Robusta Coffee (*Coffeacanephora*) Down Regulation TNF-α Expression in Carotid Artery Endothelial Cell of Hyperlipidemia Rat Model

Nadie Fatimatuzzahro[*], Rendra Chriestedy Prasetya and Kanwangwang Dwi Nada Anggara

Departmentof Biomedic, Faculty of Dentistry, Universitas Jember, Jember 68121, Indonesia

([*]**Corresponding author's e-mail: nadie.fkg@unej.ac.id**)

Abstract

Hyperlipidemia is a cause of atherosclerosis, that characterized by an abnormal elevation of cholesterol, especially Low Density Lipoprotein (LDL). It leads to foam cell formation, andcorrelated with the increased level of pro-inflammatory cytokine TNF-α.Inflammatory is assumed to be inhibited by natural ingredients which have antiinflammatory effect. One of the natural product which predicted to have antiinflammatory effect is *robusta coffee*. This research aimedto investigate the effect of robustacoffee on TNF-α expression in hyperlidemiarat model. Fiveteenmale Wistar rats were randomly divided into 3 groups: Control (K), Hyperlipidemia (H), and coffeerobusta(C). Hyperlipidemia induced by feeding the yolks of duck and pork oil. Infraorbital blood was taken for examination of LDL levels andthe rats were sacrified at 29 day after the treatment. Carotid arteries were then processed histologically. Anova and LSD test results showed levels of LDL in the coffee group were significantly different from the hyperlipidemia group ($p < 0.05$).Immunohistochemical (IHC) analysis was performed using rabbit anti rat TNF-α polyclonal antibody. Lipid deposition in carotid artery observation within Sudan IV staining.Resultshowed *robustacoffee* induced lower expression of TNF-α and less lipid deposition in subendotelial layer. StrongTNF-αpositive staining seems on hyperlipidemia group. *Robusta coffee*down regulationTNF-α expressionin carotid artery ofhyperlipidemia rat model.

Keywords: *Robusta coffee* (*Coffeacanephora*), Low Density Lipoprotein (LDL), TNF-α, Hyperlipidemia rat model

Introduction

Hyperlipidemia is one of the atherosclerosis causes, which is characterized by the increasing of Low Density Lipoprotein (LDL) cholesterol. LDL has the highest cholesterol content compared to others [1]. Hyperlipidemia causes the thickening of lining blood vessel wall which consequently inhibits the blood flow, reduces the elasticity of blood vessels and stimulates blood clotting. Several previous studies have proved the relationship between high serum lipid levels and the incidence of atherosclerosis [2].

The first step in the formation of atherosclerosis begins with the lesions in endothelial cells that covers the artery lumen due to accumulation of lipids in the blood vessels. This will trigger an inflammatory and immune reaction, resulting in the release of vasoactive peptides [2]. Monocytes attach to endothelial cells and enter the tunica intima. They transform into macrophages which will express the receptors to phagocyte Low Density Lipoprotein (LDL). The oxidized-LDL through LDL scavanger receptors causes macrophages to be full of fat called foam cells. This contributes to the inflammatory response which is characterized by an increase in proinflammatory cytokines, one of them is TNF-α. TNF-α induce expression of adhesion molecules and recruitment of monocytes in atherosclerosis formation [3].

Antioxidants and anti-inflammation, however, can be utilized for prevention and treatment of atherosclerosis.One source of antioxidants and anti-inflammatory that is easily obtained with minimal side effects comes from herbal plants, is coffee [4]. Jemberis a region of coffee plantations and the most widely produced is *Robusta coffee*. In addition, *Robusta coffee* has the highest antioxidant content compared to other types of coffee [5].

