

DOI: <http://dx.doi.org/10.33846/hn40303>  
<http://heanoti.com/index.php/hn>



RESEARCH ARTICLE

URL of this article: <http://heanoti.com/index.php/hn/article/view/hn40303>

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## The Effect of Coffee Consumption on Superoxide Dismutase (SOD) Enzyme Expression in Wistar Rats Induced by Hyper LDL

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### ABSTRACT

One of the causes of stroke is atherosclerosis. The incidence of occurrence ischemic stroke caused by atherosclerosis is 95%. Atherosclerosis is caused by oxidative stress in the endothelium due to the formation of Reactive Oxygen Species (ROS). Hyperlipidemia (hyper LDL) is one of the risk factors of elevated ROS. One of the enzymes that play a role in preventing the formation of ROS is superoxide dismutase (SOD). Coffee has a powerful antioxidant in which one of its active ingredients is chlorogenic acid that capable to inhibiting ROS formation. Fifteen wistar male divided into 3 groups. Group I (Control) were given standart fed, Group II (Hyperlipidemia) were given standart fed and hyperlipid diet, Group III (Coffee) were given standart fed and hyperlipid diet + coffee consumption. Treatment were done everyday for 30 days. Blood serum in each group was taken on day 30 and examined by ELISA superoxide dismutase (SOD). The results showed that superoxide dismutase levels in coffee group was significantly higher than hyperlipidemia group ( $p = 0,006$ ). This study proves that coffee consumption can increase the production of superoxide dismutase enzyme.

**Keywords:** atherosclerosis; coffee; hyperlipid; SOD

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### INTRODUCTION

#### Background

Atherosclerosis is condition where the arteries become narrowed and hardened due to build up of plaque around the artery wall. Atherosclerotic plaque is cholesterol fatty acid have been successfully in unreaveiling some of the mechanism involved in the formation and rupture of this plaque.<sup>(1)</sup> 90 % prevalence ischemia stroke commonly caused by brain blood vessels atherosclerosis. Atherosclerosis is a inflammation process caused by agent or injury in cell. One can cause inflammation in human is *Low Density Lipoprotein (LDL)*. *Low Density Lipoprotein* particle is a cholesterol rich, trygliceride poor particle. LDL is composed of a hydrophilic surface layer of phospholipid, free cholesterol and hepatically derived apo B 100 to package the particle and add stability. The core of particle includes esterified cholesterol and trygliceride together with fatty acid tails of the phospholipid.<sup>(1)</sup>

Classical risk factors such as hypercholesterolemia lead to increased oxidative stress. Increased oxidative stress is believed to be an initial and important step in the development of endothelial dysfunction and atherosclerosis.<sup>(2)</sup> Superoxide Dismutase (SOD) is a kind of antioxidant that inhibit reactive oxygen species accumulation and remove free radicals. Several kind of supplement containing antioxidant that can suppress oxidative stress such as selenium or vitamin as well as antioxidant enzyme such as Superoxide Dismutase (SOD), catalase (CAT) or glutathione peroxidase (GPx), which have longer lasting effect because of lower rate of exhaustion.<sup>(3)</sup> Imbalance process between ROS production and antioxidant defence resulting in the

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accumulation of oxidative product is called oxidative stress. Oxidative stress causes oxidative burst leading to lipid peroxidation in endothelium. Oxidized low-density lipoproteins can be deposited on blood vessel walls which leads to atherosclerosis and cardiovascular disease.<sup>(4)</sup> Beneficial health effects of coffee are usually attributed to its high antioxidant activity (ability to inhibit the process of oxidation). Coffee has essential components such as caffeine, chlorogenic acid and other polyphenols. The major polyphenols in coffee is chlorogenic acid (CGA) an ester of caffeic acid and quinic acid. In vitro study noted that chlorogenic acid (CGA) from coffee can inhibit free radical product.<sup>(5)</sup> We hypothesize that the bioactive ingredients in coffee relating to vascular disease are the polyphenols which in the body may be acting as protective antioxidants such as superoxide dismutase (SOD).

