EFFECTS OF THE ADMINISTRATION OF BREWED ROBUSTA COFFEE LEAVES ON TOTAL ANTIOXIDANT STATUS IN RATS WITH HIGH-FAT, HIGH-FRUCTOSE DIET-INDUCED METABOLIC SYNDROME

Nurmasari Widyastuti, Gemala Anjani, Vita Gustin Almira, Suci Eka Putri, Amali Rica Pratiwi

ABSTRACT
Robusta coffee (Coffea canephora) leaves contain phytochemical compounds and have antioxidant and anti-diabetic effects. This study investigated the effect of brewed Robusta coffee leaves on the total antioxidant status in metabolic syndrome rats. Metabolic syndrome in rats was induced by high-fat-fructose diet containing pork oil (20%), cholesterol (1.5%), cholic acid (0.5%), standard feed (80%), and fructose (1 mL per 200 g BW). The animals were categorized into normal control group (K1), metabolic syndrome control group without treatment (K2), mangiferin treatment group (X1), brewed Robusta coffee leaves 0.09 g per 200 BW group (X2), brewed Robusta coffee leaves 0.18 g per 200 BW group (X3), and brewed Robusta coffee leaves 0.36 g per 200 BW group (X4). Each dose of the coffee leaves was brewed with 3.6 mL of water at 70 °C for 10 min. The intervention was administered for 28 days. There was a significant increase in the total antioxidant status (p <0.000) in all the groups. In conclusion, the administration of brewed Robusta coffee leaves increased the total antioxidant status in metabolic syndrome rats.

Key words: metabolic syndrome; Robusta coffee leaves; total antioxidant

INTRODUCTION
Metabolic syndrome is a cluster of metabolic disorders characterized by hyperglycemia, hypertension, obesity, low high-density lipoprotein (HDL), and hypertriglyceridemia (Srikanthan et al., 2016). Obesity and insulin resistance are the known risk factors for metabolic syndrome. The prevalence of metabolic syndrome has been increasing every year. From 2009 to 2013, the prevalence of metabolic syndrome increased from 28.84% to 30.52% in adults >30 years of age in Korea (Lee et al., 2018). In Indonesia, its prevalence by the province in 1995 – 2007 was about 21.66%, with the highest prevalence noticed in Jakarta (Herningtyas and Ng, 2019).

The main mechanism of obesity and insulin resistance is oxidative stress (Hurrle and Hsu, 2017; Marseglia et al., 2015), which damages both insulin secretion by the pancreatic β-cells and glucose transport in the muscle and adipose tissues (Marseglia et al., 2015). Oxidative stress is caused by increases in lipid peroxide, malondialdehyde (MDA), carbonyl protein, and oxide xanthine activity because of an imbalance between free radicals and antioxidants (Mancini et al., 2015; Tangvarasittichai, 2015). The body has natural defenses in the form of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), dan glutathione peroxidase (GPx). Besides, consumption of a diet high in antioxidants provides defence against oxidative stress (de Souza Cardoso et al., 2018). Robusta coffee leaves are consumed by the people of West Sumatra, Indonesia, as a healthy beverage (Rasyid, Sanjaya and Zulhamrni, 2013). These leaves contain caffeine, chlorogenic acid, flavonoid, and mangiferin, which have antioxidant and anti-diabetic properties (Chen, Ma and Kitts, 2018).

In this study, we evaluated the effect of administration of brewed Robusta coffee leaves processed using the Japanese style green tea process (JGTP) (Che, Ma and Kitts, 2018) and mangiferin on the total antioxidant status in a rat model of high-fat-fructose diet (HFFD)-induced metabolic syndrome.

Scientific hypothesis
Brewed Robusta coffee leaves increase the total antioxidant status in rats with metabolic syndrome.

