Effect of feeding coconut sugar-mix coffee rich in antioxidants on blood pressure, serum SOD and MDA of sprague dawley rats

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Abstract. High exposure to cigarette smoke triggers oxidative stress, thereby the intake of foods rich in antioxidants is needed. Coconut sugar-mix coffee enriched with antioxidants from red palm oil is one of the alternatives to increase the supply of antioxidants. This study aimed to determine the effects of feeding coconut sugar mixed coffee rich in antioxidant (CMCRA) on blood pressure, serum SOD and MDA of Sprague Dawley rats. Six groups of rats (n = 6) received the following treatments: one group of rats were given 0.45 g/day of the CMCRA without exposure to cigarette smoke (positive control/P1) while five groups of rats were exposed to cigarette smoke from two cigarettes per day during the study period and given distilled water (negative control/P2), 0.45 g/day of CMCRA (P3), 0.90 g/day of the CMCRA (P4), 1.35 g/day of the CMCRA (P5), and 0.45/day of cane sugar mixed coffee (P6). The observations were conducted on body weight, blood pressure, serum SOD levels, and serum MDA levels. The results of the study showed that the delta increase in blood pressure in the CMCRA groups was lower (5.8–28.6 mmHg) than the cane sugar coffee group (79.2 mmHg) or the negative control group (103.6 mmHg). The decrease in serum SOD levels in the tested coffee groups (6.75–44.62%) was lower than the market-sold coffee group (45.97%) or the negative control group (60.44%). The delta increase in serum MDA levels was lower in the tested-coffee groups (0.51–5.73 nmol/mL) compared to the cane sugar coffee group (6.69 nmol/mL) or the negative control/distilled water group (8.28 nmol/mL). The coffee drinks enriched with antioxidants from red palm oil have the potential to suppress oxidative stress in Sprague-Dawley rats exposed to cigarette smoke.

Keywords: antioxidant, coffee drink, SOD, MDA

1. Introduction
Indonesia is the best-quality of coffee producing countries in the world with the estimated production of coffee in 2015 is about 739,005 ton [1]. Furthermore, it is reported that the coffee consumption of the people reaches 1.2 kg/capita/year. Most coffee drinkers are also smokers, in which cigarette is one of the risk factors for the development of degenerative diseases such as cancer.

The number of active smokers in Indonesia from the age of 10 years and above reached 58,750,592 people [2]. Cigarette smoke contains many harmful components such as free radicals, tar, benzoyprene,
and nitrosamine, which can trigger many of degenerative diseases. Smoking is the main risk factor for heart attacks, stroke, chronic obstructive pulmonary disease/COPD (including emphysema and chronic bronchitis), and cancer (especially lung cancer, oropharyngeal cancer, and pancreatic cancer). It also causes peripheral vascular disease and hypertension [3].

Nicotine in tobacco and tar, benzopyrene, acrolein, and nitrosamine in cigarette smoke are carcinogenic components that cause cancer [4]. Components in cigarette smoke can also increase oxidative stress, which is also a trigger for degenerative diseases such as diabetes and hypertension [5]. The global death rate from cigarettes is estimated to reach 10 million people by 2030, in which 70% of them are from developing countries [6]. Smoking can also cause the oxidation of glutathione (GSH, an antioxidant that protects DNA from the damage caused by ROS), decrease the antioxidant levels in the blood, and increase the release of superoxide radicals or commonly called as oxidative stress [7].

Increased oxidative stress also occurs in obese and diabetic individuals [8];[9]. Increased susceptibility to oxidative DNA damage has also been reported in type 2 diabetes. Therefore, the role of antioxidants becomes important as an oxidation chain-breaker agent. Some studies have stated that vitamin E improves oxidative stress and hepatocellular function and decreases plasma glucose concentration through its role as an antioxidant [10]. There were reported that added that vitamin E intake significantly reduced the risk of type 2 diabetes [11].

Therefore, the development of mixed coffee with the addition of red palm oil that contains high antioxidants is one the appropriate steps to provide a functional drink to suppress oxidative stress and prevent the development of various degenerative diseases triggered by oxidative stress.