The content of polyphenols, especially chlorogenic acid and caffeine in *Robusta coffee* which functions as an antioxidant can reduce toxic free radicals and increase immunity against disease in the

body [5]. At a certain dose setting, chlorogenic acid has been shown to reduce the risk of high blood cholesterol levels [6]. Chlorogenic acid has anti-hypertensive activity by reducing the occurrence of oxidative stress, which has an effect on blood pressure reduction through the increased endothelial function and bioavailability of nitric oxide in artery blood vessels [7]. Antioxidants which is contained in *Robusta coffee* can help the body prevent the damaging effects of free radicals and inhibit the formation of foam cells [8]. Our preliminary study showed that robustacoffee reduced LDL blood level [9].

Materials and methods

Theexperimental procedure has obtained the ethical clearance at Health Research Ethics Committe, Faculty of Dentistry, Gadjah Mada University, Indonesia. The sample consisted of 15 wistar rats,divided to 3 research groups (5 ratseach group) K: the group that given standard feed (control). H: Group that given a high-fat diet (Hyperlipidemia rat model) and C: A group that given a high-fat diet + brewed *robustacoffee*. Hyperlipidemia was induced by high-fat diet with a mixture of 2 grams of duck egg yolks and 3 g of pork oil [10]. Brewed coffee was prepared by dissolving 10 g of *robusta coffee* powder in 200 mL of boiling water [11].

The treatment procedure was carried out for 28 days. On the 29th days, rats were maintained 12 h before blood examination for LDL levels, then wereperformed euthanasia,and carotid rats were taken for histological preparations. Immunohistochemical staining was performed to show TNF-α expression in carotid artery endothelial cells. Observation of lipid deposition in carotid artery withinSudan IV staining.

Results and discussion

The lowest LDL level was 23.70 ± 1.5 mg/dL obtained in the control group and the highest was 43.25 ± 3.2 mg/dL found in the hyperlipidemia group. Based on the One-Way ANOVA test there were significant differences in LDL cholesterol levels between the control group and the treatment group ($p = 0.01$). The results of the Post Hoc LSD test showed that the control and coffee were significantly different from the hyperlipidemia group, but there were no differences between the control and coffee group. It is proven that the group that was given brewed coffee had the effect of lowering LDL levels.This is consistent with cohort studies which proves that coffee consumption was not associated with plasma lipid changes, so the adverse effect of consumption appears unrelated to lipid profile [12].

The results showed lipid deposition in the coffee group was less than the hyperlipidemia group (**Figure1**). Chlorogenic acid in *robusta coffee* has the ability to scavenge free radicals and release single oxygen, so that it can prevent LDL oxidation and oxidative damage to nucleic acids [13]. Previous studies have shown that caffeine can protect endothelial cells from free radical production by increasing the expression of Nitric Oxide (NO) [14]. Saponin has been shown to inhibit the process of lipid oxidation by preventing the formation of reactive oxygen species (ROS) which affects the formation of foam cells [15]. Flavonoids can also inhibit LDL oxidation by suppressing/reducing the formation of ROS by inhibiting enzymes or binding elements left over from free radical production [16]. As a result of these antioxidant effects, the formation of oxidized LDL (LDL-ox) is reduced as well as the formation of foam cells originating from macrophages that phagocyte LDL-ox through the scavenger receptors. Reducing foam cells, can reduce lipid deposition formation.
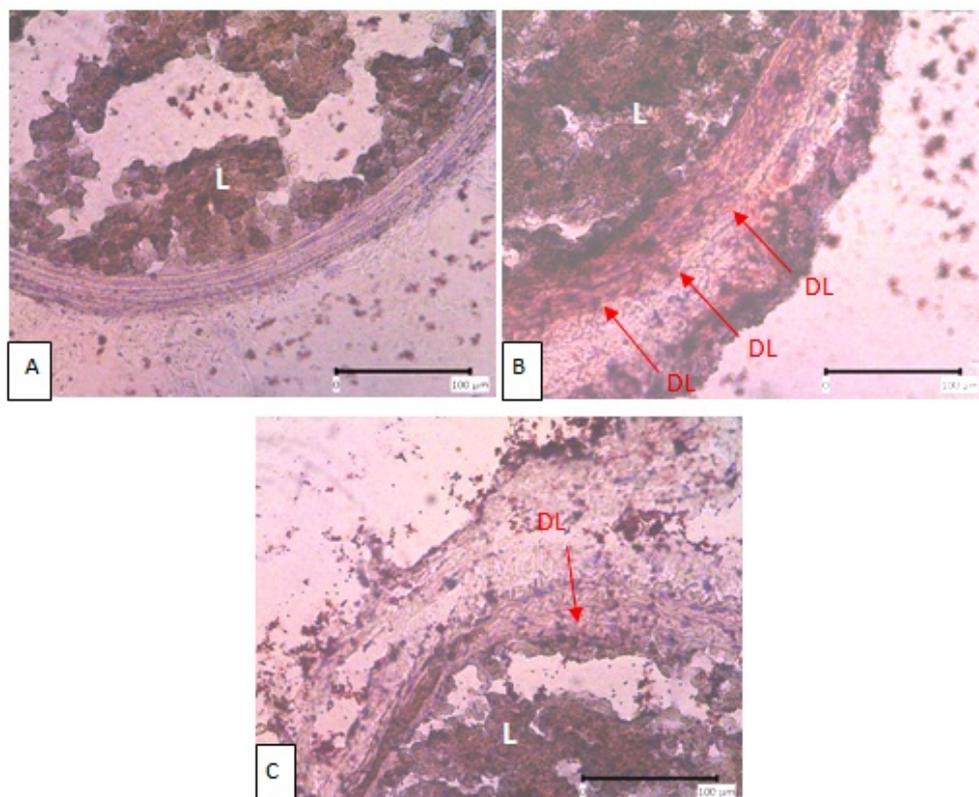
Figure 1 Lipid deposition in carotid artery within Sudan IV staining (400×). B) Hyperlipidemia group has the most deposition lipid (DL) in subendotelial layer compared to other groups. A) Control group. C) Coffee Group. L: Lumen.