MATERIAL AND METHODOLOGY
This study is included in the research project Study of The Administration of Brewed Robusta Coffee Leaves for Metabolic Response In Vivo in Metabolic Syndrome supported by funding received from Faculty of Medicine, Universitas Diponegoro 2019.
The processing of Robusta coffee leaves using JGTP

Robusta coffee leaves were picked from 2nd, 3rd, and 4th leaves of each branch of Robusta coffee plants. Leaves were sorted and then blanched for 75 s. They were then dipped in water and placed in a seder for ±15 min to separate the leaves from the midrib. Next, the leaves were crushed using a crushing-tearing-curving machine 3 times. Finally, the leaves were dried for 4 – 5 h in a rack drier machine at 80 °C. The dried leaves were stored in airtight containers. The process was carried out in a mini-processing green tea processing laboratory at The Tea Quality Processing & Testing Laboratory in The Tea and Quinine Research Center Gambung, Bandung, Indonesia.

Animal treatments

Six-week-old male Wistar rats (n = 36), each weighing 150 – 200 g, were acquired from the Centre for Food and Nutrition of Universitas Gadjah Mada, Yogyakarta-Indonesia. The animals were provided standard feed of Comfeed II at 20 g per rat per day and water ad libitum. Body weight gain was recorded weekly and the remaining food was weighed daily. The experiments were approved by The Ethical Committee of Medical Research of Faculty of Medicine, Universitas Diponegoro (No. 16/EC/H/FK-UNDIP/III/2019), Indonesia. Rats were randomly divided into six groups (n = 6 per group): healthy control (K1), metabolic syndrome without treatment (K2), mangiferin 20 mg.kg⁻¹ BW (X1), brewed Robusta coffee leaves 0.09 g per 200 g BW (X2), brewed Robusta coffee leaves 0.18 g per 200 g BW (X3), and brewed Robusta coffee leaves 0.36 g per 200 gBW (X4) groups. All animals, except the K1 group animals, were fed a HFFD for 14 days. This diet contained pork oil (20%), cholesterol (1.5%), cholic acid (0.5%), and standard feed (80%) and was administered orally, while fructose 1 mL per 200g BW was administered by sonde. Metabolic syndrome was defined when the rats had fasting blood glucose ≥110 mg.dL⁻¹, triglycerides >150 mg.dL⁻¹, and HDL <40 mg.dL⁻¹.

Brewed Robusta coffee leaves were administered daily through a gastric sonde. The doses were brewed in 3.6 mL of water at 70 °C for 10 min. Mangiferin was dissolved in 3.6 mL of water and administered through a gastric sonde. The intervention was performed for 28 days.

Blood sample analyses

Fasting blood glucose, triglyceride, and HDL measurements as criteria of metabolic syndrome were performed after 14 days of HFFD administration. Fasting blood glucose, triglyceride, and HDL were analysed by GOD-PAP, GPO, and CHOD-PAP methods respectively. Measurement of total antioxidant status was performed before intervention and at the end of intervention. Total antioxidant status was analysed by ABTS method. Blood sampling to analyze fasting blood glucose, triglyceride, HDL, and total antioxidant status through plexus retroorbital. Blood serum was analyzed in the Centre for Food and Nutrition of Universitas Gadjah Mada Yogyakarta-Indonesia.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistic 22 software. Data are presented as mean ±SD or median. Paired t-test and one-way analysis of variance were used for parametric results; differences between the groups were evaluated using the post-hoc test. Wilcoxon, Kruskal-Wallis, or Mann-Whitney test was used, as appropriate, for non-parametric results.

RESULTS AND DISCUSSION

Oxidative stress occurs when more oxidizing species are produced than the amount of antioxidants present in the body (Morillas-Ruiz and Hernández-Sánchez, 2015). Insufficient antioxidant levels in the body could be overcome by dietary antioxidants (Mirończuk-Chodakowska, Witkowska and Zujko, 2018; Yadav et al., 2016).