Red palm oil contains beta-carotene between 500–700 ppm [12], vitamin E (560–1000 ppm) that consists of tocopherols (18–22%), tocotrienols (78–82%) [13]; [12] Sundram 2003, and lycopene (18.5–38 ppm) [14]; [15]. Tocopherol is a quite powerful antioxidant, and it acts as a radical scavenger [12] especially α-tocopherol that is a radical chain-breaking antioxidant [16]. Tocotrienol is an antioxidant that is more effective than tocopherol because unsaturated side chains facilitate better penetration [17]. Animal studies show that palm tocotrienol improves blood glucose, dyslipidemia, and oxidative stress in diabetic rats. It can prevent the development of changes in vascular walls occurring in DM [18]; [19].

The mixed coffee on the market is a mixture of coffee powder and cane sugar, which is known to have a high glycemic index value. The use of coconut sugar in the production of mixed coffee will improve its glycemic index value. Coconut sugar is made by evaporating coconut sap. At its production stage, there is vegetable oil added for defoaming purposes. The addition of red palm oil that is rich in beta-carotene (provitamin A) and antioxidants (tocopherol and tocotrienol) in the production stage not only improves the functional value but will also improve the competitive value of the product. In a recent study, the addition of 0.3% of red palm oil, did not change the hedonic characteristics of coconut sugar rich in antioxidant, and this product contained beta-carotene of 1337–3946 µg/100 g [20]. The storage using double packaging to protect against the exposure to light can slow down the beta-carotene reduction (15.38%) better than single packaging (22.05%) [21]. In another our research the 2-wk intervention on experimental rats could increase liver retinol reserves as an indicator of vitamin A status beyond the cut-off point value and could increase immunoglobulin G levels to almost 3-fold, which was an indicator of increased immunity [22]. Besides that, the intervention also provided a positive response to weight gain in vitamin-A-deficient rats [23].

Developing of high-in-antioxidants mixed coffee with the addition of 0.3% of red palm oil and 10% of coffee powder with a temperature of 108 °C during the addition was a combination with the best result based on chemical and sensory aspects. The product’s characteristics were as follows: 5.33% of water, 40.17% of yield, 704.83 µg/100 g of beta-carotene, 2.88% of total phenols, 77.55% of DPPH antioxidant activity, and 0.71% of tocopherols (vitamin E) [23].

The mixed coffee enriched with antioxidants from red palm oil contained active components such as tocopherols, beta-carotene (provitamin A), lycopene, and phenols that were antioxidant components. However, there has been no study on antioxidant activity in the mixed coffee in suppressing oxidative stress. Thus, this study had been conducted to determine the effects of intervention using coffee drinks
enriched with antioxidants from red palm oil on blood pressure, serum superoxide dismutase (SOD) levels, and serum malondialdehyde (MDA) levels of Sprague-Dawley rats exposed to cigarette smoke.

2. Methods
This study was conducted after obtaining approval from the Medical/Health Research Bioethics Committee of the Faculty of Medicine of Sultan Agung Islamic University, Semarang Number 25/1/2018/Komisi Bioetik.

2.1. Preparation of high-in-antioxidants mixed coffee drink

2.1.1. Coconut sap purification. The coconut sap obtained from the result of sap tapping was purified by stages, which was preceded by 60-mesh filtration. The coconut sap was then heated until boiling and then cooled. The precipitate formed was separated by decantation. The subsequent purification was refiltration with a 200-mesh filter.

2.1.2. Heating coconut sap. The purified coconut sap was heated. After reaching the temperature of 102 °C, 0.3% of red palm oil was added. The addition of 10% of coffee powder was performed at 108 °C. The amount of coffee powder and red palm oil added were corrected with the standard of sap’s Brix degree of 20 [24]. After reaching the final cooking temperature (endpoint) of 119 °C, the heating was stopped and continued with the solidification process (continuous stirring until the semi-solid mass was formed). The next stage was the granulation phase performed by grinding until the granules were formed, and the last stage was sieving using a 16-mesh screen. The coffee produced was packed using aluminium foil before being used for the testing in the experimental rats.

2.2. Preparation of experimental animals
This study was conducted at the Animal Research Laboratory and Nutrition Laboratory of the Centre of Food and Nutrient Studies at UGM, Yogyakarta. This study used 30 male albino rats of Sprague-Dawley strain at the age of three months with a weight range of 180–197 g, which were obtained from the Animal Research Laboratory of UGM. The rats were initially adapted for three days by feeding them with AIN-93 M standard feed and distilled water ad libitum. After the adaptation period was completed, the rats were divided into six groups (n = 5). One group of rats was given 0.45 g/day of the tested coffee without exposure to cigarette smoke (positive control/P1) while five groups of rats were exposed to cigarette smoke from two cigarettes/day during the study period and each of them was given the following treatments: distilled water (P2), 0.45 g/day of the tested coffee (P3), 0.90 g/day of the tested coffee (P4), 1.35 g/day of the tested coffee (P5), and 0.45 g/day of cane sugar mixed coffee (P6). The duration of intervention was 30 days (four weeks).

