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The Effect of Robusta Coffee (*Coffea Canephora*) on Expression Nuclear Factor-Kappa B (NF-kB) in Coroner Artery of Hyperlipidemia Rat

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Abstract

Background: The condition of hyperlipidemia can lead to the occurrence of oxidative stress which is characterized by the increasing number of Reactive Oxigen Species (ROS). ROS will activate the Nuclear Factor-Kappa B (NF-κB) signaling pathway. Oxidative stress and inflammatory conditions can be inhibited by natural ingredients that contain antioxidants and anti-inflammatory. Antioxidant of caffeine can reduce and protect cells from free radicals such as ROS, then the progression of atherosclerosis can be inhibited.

Objective: To determine the effect of Robusta coffee (Coffeacanephora) on NF-κB expression in male wistar coronary arteries induced by high-fat diet.

Methods: Fifteen male wistar rats were divided into three groups: control group, hyperlipidemia group, and coffee treatment group. High fat diet for the hyperlipidemia group is made by mixing yolks of duck and pork oil. Infraorbital blood was taken for examination of LDL levels and rats were sacrificed at the 29th day, heart organs then carried out for tissue processing histologically. Observation and measurement of NF-κB expression performed by immunohistochemically.

Results: The results showed that the coffee treatment group expressed NF-κB in coronary artery endothelial cells was less than the hyperlipidemia group ($p < 0.05$). LDL level in the coffee group were significantly lower than the hyperlipidemia group ($p < 0.05$). Coffee ingredients has anti-inflammatory and antioxidant properties which can decrease in NF-κB expression.

Conclusion: Robusta coffee can reduce NF-κB expression in coronary artery endothelial cells hyperlipidemia rats.

Keywords: Robusta Coffee; Hyperlipidemia; Ldl; Coronary Artery; Ros; Nf-Kb

Abbreviations

ROS: Reactive Oxigen Species; HDL: High Density Lipoprotein; VCAM: Vascular Cell Adhesion Molecule; ICAM: Inter Cellular Adhesion Molecule; NIK: NF-κB-inducing Kinase; HR: Hydroxyl Radicals.

Introduction

Hyperlipidemia is one of the factors causing cardiovascular disease which is the main cause of mortality in developing countries [1,2]. Hyperlipidemia is abnormality of lipid levels in the blood which is characterized by increased levels of LDL (Low Denity Lipoprotein) and triglycerides, and decreased levels of HDL (High Density Lipoprotein) [3]. Unhealthy lifestyles, such as consumption of a high-fat diet can lead to hyperlipidemia. High levels of fat can be found in animal fat and egg yolks [4].

The condition of hyperlipidemia can reduce oxidative stress which is characterized by an increased number of Reactive Oxigen Species (ROS) [5-7]. ROS will activate the

Nuclear Factor-Kappa B (NF-κB) signaling pathway [8]. NF-κB is a transcription factor that regulates the activation of a number of genes that play an important role in immunity and inflammation. The role of NF- κB is very important in the development of chronic inflammatory diseases [9]. NF-κB is activated by target genes that regulate cell proliferation, release of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), and activated adhesion of molecules such as Vascular Cell Adhesion Molecule 1 (VCAM-1) and interleukin 1 beta (IL-1β), and activated adhesion of molecules such as Vascular Cell Adhesion Molecule 1 (VCAM-1) and Inter Cellular Adhesion Molecule 1 (ICAM-1) [10]. NF-κB was found in almost all cells involved in the process of development, cell growth, immune response, and inflammatory processes. Expressions of NF-κB was not found in normal animal's arteries, but in inflamed arteries [11].

Conditions of oxidative stress and inflammation can be inhibited by administering natural ingredients that contain antioxidants and anti-inflammatory. Coffee contains bioactive compounds that has function as antioxidants and anti-inflammatory. People who consume 3 cups of coffee / day have a 21% lower risk of heart attack [12,13]. Previous studies have shown that consumption of Robusta coffee brewing can reduce blood LDL levels [14]. In general, there are two types of coffee that are widely cultivated and have economic value, namely Arabica coffee (Coffea arabica) and Robusta coffee (*Coffea Canephora*). Robusta coffee has caffeine content two to three times higher than Arabica coffee and has the highest chlorogenic acids content among other types of coffee [15]. Robusta coffee has the most antioxidant content, which is around 200-550 mg / cup compared to other anti-oxidants such as vitamin C, beta carotene, and alpha tocopherol [16].