In this study, body weight of the animals significantly increased after treatment in all the groups (p <0.05). The X2 group (34.33 ±1.03 g) experienced the highest weight gain after the administration of brewed Robusta coffee leaves compared to other treatment groups (Table 1). The X1 group (22.00 (22.00 – 26.00) g) also experienced weight gain. The weight gain was the highest in the X2 group (43.50 ±1.64 g). The body weight between all groups significantly different before and after treatment (Table 2). Statistically, the body weight in the X4 group was not significantly different from that in the K1 group, as well as the body weight in the X3 group was not significantly different from that in the X4 group. This indicated that the increase in body weight in the X3 group was similar to that in the K1 group.

HFFD administration in rats has been known to cause weight gain and increases in blood glucose, triglyceride, LDL, and cholesterol levels (Nugroho et al., 2012). In this study, rats were administered HFFD for 14 days to achieve metabolic syndrome. In a previous study, HFFD administration caused hyperglycemia and dyslipidemia (Octavia, Djamiatun and Suci, 2017). In another study, a high-fat diet increased fasting blood glucose and a high-fructose diet increased triglyceride levels in rats (Huang et al., 2004). The administration of a high-fat diet increased reactive oxygen species (ROS) and decreased antioxidant enzyme activity (Du et al., 2012; Sreekumar et al., 2002). A high-fructose dietary induces oxidative stress by decreasing the antioxidant defense system (Zhang, Jiao and Kong, 2017).

Rats achieved metabolic syndrome condition with fasting blood glucose (131.14 ±2.13 – 132.70 ±1.48 mg.dL⁻¹), triglycerides (153.49 ±1.96 – 157.18 ±4.88 mg.dL⁻¹), and HDL (24.59 ±1.96 – 26.22 ±1.69 mg.dL⁻¹) as seen in Table 3. Fasting blood glucose and triglyceride levels in the K2, X1, X2, X3, and X4 groups were increased compared to those in the K1 group. Meanwhile, HDL level in the K2, X1, X2, X3, and X4 groups was decreased compared to that in the K1 group.
After the administration of brewed Robusta coffee leaves, the TAS increased in the treatment groups. The higher the doses administered, the greater was the increase in the TAS. The higher TAS levels were found in the X2, X3, and X4 (p = 0.010), X1 (p = 0.000), X2 (p = 0.027), X3 (p = 0.027), and X4 (p = 0.000) groups. Meanwhile, there was no significant difference in TAS in the K2 group (p = 0.063) before and after treatment. Significantly increased TAS levels were found in the X2, X3, and X4 groups, implying that three doses of brewed Robusta coffee leaves could significantly increase TAS levels in rats with metabolic syndrome (Table 4).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre Mean ±SD</th>
<th>Post Mean ±SD</th>
<th>Δ Mean ±SD</th>
<th>p</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 (n=6)</td>
<td>183.00 ±2.82</td>
<td>208.50 ±3.27</td>
<td>25.50 ±1.37</td>
<td>0.000a</td>
<td>0.000a</td>
</tr>
<tr>
<td>K2 (n=6)</td>
<td>195.83 ±2.04</td>
<td>239.33 ±2.16</td>
<td>43.50 ±1.64</td>
<td>0.000a</td>
<td>0.000a</td>
</tr>
<tr>
<td>X1 (n=6)</td>
<td>197.50 ±5.68</td>
<td>220.50 ±5.95</td>
<td>22.00</td>
<td>0.024b*</td>
<td></td>
</tr>
<tr>
<td>X2 (n=6)</td>
<td>196.50 ±3.78</td>
<td>230.83 ±3.37</td>
<td>(22.00 – 26.00)d</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>X3 (n=6)</td>
<td>198.33 ±4.67</td>
<td>226.83 ±4.99</td>
<td>28.50 ±1.87</td>
<td>0.000a*</td>
<td></td>
</tr>
<tr>
<td>X4 (n=6)</td>
<td>201.67 ±3.32</td>
<td>228.17 ±4.70</td>
<td>26.50 ±1.87</td>
<td>0.000a*</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: The Average Body Weight Before and After Treatment.

