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Robusta Coffee Decreased Malondialdehyde Levels in Wistar Mice Experiencing Oxidative Stress

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ABSTRACT

Coffee drinking is a habit that still doubtful for it's benefits. Many active ingredients in robusta coffee (*Coffea canephora*) are very useful for health, especially the antioxidant content that can ward off free radicals that absorbed in the body. Free radicals can be caused by psychological stress exposure. Excessive amounts of radicals in the body cause oxidative stress. This study aimed to see the robusta coffee effect on decreasing levels of Malondialdehyde (MDA) with "randomized post test only control group" design with 25 samples of white male rat strain wistar (*Rattus norvegicus*). Samples were divided into 5 groups: control groups and randomized treatment groups. Treatment given in psychological stress and giving robusta coffee drink with single dose. This study used "one way anova" test and followed by "pos hoc tukey HSD" test for statistics. Robusta coffee had an effect in MDA levels changing. There was a MDA levels decreasing in 1 hour after robusta coffee drinking in experimental rats with oxidative stress conditions that were exposed by psychological stress.

Keywords: Robusta coffee, Malondialdehyde, Oxidative stress

INTRODUCTION

There were lots of researches on oxidative stress, the cause of pathological conditions in degenerative diseases. Degenerative diseases incidence is increasing, especially in developed countries. This is in line with the report presented by WHO in 2008 and 2010 showing an increase, in example: degenerative diseases was causing deaths from 13% of morbidity (WHO, 2010).

Free radicals, as the main factor for causing oxidative stress, can come from within and outside the body (Phaniendra et al, 2015). Psychological stress is the source of free radical formation that comes from within the body, in which the stress triggered from outside the body. The incidence of psychological stress increases with the increase in human needs. Either it was because of excessive workload, work time that exceeds the body's ability, and shift work patterns. This leads to a decline in kinship relationships, disruption of sleep quality, and ultimately affects the onset of depression, anxiety, and psychological stress (Kristanti, 2013; Maramis, 2009).

Psychological stress that occurs protracted can cause increased ROS (Reactive Oxygen Species) in the body. Increased ROS that is not offset by body resistance to ROS, as an increase in endogenous antioxidants, can lead to oxidative stress (Yoshikawa and Naito, 2002). Oxidative stress triggers the pathological state of chronic degenerative diseases, such as Diabetes Mellitus (DM), coronary heart disease (CHD), osteoarthritis (OA), carcinogenesis, aging process faster than expected, neurodegenerative diseases (Parkinson's and Alzheimer's), and many other diseases caused by oxidative stress (Pham-Huy et al, 2008; Ullah et al, 2016; Vichova and Motovska, 2013; Naoko et al, 2016; Klaunig et al; 2010; Naidoo and Birch-Machin, 2017; Geon et al, 2015).

Anticipation needs to be done to overcome oxidative stress by triggering the increase or activation of enzymatic antioxidants in the body with functional food consumption and natural ingredients use, such as herbs (Lobo et al, 2010). Robusta coffee (*Coffea canephora*) is an agricultural product from Indonesia that has been widely consumed by the world community. During this time, there is a lot of controversy about coffee consumption although there are benefits from coffee beans. The content of antioxidants in robusta coffee is expected to cope with the state of oxidative stress (Rostagno et al, 2015). Therefore, it is necessary to do research on how fast coffee consumption can reduce the state of oxidative stress, especially those triggered by psychological stress exposure.

METHODS

This research was conducted in Biochemistry Laboratory, Faculty of Medicine Airlangga University, Surabaya, in August-September 2017. This research was a type of laboratory experimental research with design

research Randomized Post Test Only Control Group Design. So, the determinations of experimental animals to be used in this research were done in randomly simple way and it compared the experimental groups with the control groups. There were 5 groups of experimental animals consisting of 2 control groups: normal control and stress control, and 3 experimental groups. The experimental animals used in this study were 25 male white rats of wistar strains (*Rattus norvegicus*). Each group consisted of 5 white male rats. Age of mice were ± 2-3 months with body weight 150-200 gram.