The active ingredients of robusta coffee include polyphenols (caffeic acid, chlorogenic acids, ferulic acids) and alkaloids (caffeine) [1,17]. Polyphenols have anti-inflammatory properties, it can reduce the secretion of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). Chlorogenic acids also have antioxidant properties by inhibiting oxidative stress thereby preventing endothelial dysfunction [18,19]. The antioxidant properties of caffeine can reduce and protect cells from free radicals such as ROS so as to inhibit the progression of the development of atherosclerosis [20,21]. This study aimed to determine the effect of robusta coffee (Coffeacanephora) on NF-κB expression in the coronary arteries of male wistar rats induced by a high-fat diet.

Materials and Methods

Thisexperimental procedure has obtained the ethical clearance at Health Research Ethics Committe, Faculty of Dentistry, Jember University, Indonesia. Fifteen wistar rats,divided to 3 groups; the control group, the hyperlipidemia group, and the coffee treatment group.Hyperlipidemia was induced by high-fat diet with a mixture of 2 grams of duck egg yolks and 3 grams of pork oil [10]. Brewed coffee by dissolving 10 grams of robusta coffee powder in 200 ml of boiling water [11]. The dose conversion to mice the dose of coffee becomes 10 grams x 0.018 = 0.18 grams / head / day. Coffee powder as much as 0.18 grams is dissolved in 3.6 ml of boiling water.

On the 29th days,rats were maintained 12 hours before blood examination for LDL levels, then were sacrified and heart organ were taken for histological preparations. The specimens were embedded in paraffin, and sectioned serially, removing coronary arteries were done by taking longitudinal pieces below 3 mm from the atrial and ventricular borders [22,23]. Immunohistochemistry procedure was performed using rabbit anti rat NF-kB polyclonal antibody (Santa Cruz). The specimens were observed under a microscope with 400x magnification in three different field. NF-kB expression was evaluated by counting the cytoplasm of its brown color coronary artery endothelial cells.

Results and Discussion

The highest LDL levels of 43.00 ± 3.60 mg / dL were measured in the hyperlipidemia group while in the coffee treatment group the LDL levels were lower at 30.33 ± 1.53 mg / dL. The lowest LDL level measured in the control group was 23.27 ± 0.25 mg / dL (Figure 1).

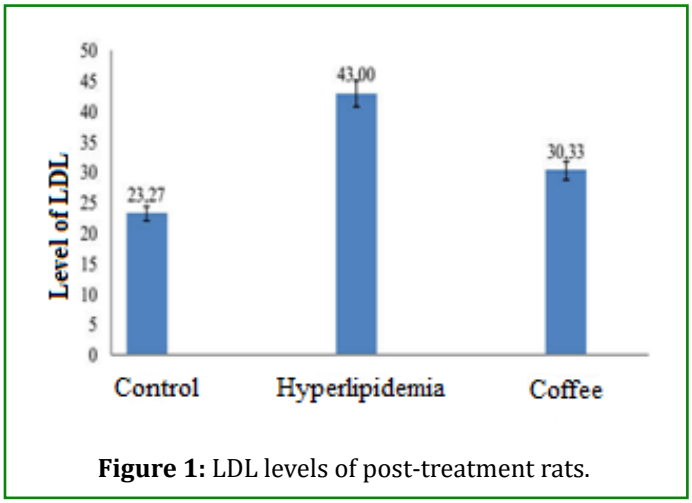

Figure 1: LDL levels of post-treatment rats.

NF-κBexpression in coronary artery endothelial cells (Figure 2) were more and the intensity was strongest in the hyperlipidemia group compared with the coffee and control groups. In the hyperlipidemia group, the amount of brown color in the cytoplasm of endothelial cells was greater than

in the control and coffee groups. The least amount of brown color in the cytoplasm of coronary artery endothelial cells occurs in the control group.