The observations were made by measuring blood pressure using a sphygmomanometer and measuring serum SOD and MDA levels using the thiobarbituric acid reactive substances (TBARS) method. The resulting data were analyzed by ANOVA. If there was variance, the analysis was followed by Duncan Multiple Range Test.

3. Results and discussion

3.1. Characteristics of the coconut sugar mixed coffee
The tested coffee drinks used in this study were the coconut-sugar mixed coffee with the addition of 0.4% of red palm oil and 10% of coffee powder, which was the best product of the first-year study, and the mixed coffee on the market (cane sugar coffee) as the control. Based on the analysis of the samples of the tested mixed coffee and the control mixed coffee, the results obtained are presented in table 1.
### Tabel 1. Characteristics of coffee drinks used in this study.

| Components                  | Tested Coffee | Cane Sugar Coffee |
|-----------------------------|---------------|-------------------|
| Water content (%)           | 4.607         | 1.351             |
| Ash content (%)             | 2.615         | 0.99              |
| Protein content (%)         | 5.179         | 2.6855            |
| Fat content (%)             | 3.336         | 2.939             |
| Crude fiber content (%)     | 17.69         | 18.1865           |
| Glucose content (%)         | 0.9755        | 0.641             |
| Total sugar content (%)     | 71.134        | 88.205            |
| Carbohydrates by difference (%) | 84.263        | 92.0345           |
| Total phenols (%)           | 0.65845       | 0.845             |
| Total tocopherols (%)       | 0.1238        | 0.0195            |
| Beta-carotene (µg/100 g)    | 454.157       | 99.11765          |

3.2. The effect of feeding mixed coffee on blood pressure of the experimental rats

The study was conducted on six groups in which five groups of rats were exposed to cigarette smoke from two cigarettes per day during the study, and one group of rats was not exposed to cigarette smoke. Each group was given a drink with the following conditions: group 1 (without exposure to cigarette smoke) was given 0.45 g of the tested coffee/CMCRA (P1), group 2 was given distilled water and exposure to cigarette smoke (P2), group 3 was given 0.45 g of CMCRA and the exposure (P3), group 4 was given 0.90 g of the tested coffee and the exposure (P4), group 5 was given 1.35 g of the tested coffee and the exposure (P5), group 6 was given 0.45 g of cane sugar coffee and the exposure (P6).

![Figure 1. Changes in rats' blood pressure during the study.](image-url)

The results of this study showed that the blood pressure in all groups of rats was almost the same at the beginning after the adaptation period, which ranged from 92.4 mmHg to 97.6 mmHg (‘figure 1’). At the end of the study, the end line data showed that the group that was not exposed to cigarette smoke and given 0.45 g/day of the tested coffee (CMCRA) for four weeks had the lowest delta change in blood pressure (0.2 mmHg) while the highest was found in rats that were given distilled water and exposed to cigarette smoke (103.6 mmHg) (‘figure 2’). Group which accept mix coffee with coconut sugar (tested coffee) had the lower increase in blood pressure (5.8 – 28.6 mmHg) than the group which accept coffee mix with cane sugar (79.2 mmHg). Furthermore, the higher the doses of tested coffee, the lower increasing of blood pressure. The average of blood pressure at the beginning up to the end in the groups exposed to cigarette smoke which given of 0.45 g/day of cane sugar coffee was 92.4–171.6 mmHg with...
a delta of 79.2 mmHg, given 0.45 g/day of the tested coffee was 93.2–121.8 mmHg with a delta of 28.6 mmHg, given 0.90 g/day of the tested coffee was 93.6–108.8 mmHg with a delta of 15.2 mmHg, and that were given 1.35 g/day of the tested coffee was 95.4–101.2 mmHg with a delta of 5.8 mmHg.

Figure 2. Delta changes in rats’ blood pressure during the study period.