Psychological Stress

The rats were acclimatized for 7 days then randomly grouped into 5 groups. Each group consisted of 5 male white rats of wistar strains. The Normal Control Group (K1) didn't get any treatment. Stress Control Group (K2) and Experiment Group (P1, P2, P3) were given psychological stress treatment. Psychological disorders were given by disturbing the rats sleep patterns. The rats were awakened in a shocked way and given bright daylight lighting, while rats were left to sleep by turning off the lights at night. This treatment was given for 1 x 24 hours.

Robusta Coffee

Robusta coffee used for this study was came from PTPN XII Durjo, Jember, East Java. 200 ml of robusta coffee contained 2.46% caffeine, 5.92% polyphenol, and 4.82% flavonoids. Robusta coffee examination was done at the Central Health Laboratory Surabaya in 2017. Robusta coffee steam came from pure coffee type robusta previously roasted at a temperature of 150°C, milled to smooth, and used as a cup of coffee 20 grams in 400 ml of water which was comparable to 2 cups of coffee, and converted in doses of rats by 0.018. Robusta coffee was given at one drink. The coffee was given shortly after the end of psychological stress treatment. The groups that were given the robusta coffee treatment was only Experimental Groups (P1, P2, and P3).

Measurement of Malondialdehyde (MDA)

Blood samplings for MDA concentration were performed in the P1 experimental group at 1 hour after the Robusta coffee treatment, while P2 group at 6 hours after the coffee treatment, and P3 group at 24 hours after the Robusta coffee treatment. For the stress-control group, blood samplings were performed shortly after the stress treatment ended, whereas the normal control group was no restriction on blood-taking time.

MDA examination levels was performed on rat animal serum because the examination was a major marker of oxidative stress. MDA is the resultant compound of lipid peroxidase reactions. The method of examination of MDA levels was done by Thiobarbituric Acid Reactive Substance (TBARSC) technique. The results was showed using spectrophotometry (Rich et al., 2007).

Data Analysis

The data were analyzed by descriptive analysis of the research characteristics subjects, then analyzed the difference with One Way Anova statistical test and continued with Tukey HSD test. The difference was considered significant if the p value < 0.05 with 95% confidence interval. Previously, the data should be fulfill One Way Anova test criteria of normal distribution with One Sample Kolmogorov-Smirnov normality test and homogenous data variations by performing the Lavene Test.

RESULTS

Based on the research, the average malondialdehyde (MDA) levels of all experimental groups as Table 1.

Table 1. Average MDA (nmol/ml) and Standard Deviation of Control and Experimental Groups on Wistar Strain White Rats

Group	n	Malondialdehyde (MDA) (nmol/ml)
		Mean ± SD
K 1	5	3.77680 ± 0.229757
K 2	5	4.91680 ± 0.651671
P 1	5	3.86180 ± 0.541284
P 2	5	3.77020 ± 0.666323
P 3	5	3.76840 ± 0.549536

Information:
K1: Normal Control Group (no treatment); K2: Stress Control Group (with only stress treatment); P1: Experiment Group 1 (blood sampling in 1 hour after the Robusta coffee treatment); P2: Experiment Group 2 (blood sampling in 6 hour after the Robusta coffee treatment); P3: Experiment Group 3 (blood sampling in 24 hour after the Robusta coffee treatment)

Based on the table, MDA level in the Normal Control Group (K1) was 3.77680 ± 0.229757 nmol/ml. The highest MDA level was in the Stress Control Group (K2) with 4.91680 ± 0.651671 nmol/ml. MDA level of the Experimental Group P1 was close to the value of the Normal Control Group (K1) to 3.86180 ± 0.541284 nmol/ml. MDA level of the P2 Experimental Group was 3.77020 ± 0.666323 nmol/ml and MDA level in the P3 Experimental Group was 3.76840 ± 0.549536 nmol/ml.

MDA levels in all groups had a normal distribution ($p > 0.05$) using a simple Kolmogorov-Smirnov single test. Normal Control Group (K1) with $p = 0.780$, Stress Control Group (K2) with p value = 0.734, Experiment Group P1 with p value = 0.658, Experiment Group P2 with p value = 0.500, and Group Experiment P3 with p value = 0.515. Homogenity test by using Lavene Test on MDA data showed homogeneous data on all groups of experimental animals with $p = 0.402$ ($p > 0.05$). One Way Anova test in all experimental groups on MDA content showed $p = 0.013 < 0.05$. So, it could be stated that there was significant difference of MDA level to giving robusta coffee in all group of experiment animals.

