The effect of feeding antioxidant rich coffee on glucose blood response, MDA and SOD on diabetic rats induced with streptozotocin

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Abstract. This study aimed to examine the effects of antioxidant rich coffee on blood pressure, glucose blood response, levels of MDA and SOD serum, and liver tissue of Wistar diabetic rats. The study used 24 Wistar rats in which 18 rats were induced with Streptozotocin of 60 mg/kg BW for 4 days to make them diabetic (fasting blood glucose level >250 mg/dL). Rats than divided into 4 groups: 1) control group (non-diabetic group), given distilled water, 2) diabetic control group, given sugar cane coffee of 0.45 gram/200 g BW/day, 3) diabetic group, given antioxidant rich coffee of 0.45 g/200 g BW/day, 4) diabetic group, given antioxidant rich coffee of 0.90 gram/200g BW/day. Weekly observations were made on blood pressure, fasting blood sugar levels, body weight and blood pressure, as well as serum and tissue levels of SOD and MDA. The results showed that coffee rich in antioxidant significantly reduced oxidative stress in diabetic rats with lower blood pressure, serum MDA values, and the tissue MDA value than that in the diabetic rats given sugar cane coffee. This illustrates that consuming 0.90 g/day antioxidant rich coffee in mice, or the equivalent of 60 g/day in humans, can reduce oxidative stress in diabetes.

1. Background
Diabetes is a multisystem disease that caused metabolic disorder and characterised by elevated blood sugar level. It resulted from defects in insulin production and/or insulin action, and impaired function in the metabolism of carbohydrates, lipids, and proteins which leads to macro and microvascular complications. Blood glucose elevation or hyperglycemia is a general effect of uncontrolled diabetes leading to severe damages to the heart, blood vessels, eyes, kidney as well as nerves [1]. Many studies report that there is an important relationship between some diseases and oxidative stress, because these participate in vital processes, such as glucose homeostasis, inflammation, and also cell survival. In Diabetes Mellitus (DM), hyperglycemia induces increased oxidative stress through several biochemical processes which causes the progression and complications of DM due to increased free radicals and decreased antioxidant enzymes leading to an increase in lipid peroxidation. In-vivo studies report the role of hyperglycemia in the generation of oxidative stress leading to endothelial dysfunction in diabetic patients blood vessels [2].

Oxidative stress is the outcome of an imbalance between the production and neutralization of reactive oxygen and nitrogen species (RONS) such that the antioxidant capacity of cell is overwhelmed, and
results in the development of pathological condition among which one is diabetes [3-6]. Its also has been reported as a known pathway in the pathogenesis of diabetic complications. Elevated levels of ROS cause damage to lipids, proteins, and DNA, and it is associated with several diseases including cancer, DM, cardiovascular, neurodegenerative diseases, and MDD [7]. Many researchers also report that in diabetic patients, increased blood glucose trigger free radical generation and fail the defense mechanism of the body, becoming incapable of counteracting the elevated levels of reactive oxygen species (ROS) generated and as a result an imbalance between oxidants and pro-oxidants leads to the induction of oxidative stress [8,9].

Due to oxidative stress produced in metabolic abnormalities of diabetes, mitochondrial superoxide is over produced in endothelial cells of both the large and small vessels and in the myocardium. In diabetic patients, insufficient insulin secretion condition was increase lipase activity consequently increase the lipolysis and led to elevated levels of free fatty acid in plasma and liver [10]. The level of glucagon also elevates in diabetic patients and this increases the release of free fatty acids. Free fatty acids are metabolized to acetyl CoA and finally to CO2 and H2O via citric acid cycle. In the patient with insulin deficiency, the capacity of this process is rapidly elevated in which the acetyl CoA converted to acetoacetyl CoA and finally to acetoacetic and hydroxy-butyric acids. Insulin apparently also affects either formation or clearance of VLDL and LDL, since levels of these particles and levels of cholesterol are often elevated in poorly controlled hyperglycemic patients.

There is an important relationship between some diseases and oxidative stress, because they participate in vital processes, such as inflammation, glucose homeostasis, and cell survival [11]. In DM, hyperglycemia induces increased oxidative stress through several biochemical processes, including the glucose self-activation, increase of glycation and diacylglycerol, and the activation of protein kinase C and polyol pathways [12]. For that, individual with DM requires adequate intake of antioxidants to prevent the development of degenerative diseases triggered by free radicals.