(P1 = 0.45 g of tested coffee, not exposed; P2 = distilled water, exposed; P3 = 0.45 g of tested coffee, exposed; P4 = 0.90 g of tested coffee, exposed; P5 = 1.35 g of tested coffee, exposed; P6 = 0.45 g of cane sugar coffee, exposed).

It can be seen that the increased blood pressure of the rats exposed to cigarette smoke can be further suppressed along with the increasing dose of coconut-sugar mixed coffee given. This result is probably caused by the higher content of antioxidant components such as tocopherols and beta-carotene (0.1238% and 454.16 µg/100 g) than the cane sugar coffee/cane sugar mixed coffee (0.0195% dan 99.12 µg/100 g). Antioxidants play a role in reducing oxidative stress so that the blood pressure does not increase. 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 [25].

3.3. The effect of feeding mixed coffee on serum SOD levels
Superoxide dismutase (SOD) is an enzymatic antioxidant that works to protect cells from the damage caused by reactive oxygen species (ROS) that have the potential to damage cells such as superoxide and hydroxyl radicals. It can be seen that the serum SOD levels were relatively unchanged in the group of rats not exposed to cigarette smoke while the SOD levels decreased in the groups of rats exposed to cigarette smoke. The exposure to cigarette smoke causes an increase in oxidative stress; thereby, the endogenous antioxidant enzyme (SOD) will encounter the attack of ROS produced by cigarette smoke. The intervention using mixed coffee drinks that contain antioxidants (beta-carotene, tocopherols, and lycopene) could reduce the decrease in the amount of serum SOD.

Notes: P1 = 0.45 g of tested coffee, not exposed; P2 = distilled water, exposed; P3 = 0.45 g of tested coffee, exposed; P4 = 0.90 g of tested coffee, exposed; P5 = 1.35 g of tested coffee, exposed; P6 = 0.45 g of cane sugar coffee, exposed.
The decrease in serum SOD levels in the tested coffee groups (6.75–44.62%) was lower than the cane sugar coffee group (45.97%) or the negative control group (60.44%). The higher the dose of the tested coffee given, the lower the decrease in serum SOD levels due to exposure to cigarette smoke (‘figure 3’). The tested coffee contained antioxidants (i.e., vitamin E/tocopherols and beta-carotene) derived from red palm oil added to the production process of high-in-antioxidants mixed coffee. Thus, the higher the dose of the tested coffee, the higher the intakes of vitamin E and beta-carotene. These results were in line with a researcher who stated that the administration of vitamin E could improve the serum antioxidant status, among others, by increasing the amount of serum SOD [26]. Another research reported that vitamin E intervention in patients with chronic periodontitis could improve SOD activity [27].

3.4. The effect of feeding high-in-antioxidants mixed coffee on serum MDA levels

The high-in-antioxidants mixed coffee given to the rats exposed to cigarette smoke could significantly suppress serum MDA levels better than the rats that were given cane sugar coffee or distilled water. The addition of red palm oil rich in beta-carotene, tocopherols, and other antioxidant components in the production of the mixed coffee increased the antioxidant content of the product. Therefore, the product could suppress the oxidative reaction, which was indicated by the lower serum MDA levels in the groups given the tested coffee than the groups receiving cane sugar coffee or distilled water (figure 4).

The research results showed that the delta increase in serum mda levels was lower in the groups that were given the tested coffee (p3, p4, p5) (0.51–5.73 nmol/ml) than the cane sugar coffee group or p6 (6.69 nmol/ml) or the which given distilled water or p2 (8.28 nmol/ml) (‘figure 4’). The deltas changes in MDA level of The tested coffee contained vitamin E (tocopherols) and beta-carotene that could counteract free radicals from the 2-h cigarette smoke exposure given to the experimental rats every day, thereby decreasing the amount of MDA produced. In line with this research there were reported that the 300 mg/day vitamin E supplementation for three months in patients with diabetic retinopathy could decrease serum MDA levels, which mean that it could suppress oxidative stress in diabetic patients.[27].
4. Conclusion
High-in-antioxidants mixed coffee which used coconut sugar as a sweetener had a better anti-oxidative-stress effect as indicated by the lower delta increase in serum MDA levels of the tested-coffee groups (0.51–5.73 nmol/mL) than the cane sugar coffee group (6.69 nmol/mL) and the lower serum SOD levels of the tested-coffee groups (6.75–44.62%) than the cane sugar coffee group (45.97%).

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