To find out which groups differed significantly, then Tukey HSD test was significant, if the value of $p < 0.05$. Tukey HSD test results on MDA levels could be shown in Table 2.

Table 2. P Value Tukey HSD Test Malondialdehyde (MDA) Levels

Group	K 1	K 2	P 1	P 2	P 3
K 1	-	0.028	0.999	1.000	1.000
K 2	0.028*	-	0.046	0.027	0.026
P 1	0.999	0.046*	-	0.999	0.999
P 2	1.000	0.027*	0.999	-	1.000
P 3	1.000	0.026*	0.999	1.000	-

Information:
K1: Normal Control Group (no treatment); K2: Stress Control Group (with only stress treatment); P1: Experiment Group 1 (blood sampling in 1 hour after the Robusta coffee treatment); P2: Experiment Group 2 (blood sampling in 6 hour after the Robusta coffee treatment); P3: Experiment Group 3 (blood sampling in 24 hour after the Robusta coffee treatment)

Based on Table 2, it was known that there was a difference between Normal Control Group (K1) and Stress Control Group (K2) with significant value p = 0,028. In addition, there was a difference between Stress Control Group (K2) and group P1, group K2 with group P2, and group K2 with P3 group with significant value $p < 0.05$ in p value 0.046; 0.027; and 0.026. This could be explained with the graph in Figure 1.

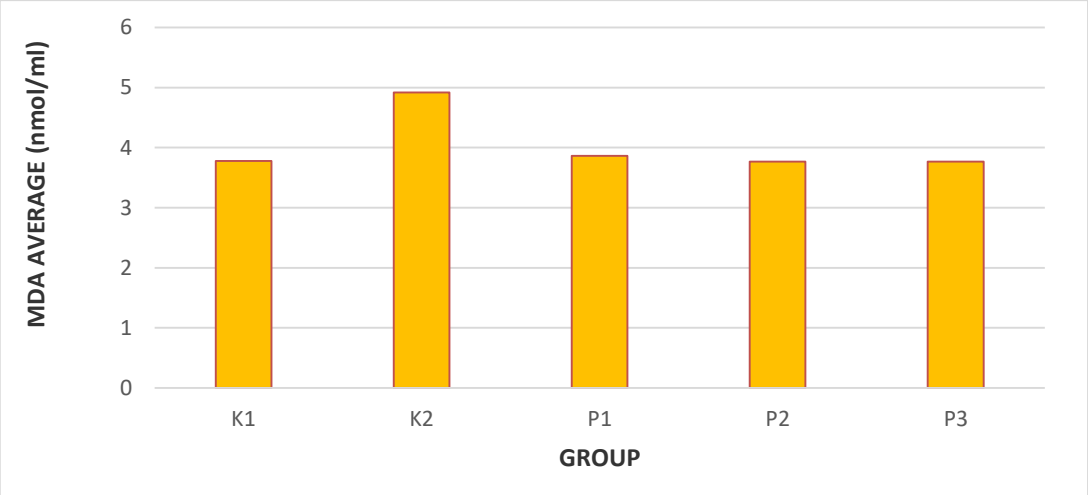

Figure 1. Average of Malondialdehyde (MDA) Levels in Experimental Group

Based on figure 1, it was known that the Normal Control Group (K1) and Group experiments P1, P2, and P3 were different from the Stress Control Group (K2). There was a noticeable difference in the Stress Control Group (K2), where the mean MDA content was higher than the Normal Control Group (K1) and the highest of all the study groups. Mean of MDA concentration in Experiment Group P1, P2, and P3 had values that weren't much different from Normal Control Group (K1).

From the data of MDA levels in all animal groups in this study, it was found that the Stress Control Group (K2) had the highest levels of MDA compared to all other groups, while the Normal Control Group (K1) and the entire Treatment Group (P1, P2, P3) had an unequal mean rate.