Developing coffee drink rich in antioxidant with coconut sugar as sweeteners is an alternative to meet adequate need of antioxidant. Using coconut sugar, a food that categorized as Low GI, is beneficial for diabetic as it keeps balance on blood sugar level. Low-GI diets will improve glycaemic control [13]. To solve the problem on oxidative stress in diabetic is by developing functional foods rich in antioxidant and low glycaemic index such as the mix coffee drink which produce by modify coconut sugar processing and addition red palm oil to increase antioxidant content. Red Palm Oil (RPO) is a rich source of antioxidant such as tocopherols (18–22%), tocotrienols (78–82%), lycopene (18.5–38 ppm), betacarotene (500–700 ppm) [14]. Tocopherol is a quite powerful antioxidant, and it acts as a radical scavenger especially α-tocopherol and play role as a radical chain-breaking antioxidant [15]. Coffee powder is also contains many antioxidant compounds such as phenol; melanoidin, N-methylpyridinium, and Chlorogenic acid.

High antioxidants coffee drink with the addition of RPO of 0.3% and coffee powder of 10 % that added on temperature of 108°C is the best combination based on chemical and sensory aspects [16]. Research on normal rats exposed to cigarette smoke showed that consumption of antioxidant rich coffee with palm sugar sweeteners had a better hypoglycaemic effect and antioxidative stress than mix coffee with cane sugar [17]. But it has not been studied in diabetic rats. The aim of this study was to examine the effects of antioxidant rich coffee on blood pressure, glucose blood response, levels of MDA and SOD serum and liver tissue of Wistar diabetic rats induced with Streptozotocin.

2. Methods
Study was conducted at the Animal Research Laboratory and Nutrition Laboratory of the Centre of Food and Nutrient Studies at UGM, YK, after obtaining approval from the Medical/Health Research Bioethics Committee of the Faculty of Medicine of Sultan Agung Islamic University, Semarang.
2.1. Preparation of rich antioxidant coffee mix

2.1.1. Purification of coconut sap. The tapped coconut sap was purified by several stages of filtration through screen of 60-mesh and then heated until boiling and turn cooled. Separation the formed precipitate was done by decantation and re filtration with a 200-mesh filter.

2.1.2. Coconut sap heating. The purified coconut sap was heated up to 102°C, and then 0.3% of red palm oil was added and heated until temperature reached 108°C. Then, 10% of coffee powder was added. The amount of red palm oil and coffee powder added were corrected with the standard of sap’s Brix degree of 20 [17]. After the temperature cooking reached 119°C, the heating was stopped and continued with the solidification process (continuous stirring until the semi-solid mass was formed). The granulation phase was the next stage, it performed by grinding to form the coconut sugar granules. The last stage was sieving using a 16-mesh screen. The coffee produced was packed using aluminum foil before used for the testing in the experimental rats.

2.2. Animal handling

This research was conducted at the Animal Research Laboratory and Nutrition Laboratory of the Centre of Food and Nutrient Studies at UGM, Yogyakarta. This study used 24 male albino rats, Sprague-Dawley strain, at the age of three months with a weight range 184-203 g. Those rats were obtained from the Animal Research Laboratory of Gajah Mada University. The rats were initially adapted for three days by feeding them with the standard feed Comfeed II AD and distilled water ad libitum. After the adaptation period was completed, a total of 18 rats were induced with Streptozotocin of 60 mg/kg BW for 4 days up to be a fasting blood glucose level of > 250 mg/dL (diabetic rats group) and other 6 rats (non-diabetic) were used as the control group. Rats than grouped into 4 groups (n=6): 1) control group (non-diabetic group) was given distilled water (P1), 2) diabetic control group that given sugar cane coffee of 0.45 gram/200 g BW/day (P2), 3) Diabetic group that given antioxidant rich coffee of 0.45 g/200 g BW/day (P3), 4) Diabetic group that given antioxidant rich coffee of 0.90 gram/200g BW/day (P4). Intervention was carried out for 4 weeks. The observations were made every week by measuring blood pressure using a sphygmomanometer, fasting blood sugar levels, as well as serum and tissue levels of SOD and MDA using the thiobarbituric acid reactive substances (TBARS) method. The data were analyzed by ANOVA. If there was significance variance, the analysis was followed by Duncan Multiple Range Test.

3. Results and discussion

3.1. Respond on body weight

![Figure 1](change_on_rat_body_weight.png)

Note: (P1=normal group; P2= diabetic control group, P3= diabetic test group1; P4= diabetic test group 2)

**Figure 1.** Change on rat body weight
Rat body weight in all treatments along the study was increased (Figure 1). The average body weight of the rats before treatments was 182.2 g - 200 g. The administration of a coffee mixture that was rich in antioxidants increased the body weight of diabetic rats between 7.8 - 22.7 g. In the diabetic control group and mix coffee with sugar cane group had lower increasing weight gain. In contrast, the normal rat group or non-diabetic rat group, which given distilled water, had the highest increasing body weight gain of 28.3 g.