Note: normal control group (K1); metabolic syndrome control group without treatment (K2); mangiferin treatment group (X1); brewed Robusta coffee leaves 0.09 g/200BW group (X2); brewed Robusta coffee leaves 0.18 g/200BW group (X3); brewed Robusta coffee leaves 0.36 g/200BW group (X4); *p<0.05 = significantly different; a* = paired t-test p<0.05 = significantly different; b* = Wilcoxon test p<0.05 = significantly different; c* = kruskal-wallis test p<0.05 = significantly different; d = abnormal distribution data, displayed in median (min-max).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Δ BW (g) Mean ±SD</th>
<th>p Value</th>
<th>X1</th>
<th>X2</th>
<th>X3</th>
<th>X4</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>25.50 ±1.37</td>
<td>-</td>
<td>0.004a</td>
<td>0.026a</td>
<td>0.004a</td>
<td>0.019a</td>
</tr>
<tr>
<td>K2</td>
<td>43.50 ±1.64</td>
<td>-</td>
<td>0.003a</td>
<td>0.004a</td>
<td>0.004a</td>
<td>0.004a</td>
</tr>
<tr>
<td>X1</td>
<td>22.00</td>
<td>(22.00 – 26.00)</td>
<td>-</td>
<td>-</td>
<td>0.004*</td>
<td>0.004*</td>
</tr>
<tr>
<td>X2</td>
<td>34.33 ±1.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.004*</td>
</tr>
<tr>
<td>X3</td>
<td>28.50 ±1.87</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>X4</td>
<td>26.50 ±1.87</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Mann-Whitney Test Results for Weight Change Before and After Treatment.

Note: normal control group (K1); metabolic syndrome control group without treatment (K2); mangiferin treatment group (X1); brewed Robusta coffee leaves 0.09 g/200BW group (X2); brewed Robusta coffee leaves 0.18 g/200BW group (X3); brewed Robusta coffee leaves 0.36 g/200BW group (X4); *p<0.05 = significantly different.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose (mg/dl) Mean ±SD</th>
<th>Triglyceride (mg/dl) Mean ±SD</th>
<th>HDL (mg/dl) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 (n = 6)</td>
<td>71.29 ±1.53</td>
<td>68.77 ±5.97</td>
<td>86.36 ±2.28</td>
</tr>
<tr>
<td>K2 (n = 6)</td>
<td>132.70 ±1.48</td>
<td>157.18 ±4.88</td>
<td>25.05 ±1.84</td>
</tr>
<tr>
<td>X1 (n = 6)</td>
<td>131.81 ±1.88</td>
<td>153.75 ±3.11</td>
<td>26.22 ±1.69</td>
</tr>
<tr>
<td>X2 (n = 6)</td>
<td>131.56 ±2.57</td>
<td>156.12 ±2.48</td>
<td>26.22 ±1.30</td>
</tr>
<tr>
<td>X3 (n = 6)</td>
<td>131.14 ±2.13</td>
<td>153.49 ±1.96</td>
<td>26.22 ±2.15</td>
</tr>
<tr>
<td>X4 (n = 6)</td>
<td>132.53 ±2.36</td>
<td>156.12 ±2.77</td>
<td>24.59 ±1.99</td>
</tr>
</tbody>
</table>

Table 3: Fasting Blood Glucose, Triglyceride and HDL Level After Administration of HFFD.

Note: normal control group (K1); metabolic syndrome control group without treatment (K2); mangiferin treatment group (X1); brewed Robusta coffee leaves 0.09 g/200 BW group (X2); brewed Robusta coffee leaves 0.18 g/200 BW group (X3); brewed Robusta coffee leaves 0.36 g/200 BW group (X4).