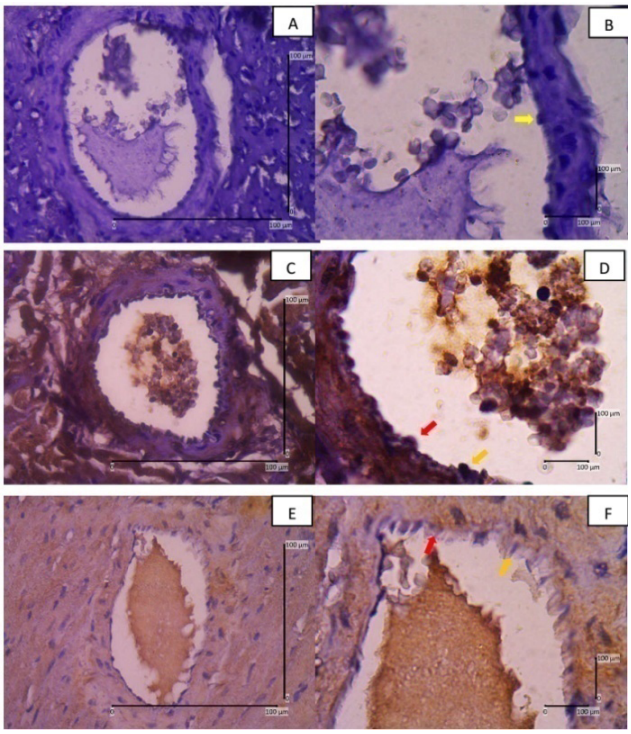

Figure 2: Immunohistochemistry of rat coronary arteries (400x and 1000x). Strongest expression showed in hyperlipidemia group. Yellow arrows indicate endothelial cells that do not express NF-κB while red arrows indicate endothelial cells that express NF-κB. (A) and (B) the control group, (C) and (D) the hyperlipidemia group, (D) and (E) the coffee group.

An increase in NF-κB expression in the hyperlipidemia group was 31.74±0.99 compared to the control group. Decreased expression of NF-κB occurred in the coffee treatment group by 18.49 ± 0.88 (Figure 3).

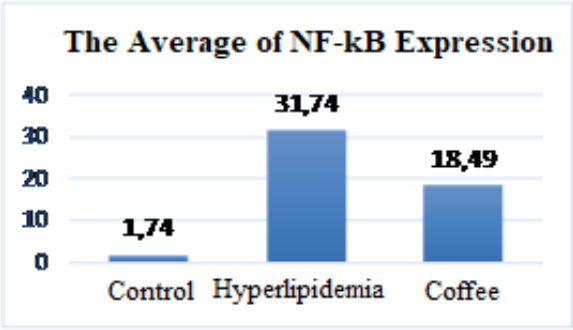

Figure 3: The average NF-κB expression.

One Way Anova test showed a significance value of 0,000 ($p < 0.05$). The results showed that there were significant differences in NF-κB expression between treatment groups. LSD test results showed a significant difference ($p < 0.05$) in all groups.

Based on the results of research that has been done, the expression of NF-κB is most numerous in the cytoplasm of coronary artery endothelial cells in the hyperlipidemia group. The intensity of the brown color in the cytoplasm of endothelial cells is the strongest among the other groups. It is due to the administration of a high-fat diet in the form of egg yolks and pork oil induces hyperlipidemia. The results of this study are in accordance with previous studies that the activation of NF-κB has been identified in endothelial cells induced by ox-LDL [24]. In the condition of hyperlipidemia, a lot of plasma LDL attaches to the walls of blood vessels and enters the intima tunica. The accumulation of LDL and the length of time the LDL stays in the intima tunica is a factor causing spontaneous oxidation I LDL which produces ox-LDL and triggers oxidative stress. Oxidative stress is characterized by increased of ReactiveOxigen Species (ROS) producion, which are highly reactive oxygen-derived oxygen radical compounds [6,25].

