflammation, and causes high blood viscosity. It is a risk factor for ischemic stroke, hemorrhagic stroke or atherosclerosis. Prevention efforts and treatment to reduce hyperlipidemia can be done by using herbal plants, one of which is robusta coffee. Robusta coffee has anti-inflammatory and antioxidant properties that are thought to be effective in reducing inflammation as indicated by reduced levels of fibrinogen. This study aimed to analyze the potential of Robusta coffee as natural anti-inflammatory and antioxidant, by analyzing the level of fibrinogen in rat induced high fat diet. Fiveteen male wistar rats were randomly divided into 3 groups including control (K), high-fat diet (H), and high-fat diet + robusta coffee (C). High-fat diet induced by feeding the yolks of duck and pork oil. The measurement of fibrinogen levels is done by calculating the differences in plasma protein levels in incubated blood (Heat Precipitating Method). Robusta coffee was reduced the fibrinogen levels in rat induced high-fat diet.

Keywords: Hyperlipidemia, LDL, fibrinogen levels, Robusta coffee

Introduction

Modern diets that contain lots of cholesterol can cause hyperlipidemia [1]. Hyperlipidemia is a pathological condition caused by abnormalities in blood lipid metabolism characterized by increased levels of total cholesterol, triglycerides, Low Density Lipoprotein (LDL) and decreased levels of High Density Lipoprotein (HDL) [2].

Hyper-LDL conditions can trigger oxidative stress due to an imbalance of pro-oxidants and antioxidants in the body. This situation can trigger a tissue inflammatory response in the form of fat or adipose cells experiencing excessive lipogenesis which will trigger the formation of reactive oxygen species (ROS) [3]. Chronic hyperlipidemia can cause an increase in vascular permeability because endothelial dysfunction causes LDL to enter the blood vessel walls more easily. It is associated with a chronic low-grade inflammatory condition with progressive infiltration of immune cells in excess obese adipose tissue [4]. The results of Prasetya's research (2016) revealed that hyper-LDL conditions can increase the formation of ROS (in *vitro*) [5].

With the release of free radicals, LDL will be oxidized and digested by macrophages to form foam cells which become precursors to the inflammatory process that occurs stimulates the production of several cytokines that can affect the regulation of the increase and decrease in acute phase proteins. One of these acute phase proteins is fibrinogen which is produced by hepatocyte cells. Fibrinogen functions to increase blood viscosity, platelet aggregation, erythrocyte sedimentation rate (ESR), and leukocyte adhesion [6]. Fibrinogen levels are one of the biomarkers of chronic inflammation. Increased levels of fibrinogen in the blood is a sign that the body is experiencing a chronic inflammation [7].

There are many community efforts to use herbal medicines as an alternative effort to overcome and control dyslipidemia, for example, coffee (Coffea Sp) [8]. Coffee drinks are the biggest source of antioxidants. In a reported in *vitro* study [8] Hodgson (2008) said that coffee antioxidants can reduce cholesterol concentrations in the blood. There are three species of coffee that are quite often cultivated, namely arabica coffee (Coffea arabika), robusta coffee (Coffea canephora), and liberika coffee (Coffea liberica) [9]. Coffee is a natural ingredient that has anti-inflammatory and antioxidant properties. This can be related to the content of caffeine, chlorogenic acid, ferulic acid and caffeic acid in coffee [10]. Several in *vitro* studies tested the content of coffee compounds, caffeine content, Ferulic acid and caffeic acid can act as anti-inflammatories because they can reduce the secretion of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 so that the inflammatory process is inhibited, while chlorogenic acid acts as an antioxidant because it inhibits the production of reactive oxygen species (ROS) through a reaction process. oxidation [11]. The caffeine content in Robusta coffee beans (Coffea canephora) is twice as high as Arabica coffee beans, and the chlorogenic acid content in Robusta coffee beans (Coffea canephora) is more than in other medicinal plants. Robusta coffee beans (Coffea canephora) contain active compounds, namely polyphenols and alkaloids. Polyphenol compounds (chlorogenic acid) in robusta coffee beans (Coffea canephora) have anti-inflammatory and antioxidant effects,

This study aimed to determine the effect of brewing robusta coffee (C. canephora) on fibrinogen levels in Wistar rats with hyperlipidemia.

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. 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, on the 57th day an examination of Low Density Lipoprotein (LDL) levels was carried out in experimental animals. The experimental animals were euthanized to take 3 ml of intracardiac blood. The blood that has been taken is calculated for fibrinogen levels using the heat precipitation method.The data obtained was tested for normality using the Shapiro Wilk test and homogeneity test using the Levene Test. After the normality and homogeneity tests were carried out, it was continued with the One-Way ANOVA test, which was then followed by the Least Significant Different (LSD) test to find out whether there were differences between the study groups.

Result and Discussion

The results of the study regarding LDL levels in wistar rats in the hyperlipidemic group (group H) which were fed a high cholesterol diet had the highest LDL levels reaching 40.4 mg/dL. The results of examining LDL levels in the coffee group (Group C) had an LDL value of 32.52 mg/dL and the control group (Group K) had the lowest LDL value of 31.65 mg/dL. (Low Density Lipoproteins) above 37.99 mg/dL.