TNF-α expressing cell appears to give a brown color in the cytoplasm endothelial cells, whereas those do not express TNF-α will give a blue color. The picture of TNF-α expression can be seen in **Figure 2**. Immunohistochemical examination showed brown staining with varying intensity which indicates the expression of TNF-α. More stronger intensity of the brown color, more stronger expression of TNF-α.The result showed that brewed coffee induced lower TNF-α expression than hyperlipidemia group. This is due to the benefit of *robusta coffee* which acts as an anti-inflammatory. Caffeine and polyphenols are to reduce the effects of histamine, bradykinin and leukotriene which can reduce capillary permeability during the inflammatory phase [7]. Polyphenols have also been shown to prevent increased production of inflammatory cytokines (IL-1, IL-6, IL-8 and TNF-α) by macrophage and lymphocyte that activated by free radicals [6]. Another research stated that caffeine and polyphenols were useful as anti-inflammatory and anticancer agents that become potential as natural ingredients for fortification of food products that are beneficial to the body as a source of antioxidants [4,17].
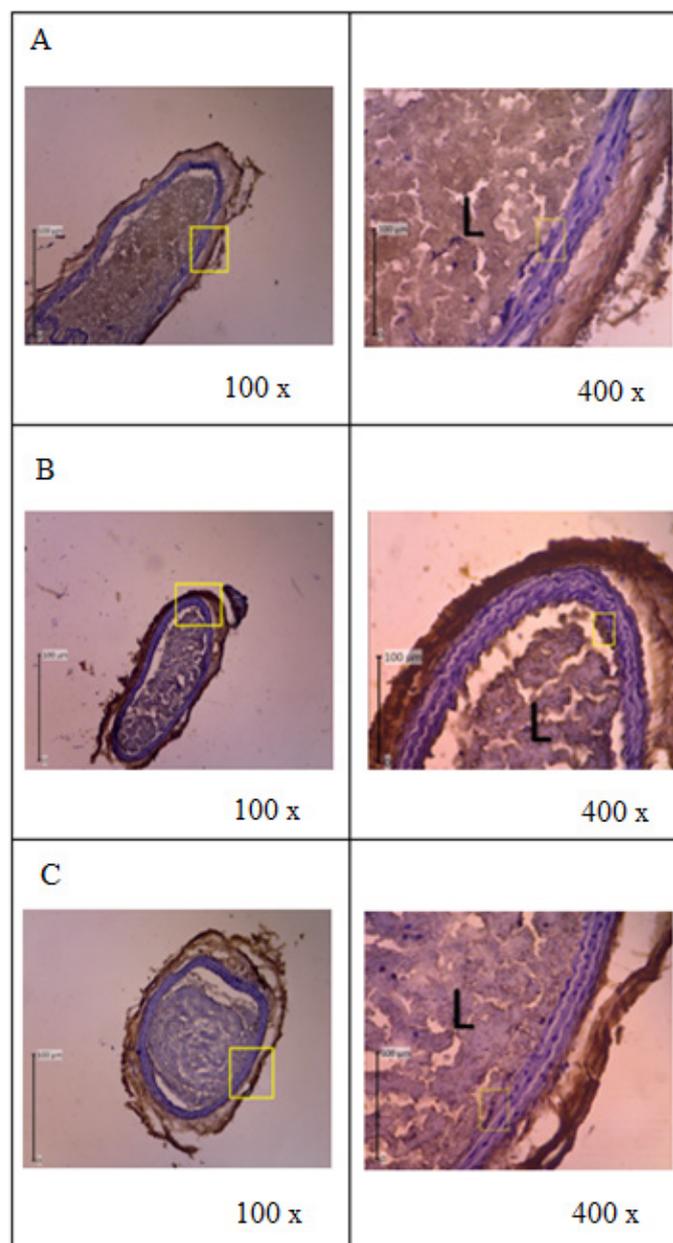
Figure 2 Carotid artery histology with IHC staining (400×). There is differential intensity of the brown stainning on endothelial tunica intima shown in the yellow area of each group. B) Hyperlipidemia groups have the strongest intensity compared to other groups. A) Control group. C) Coffee group. L: Lumen.