## METHODS

The animal studies were performed after receiving approval of the ethic study commission Faculty of Dentistry Gadjah Mada University (approval number: 001062/KKEP/FKG-UGM/EC/2017). Male wistar rats of 2-3 months old were divided into three groups (5 rats per group): control, Hyperlipidemia, Hyperlipidemia + coffee. Hyperlipidemia was induced with sondage of cholesterol feed, fat pig 3 gr/day and egg yolk 2 ml/day for 4 weeks. Rats were orally administered coffee (0.9 gr/kg/day) dissolved in 3.6 ml water 7 days/week during for 4 weeks. Cholesterol level were checked intermittently throughout the study for at least one week for 4 weeks study duration to confirm the hyperlipidemia condition. The rats were sacrificed after treatment for 30 days with intracardial blood preparation.

Blood from rats were isolated and collected by centrifugal process to separate plasma and serum. Serum that collected to analyzed by ELISA process. Cholesterol level were determined by using Enzymatic Endpoint Method with a spectrophotometer meanwhile antioxidant concentration of SOD were determined by using ELISA assay (Bioassay kit, USA) and SOD concentration were performed by optical density wave length. All values are expressed as mean  $\pm$  SD. Differences between the group were evaluated for significance with One way Anova (IBM SPSS statistic For Windows ver 19.0; SPSS Inc.Chicago,IL,USA).

## RESULTS

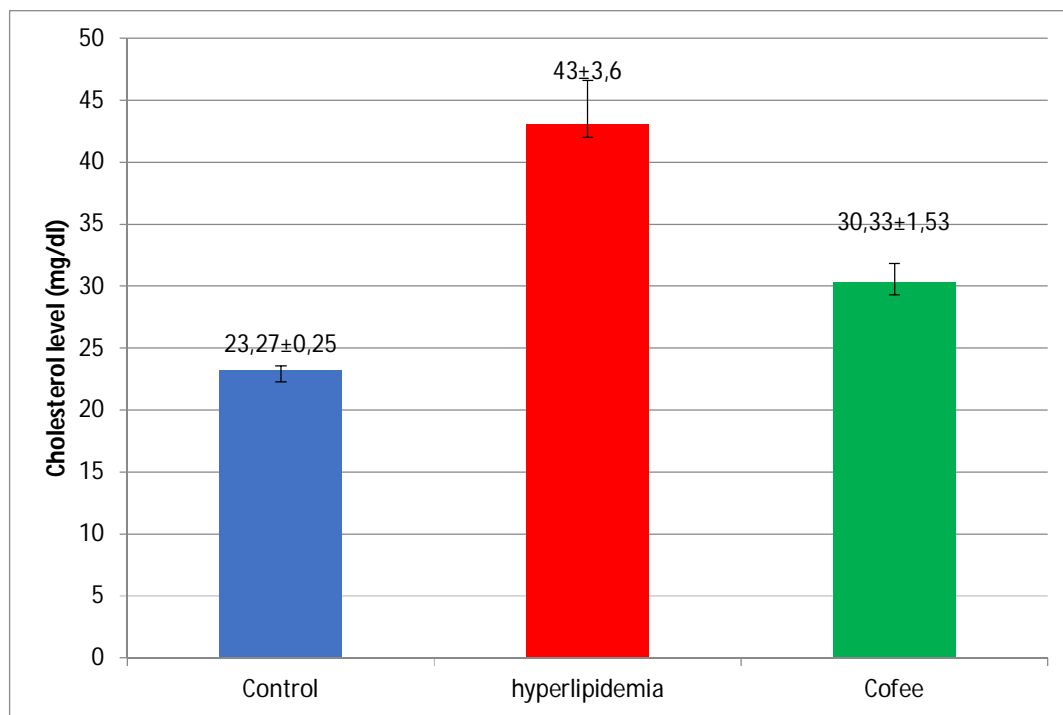


Figure 1. Cholesterol level (mg/dl)