Results level of fibrinogen is shown in Figure 1

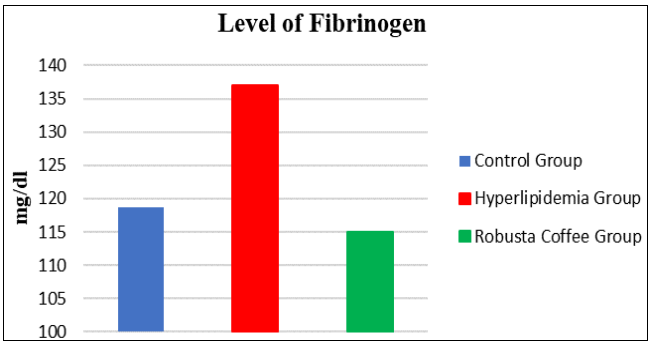

Fig 1: Histogram of the mean fibrinogen levels in the control group, the hyperlipidemic group, and the coffee group in units (mg/dl).

The research data obtained in each research group were then analyzed statistically. Data was tested for normality with the Shapiro-Wilk test and homogeneity test with the Levene test. The results of the normality test that has been carried out show that all treatment groups are normally distributed ($p > 0.05$) and the results of the homogeneity test show that all data is homogeneous ($p > 0.05$). The analysis continued with the One Way ANOVA test, obtained a significant data value ($p < 0.05$), then continued with the LSD test which showed a value ($p < 0.05$) which meant that there were significant differences between the study groups.

Based on data analysis of fibrinogen levels in the coffee group, there was a significant difference compared to the hyperlipidemia group. Robusta coffee brew is known to contain active ingredients that can act as anti-inflammatories and antioxidants, such as caffeic acid, caffeine, chlorogenic acid, and flavonoids [15].

Production of fibrinogen in the liver is stimulated by IL-6 which is a pro-inflammatory cytokine, the presence of venom can increase the activation of macrophages and endothelium to secrete Interleukin-6 (IL-6) Caffeic acid in robusta coffee can reduce mRNA and protein levels of Tumor Necrosis Factor-α (TNF-α) and IL-6 [16]. The content of chlorogenic acid in coffee can also reduce the production of pro-inflammatory mediators including TNF-α, IL-1β, IL-6, and INF-γ in macrophage cells [17]. In previous studies it was found that administration of caffeine showed inhibition of TNF-α production [18]. When the production of pro-inflammatory mediators is inhibited especially IL-6 which plays a role in the synthesis of fibrinogen in the liver, so blood fibrinogen levels also decrease. This is consistent with the results of the study which showed that the coffee group had lower fibrinogen levels compared to the hyperlipidemia group, the fibrinogen levels of the coffee group were almost the same/close to the control group.

The results showed that the fibrinogen level in the coffee group was lower than that in the hyperlipid group. Chlorogenic acid in robusta coffee has the ability to capture free radicals and release single oxygen, this can prevent LDL oxidation and oxidative damage to nucleic acids (nucleic acids) [19]. Previous studies have shown that caffeine can protect endothelial cells from free radical production by increasing the expression of Nitric Oxide (NO) [20]. The content of saponins is proven to inhibit the process of lipid oxidation by preventing the formation of Reactive Oxygen Species (ROS) which affects the formation of foam cells [21]. Flavonoids can also inhibit LDL oxidation by suppressing/reducing the formation of ROS by inhibiting enzymes or binding to the remaining elements of free radical production [22]. As a result of this antioxidant effect, the formation of oxidized LDL (LDL-ox) is reduced as well as the formation of foam cells derived from macrophages that phagocytize LDL-ox through scavenger receptors. By reducing LDL-ox levels and increasing anti-inflammatory compounds, it can reduce the inflammatory response that occurs

Feeding high cholesterol has been shown to increase LDL levels in the blood. High cholesterol feed in the form of duck egg yolk and lard is a source of cholesterol and saturated fat intake which can increase plasma cholesterol levels [23]. Increasing cholesterol absorption in the intestine will increase LDL cholesterol synthesis in the liver so that LDL cholesterol in rat blood exceeds normal limits [24]. LDL plays a role in the process of cholesterol accumulation in macrophages, smooth muscle cells, and extra cellular matrix in the blood vessels, this causes an increase in LDL to be atherogenic because it affects the function of HDL to transport cholesterol from the body to be stored in the liver [25].

The results of calculating fibrinogen levels in the hyperlipid group were greater than the coffee group and the control group. An increase in high levels of Low-Density Lipoprotein (LDL) will increase the production of reactive oxygen species (ROS/Reactive Oxygen Species) from the endothelium [26]. The function of the endothelium is affected by ROS which can inactivate Nitric Oxide (NO degradation) causing endothelial dysfunction and triggering the expression of vascular cell adhesion molecule-1 (VCAM-1). Cells that have VCAM-1 receptors, namely monocytes, will easily attach to the endothelium. Monocyte cells attached to the endothelium will then migrate through the intercellular

gap. Monocyte cells that penetrate the intima layer turn into macrophages. Macrophages phagocytize LDL-ox via several scavenger receptors. Macrophage cells that continuously phagocytize LDL-ox will eventually turn into foam cells in which there is accumulation of cellular lipids. If macrophages can no longer phagocytize LDL-ox then macrophages will undergo apoptosis and necrosis causing extracellular lipids [27].

By inhibiting the expression of inflammatory mediators, especially IL-6 which plays a role in the synthesis of fibrinogen in the liver, blood fibrinogen levels also decrease. In addition, coffee contains chlorogenic acid which is a polyphenol derivative which has antioxidant compounds that can also ward off free radicals and destroy molecules that can damage DNA [28].

Conclusions

Based on the results of this research, it can be concluded that robusta coffee (Coffea canephora) can reduce fibrinogen levels in rat induced high fat diet.

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