3.2. Blood pressure
Prior to streptozotocin (STZ) induction, blood pressure in all groups of rats was relatively similar, ranging from 88.0 to 105.3 g. After being induced by STZ, the blood pressure of rats in the diabetic group ranged from 187.3 - 189 mmHg, while in the normal group the blood pressure was relatively normal, namely 95 mmHg.

The results showed that coffee mix rich in antioxidant significantly reduced oxidative stress in diabetic rats as indicated by lower blood pressure in the group given coconut sugar mix coffee (P3 and P4) compared to sugar cane coffee (P2) (Figure 2). Diabetic rats that were given antioxidant-rich coconut sugar coffee had lower blood pressure values (101-105.3 mmHg) than that in the diabetic rats fed by sugar cane coffee (183 mmHg).

Oxidative stress will cause a decrease in the bioavailability of nitric oxide (NO), which is the main factor responsible for maintaining vascular tone. The decreased bioavailability of nitrates due to oxidative stress will lead to the incidence of hypertension [18].

![Figure 2. Change on Blood Pressure of Rats along feeding coffee rich in antioxidant](image)

3.3. Malondialdehyde level in serum and tissue of rats feeding antioxidant rich coffee
Antioxidant-rich coffee significantly reduced oxidative stress in diabetic rats which was also shown by lower serum MDA values in the group of mice given high antioxidant coconut sugar mix coffee (1.5-3.0 nmol/mL) than in the diabetic mouse group given sugar cane coffee (9.1 nmol/mL) (Figure 3). Likewise, the tissue MDA value in the group of diabetic rats given coconut sugar mix coffee drink was also lower (4.0-6.2 nmol/mL) than that in the group of mice given cane sugar mix coffee (12.1 nmol/mL). In contrast, in the normal group of rats given distilled water, the blood pressure value was relatively stable, namely 1.21 (pre) to 1.47 nmol/mL (post).

The advantages of coffee mix rich in antioxidant with coconut sugar in this research come from vitamin E (tocopherol) and beta carotene from red palm oil. Intake of tocopherol in the amount of 150 mg/kg in obese mice treated with a high-fat diet can improve insulin sensitivity and reduce oxidative stress and inflammatory responses [19]. Another research reported that vitamin E supplementation of 300 mg/day for 3 months in patients with diabetic retinopathy could reduce serum MDA levels, which means that it can suppress oxidative stress in diabetic patients [22]. Antioxidants also play a role in
suppressing oxidation events, thereby reducing the number of products resulting from oxidation, including serum MDA levels.

![Figure 3](image_url)

**Figure 3.** Level of MDA serum and tissue in rats given mix coffee rich in Antioxidant

### 3.4. Level of Super Oxide Dismutase (SOD)

Indicators for oxidative stress can also be seen from the Super oxide dismutase (SOD). The antioxidant activity of SOD in normal rats given distilled water was almost similar, in pre and post. In Diabetic rats that given mix coffee with coconut sugar (P3 and P4) experienced an increase in SOD activity better than the group given sugar cane coffee (P2) (Figure 4.)

![Figure 4](image_url)

**Figure 4.** Level of SOD serum of rats given mix coffee rich in antioxidant

In the diabetic rat group, given mixed coffee with antioxidants SOD was significantly higher in activity (49.70% /P3 and 69.94% /P4) than in the group given sugar cane mixed coffee (25.89%). The higher the dose of antioxidant-rich mixed coffee given, the higher antioxidant SOD activity. In the normal group of mice, SOD activity was relatively stable at 76.19%.

This illustrates that giving 2 doses of high antioxidant coconut sugar mix coffee, namely 0.90 g per day in mice or the equivalent of 60 grams per day in humans, can improve oxidative stress in human with diabetes. Red palm oil contains antioxidants including tocopherols (Vitamin E), tocotrienols, lycopene and beta carotene. Giving vitamin E can improve serum antioxidant status, including by
increasing the amount of serum SOD [20]. Furthermore, research on vitamin E intervention in patients with chronic periodontitis was able to improve SOD activity [21].

4. Conclusion
Addressing coconut sugar mix coffee rich in antioxidant of 0.90 g per day in diabetic rats, or the equivalent of 60 grams per day in humans, can reduce oxidative stress.

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