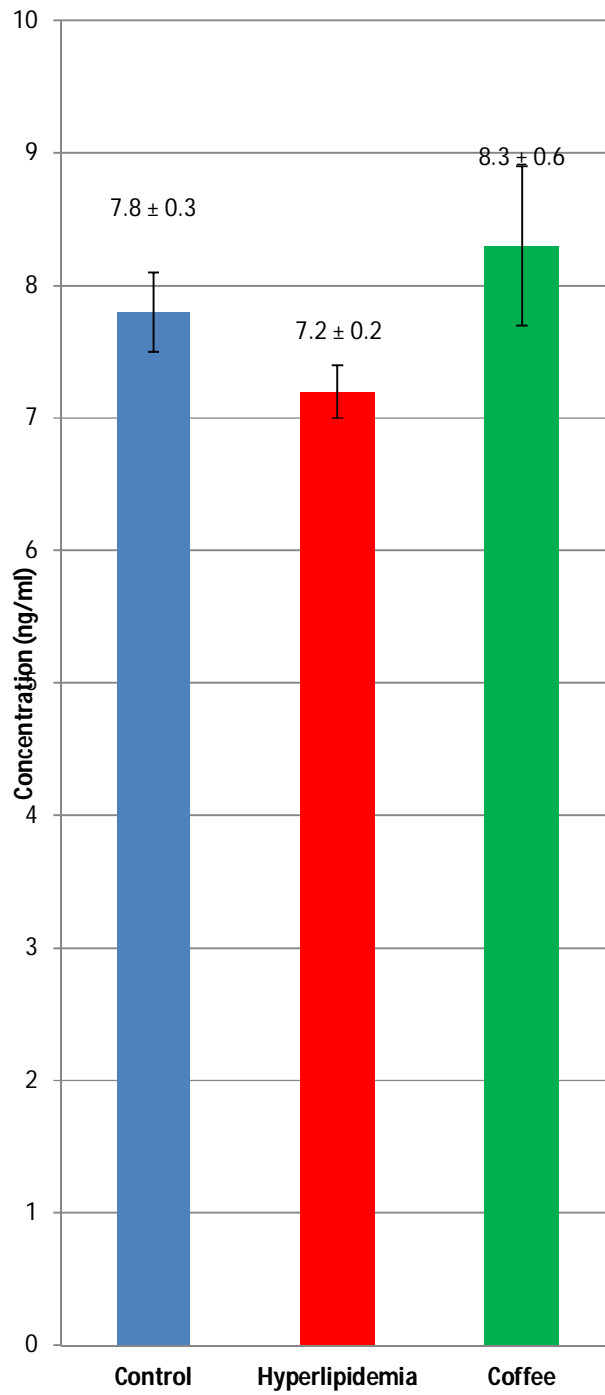


Figure 2. Superoxide Dismutase (SOD) level (ng/ml)

One way Anova test for total Cholesterol Levels show significant differences the control group and treatment group ( $p = 0.02$ ) ( $p < 0.05$ )\*. Tuckey HSD test found mean total cholesterol level the hyperlipid group was higher than other group. The result showed that coffee consumption decreased cholesterol level in coffee group (Fig 1).

SOD level among the groups  $p = 0.03$  ( $p \leq 0.05$ )\*, indicating that coffee consumption had significant effect on the SOD level. Tuckey HSD test showed SOD level coffee group is higher than hyperlipidemia group ( $p = 0.02$ ). The result showed that coffee consumption induced more production of SOD. See fig 2.

## DISCUSSION

Based on the results of the study showed cholesterol levels in rat blood serum in the hyperlipidemia group increased compared with the control and coffee group. This happened because in the hyperlipidemia group who were given a high-fat diet in the form of a mixture of duck egg yolks and fat pig which would be metabolized by the body. This is in accordance with research<sup>12</sup> which proves that the provision of a high-fat diet in the form of lard and duck egg yolk in a ratio of 3: 2 can increase rat blood cholesterol levels.