Pathogenic stimuli such as oxidative stress that produce a lot of ROS can activate NF-κB. This mechanism begins with the phosphorylation cascade process then causes the phosphorylation of IkBFurthermore the bond of NF-κB with IkB is detached and the p50 p65 heterodimer is free. The release of this bond causes NFκB to translocate into the nucleus. Increased activation of NF-κB occurs as a result of the response to conditions of continuous oxidative stress aimed at starting the process of inflation [8].

In the coffee treatment group, NF-κB expression was slightly lower than the hyperlipidemia group. The decrease is characterized by the number of cytoplasm of the endothelial brown color and the intensity of the weaker brown color compared to the hyperlipidemia group.

administered systemically reduced the activity of NF-κB [26]. Coffee contains antioxidant and anti-inflammatory bioactive compounds such as polyphenols (caffeic acid, chlorogenic acids, ferulic acids) and alkaloids (caffeine) [17-19]. Figure 4 is a scheme of inhibiting coffee compounds against the NF-κB activation pathway. It was proved that chlorogenic acids, ferulic acid and caffeic acid have anti-inflammatory properties that can inhibit NF-κB [27,28]. Chlorogenic acids inhibit NF-κB through receptors and signaling of mitogen-activated protein kinase. Chlorogenic acid and caffeic acid have also been shown to inhibit NF-κB-inducing kinase (NIK) / IkappaB kinase (IKK) and translocation of p50 and RelA [29,30].

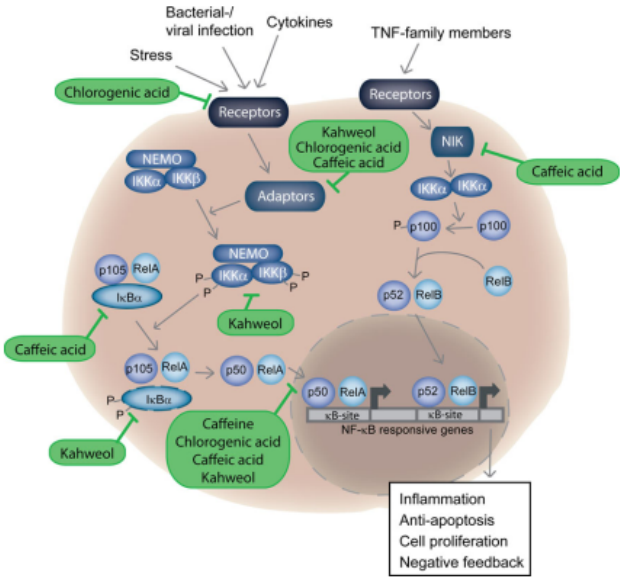

Figure 4: Scheme of Inhibiting Coffee Compounds Against the NF-κB Activation Pathway.

Polyphenol compounds have the ability as an antioxidant. Polyphenols neutralize free radicals by transferring hydrogen atoms from free radicals and releasing electrons to free radicals [31,32]. Chlorogenic acids can increase the activity of Nrf2 to transcribe genes that regulate the production of antioxidants [33]. Other studies have shown that caffeine is able to protect from ROS such as hydroxyl radicals (OH) and peroxyl radicals (ROO) [34]. If oxidative stress conditions can be suppressed, the activation of NF-κB can also be inhibited. This can occur because NF-κB is the first signaling pathway activated by ROS [8].

In the control group, the expression of NF-κB in coronary artery endothelial cells was the least compared to the coffee treatment group and the hyperlipidemia group. The control group was only given a standard diet so there was not increases of LDL in the intima tunica. LSD test results showed a significant difference between groups. Significant differences in the coffee treatment group with the hyperlipidemia group showed that brewed coffee could reduce the expression of NF-κB. Decreased expression of NF-κB occurs due to coffee content that has anti-inflammatory and antioxidant effects.

Conclusion

Based on the research that has been done, it can be concluded that the administration of brewed robusta coffee can reduce the expression of NF-κB in coronary artery endothelial cells induced by a high-fat diet.

Acknowledgment

None.

Conflict of Interest

None.

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Ethics Statement

This experimental procedure has obtained the ethical clearance at Health Research Ethics Committe, Faculty of Dentistry, Jember University, Indonesia No. 258/UN 25.8/KEPK/DL/2022.

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