TNF-α expression in coffee group was lower than the hyperlipidemia group is also thought to be influenced by the content of chlorogenic acid in *robusta coffee*. Chlorogenic acid is meant to be as an antioxidant that can neutralize Reactive Oxygen Species (ROS) produced by inflammatory cells. ROS is a very reactive free radical or oxidant. The increasing of ROS production can be the result of oxidative stress, apoptosis or cell necrosis. Chlorogenic acid in *robusta coffee* can inhibit LDL oxidation which is considered the main mechanism for endothelial dysfunction [18]. Another research which states that chlorogenic acid can reduce LDL levels by inhibiting lipolysis of triglycerides in adipose tissue thereby reducing free fatty acid transport to the liver, so that LDL levels in the blood decrease. It will inhibit NF-kB activation, resulting in decreased levels of TNF-α in circulation. NF-kB is a cell transcription factor that can control the expression of several genes including TNF-α [19].

Based on the result, the hyperlipidemia group has the strongest TNF-α expression. It was because the administration dose of 3 gr/ 200grBB/day pork oil and egg yolks 2 gr/ 200 grBB/day can significantly

increase LDL [10]. High fat diet can affect the inflammatory process in rat arteries which is characterized by lipid accumulation in the tunica. It will trigger an inflammatory reaction resulting in the release of vasoactive peptides, accumulation of macrophage and platelet outside and inside the arteries. Monocyte attached to endothelial cell, then enters the tunica intima and turns into macrophage. Macrophage expresses LDL receptors and performs phagocytosistheoxidized-LDL through LDL scavanger receptor so that macrophage is full of fat. This is called a foam cell. The formation of these foam cells will increase proinflammatory cytokines, one of them is TNF-α [3].

Conclusions

Robusta coffee down regulation TNF-α expression in carotid artery endothelial cells and reduce lipid deposition in subendotelial layer in hyperlipidemia rat model. The results of this study can provide information that *robusta coffee* is one source of anti-oxidant and anti-inflammatory leading to increase the body's immunity against disease.

Acknowledgements

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