This study showed that coffee consumption stimulates higher level of SOD in rat induced hyperlipidemia. This was probably caused by coffee ingredients can function as an antioxidant. Classical risk factors such as hypercholesterolemia or hyperlipidemia lead to increased oxidative stress. Reactive oxygen species (ROS) mediate endothelial cell apoptosis, inflammatory processes, proliferation of vascular smooth muscle cells (VSMCs), and destabilization of atherosclerotic plaques.<sup>(2,11,13)</sup> As a defense against enhanced ROS production, mammalian cells have a complex network of antioxidant enzymes, such as superoxide dismutases (SODs).

SOD is currently believed to be the main component responsible for endothelial function and integrity.<sup>(2,14)</sup> Oxidative stress can be reduced by antioxidant therapy, *i.e.*, by consumption of certain amounts of natural antioxidants contained in vegetables, fruits, berries, vegetable oils, honey, tea, coffee, cocoa, and other foods. Coffee is one of the major sources of antioxidants in people's daily diet. Antioxidant activity of coffee is related to chlorogenic, ferulic, caffeic, and *n*-coumaric acids contained in it. In roasted coffee, melanoidins (brown pigments) are synthesized—these are strong antioxidants.<sup>(5,15)</sup>

We used robusta coffee in this study, because the antioxidant activity of Robusta coffee is significantly higher than that of Arabica. A cup of coffee may have 15 to 325 mg of chlorogenic acids (CGA). CGA is an *in vitro* antioxidant with two phenolic groups for radical scavenging via proton transfers.<sup>(6,16)</sup> Perhaps there is synergism between the polyphenols in coffee as was found between CGA and catechin for LDL oxidation. Experiment demonstrates that the coffee polyphenols can bind to lower density lipoproteins, protect them from oxidation and thus be *in vivo* heart-protective antioxidants.<sup>7</sup> Some study both *in vitro* and *in vivo* proved antioxidant activity of ferulic acids. Pharmacological properties of ferulic acid are related to its high antioxidant activity, in particular, its ability to inhibit lipid peroxidation in biological membranes.<sup>(5)</sup>

Certainly caffeine is a major component in coffee. Caffeine itself has no LDL antioxidant activity. However, some of the metabolites of caffeine, namely 1-methylxanthine and 1-methyluric acid are as effective at preventing LDL oxidation.<sup>(6)</sup> The main role of SODs in all aerobic organisms is to neutralize the  $O_2^{\cdot-}$  produced in the cytosol, mitochondria and endoplasmic reticulum of cells. However, the SOD can also have a pro-oxidant effect because the dissociation of the  $O_2^{\cdot-}$  produces  $H_2O_2$ , which is toxic to cells. It plays to remove this dangerous  $H_2O_2$  that the presence of others antioxidant systems. Positive effects of SODs on cardiovascular diseases have also been shown.<sup>(3)</sup> have demonstrated that oral administration of SODs prevents aortic lipid deposition in an animal model of diet-induced atherosclerosis.<sup>(16)</sup> Other authors have shown that injection of bovine Cu/Zn-SOD reduces blood pressure in rat models of hypertension. Another studies shown that SOD prevent increasing of body weight and inhibit obesity markers such as cholesterol, triglycerides, leptin and insulin.<sup>(8,17)</sup>

Hyperlipidemia group (treatment without coffee consumption) has a less level of SOD than the other groups. Downregulation of antioxidant enzyme expression is associated with an increased vascular superoxide. Classical risk factors such as hypercholesterolemia/ hyperlipidemia lead to increased oxidative stress. A large body of evidence has been accumulated suggesting that increased oxidative stress is an initial step in the development of endothelial dysfunction and atherosclerosis.<sup>(3)</sup> Oxidative stress is linked with inflammation. Inflammation resulted by oxidative stress that generate the mediators of inflammation such as interleukin and tumor necrosis factor alfa (TNF- $\alpha$ ). Decreases in inflammation could be administration SOD as antioxidant effect.<sup>(9,18)</sup>

## CONCLUSION

In conclusion, coffee consumption can increase SOD level in rats induce hyperlipidemia. It has beneficial effects in various pathological situation.

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