DISCUSSION

Psychological stress exposure which given to experimental rats by altering sleep patterns and waking up would cause disturbances in circadian rhythms by affecting the physiological clock and synchronization with the surrounding environment. This caused the body to become susceptible to neuropsychiatric disorders and to fall under conditions of psychological stress (Karatsoreos, 2014; Hawari, 2016).

Some hormones were secreted as the body's response to the occurrence of psychological stress, including norepinephrine, epinephrine, and cortisol hormone (Guyton and Hall, 2016). All three could trigger an increase in blood pressure and speed up the heartbeat that triggered the turbulence of vascular blood flow (Lu and Kassab, 2011). This situation triggers the formation of ROS (Reactive Oxygen Species) in the body by activating the Rac-1 protein that will activate NADPH oxidase. NADPH oxidase converts O_2 to O_2^- (Dusting et al, 2005)

An increase in free radicals amount that exceed endogenous antioxidants production caused oxidative stress (Yoshikawa and Naito, 2002). Free radicals destroy important compounds in the body that affect the defense of cell integrity. Lipids are the earliest body compounds react with free radicals. ROS reacts with the fatty acid component residing in the cell membrane resulting in a chain reaction known as lipid peroxidase. MDA is a metabolite in the form of aldehyde which is the main result of lipid peroxidase by free radicals (Kaya et al, 2007). The result of this reaction is a disturbance of membrane function as well as changes in membrane flow and permeability which ultimately lead to significant cellular dysfunction.

Increased levels of MDA in the blood suggest the occurrence of oxidative stress in the body (Halliwell, 2007). This was shown in the Stress Control Group (K2) which showed an improvement when compared to the Normal Control Group (K1) and the entire Experiment Group (P1, P2, P3). This condition indicated that psychological stress exposure for 1 x 24 hours would lead to an increase in endogenous antioxidants number and causes the state to be oxidative stress. This research was supported by research conducted by Myin (2017). Students with psychological stress in facing exams experienced elevated levels of MDA with a significant positive correlation to lipid peroxidase.

A quite different situation occured in the Normal Control Group (K1) and the entire Experiment Group P1, P2, and P3, where the average MDA content had almost the same value. MDA levels decreasing occurred at 1 hour after giving robusta coffee, then decreased at 6 and 24 hours where the value equaled the mean in the Normal Group (K1). Thus, the active ingredient of robusta coffee begun to decrease MDA levels starting at 1 hours and consistently lowered MDA levels until the 24th hour. This mechanism of decreased MDA levels is played by the active ingredient of caffeine which inhibits lipid peroxidase. So, the amount of ROS could be reduced. The results of this study were supported by Amer et al's (2017) study which stated that caffeine works to decrease MDA levels and decrease TBARS (Thiobarbituric Acid Reactive Substance) which indicates reduction of oxidative stress in the membrane. In a publication performed by Devasagayam et al (1996), caffeine concentrations in millimolar could decrease lipid peroxidase in rats liver chromosome and protected the membrane against damage caused by reactive oxygen radicals, in which caffeine acted as an antioxidant. Decreased levels of MDA were also done by caffeine, reducing the production of ROS through the mechanism of inhibition of cortisol hormone released, thereby reducing the cause of blood flow turbulence that triggers the active NADPH oxidase (Lovallo et al, 2008).

Other active ingredients that also decrease MDA levels are flavonoids that reduce the amount of free radicals by scavenging, especially in superoxide anions (O2-). The content of fenolic acid coffee also contributes to the decline of this MDA marker. Of all the phenolic acids contained in Robusta coffee, the greatest part of it is chlorogenic acid (CGA). CGA inhibits oxidative stress by suppressing ROS production in cells through the optimal mechanism of oxygen scavenging and chain breaking activities in vitro (Dandan et al, 2017).

CONCLUSION

Based on the results of this study, robusta coffee given in experimental animals experiencing oxidative stress significantly reduced the levels of Malondialdehyde (MDA) until were closed by the average group level of normal rats, starting at 1 hour after robusta coffee consumption, and continued at the 6th and 24th hours.

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