Compared to the baseline level, a significant difference in TAS levels was found after the administration of brewed Robusta coffee leaves and mangiferin in the K1 (p = 0.010), X1 (p = 0.000), X2 (p = 0.027), X3 (p = 0.027), and X4 (p = 0.000) groups. Meanwhile, there was no significant difference in TAS in the K2 group (p = 0.063) before and after treatment. Significantly increased TAS levels were found in the X2, X3, and X4 groups, implying that three doses of brewed Robusta coffee leaves could significantly increase TAS levels in rats with metabolic syndrome (Table 4).

After the administration of brewed Robusta coffee leaves, the TAS increased in the treatment groups. The higher the doses administered, the greater was the increase in the TAS in the metabolic syndrome rats. Rats administered mangiferin also experienced an increase in the TAS. This may be attributed to the presence of phytochemical contents, such as mangiferin, flavonoids, chlorogenic acid, and caffeine, in brewed Robusta coffee leaves. The processing of Robusta coffee leaves by the JGTP method contributes to the retention of more number of phytochemical components (Chen, Ma and Kitts, 2018). In previous study, the extract of Robusta coffee leaves has high antioxidant activity. It is equivalent to the content of phenolic components in the leaves. The phenolic components contained in leaves contributes significantly to antioxidant capacity (Kristiningrum, Cahyanti and Wulandari 2017).
The phytochemical caffeine primarily contributes in improving the antioxidant status. The antioxidant effect of caffeine is exerted by scavenging the hydroxyl radicals (Yamagata, 2018). Caffeine directly inhibits lipid peroxidation and has a high inhibitor level against radical formation. This compound can also reduce oxidative stress and ROS, as well as protect antioxidant system (Jeszka-Skowron et al., 2016; Tellone et al., 2015). The administration of caffeine at 30 and 100 mg.kg\(^{-1}\) reduces lipid peroxidation and increases antioxidant enzyme activity (Demirtaş et al., 2012). Consumption of caffeine 5 mg.kg\(^{-1}\) body weight per day in 2 doses daily can reduce MDA and elevate the total antioxidant capacity (Metro et al., 2017).

Chlorogenic acid also acts as an antioxidant. It donates hydrogen atoms to reduce free radicals and inhibits oxidation reactions. It also oxidizes phenoxyl radicals and stabilizes them through resonance stabilization (Liäng and Kitts, 2016). Chlorogenic acid at 5 mg.kg\(^{-1}\) for 45 days causes a decrease in lipid oxidation and an increase in antioxidant endogenous enzymes in diabetic rats (Pari, Karthikesan and Menon, 2010). High chlorogenic acid shows a high level of efficiency in scavenging DPPH radicals and converting Fe\(^{3+}\) to Fe\(^{2+}\). This compound shows antioxidant activity by donating hydrogen to free radicals (Liäng and Kitts, 2014; Wu, 2007).

5-O-cafeoylquinic acid (5-CQA) is a subclass of chlorogenic acid that has a strong hydroxyl radical scavenger activity with a constant scavenger rate HO of 7.73 × 109 M\(^{-1}\)s\(^{-1}\) (Liäng and Kitts, 2014).

Flavonoids are part of antioxidants that contribute to the high antioxidant capacity and are present in fruits and vegetables. High flavonoid intake is associated with high plasma levels of total antioxidant capacity (Alipour, Rashidkhani and Edalati, 2016). Intake of flavonoids, such as flavan-3-ols, flavonols, and anthocyanins, decreases dyslipidemia, induces antioxidant capacity, and prevents insulin resistance in diabetic patients (Yamagata, 2019). Patients with type 2 diabetes administered flavonoid-enriched chocolate at 27 g per day for a year reduced insulin resistance and improved insulin sensitivity (Curtis et al., 2012). The administration of the flavonoid quercetin at 15 mg per kg per day for 4 weeks decreased lipid peroxidation and increased antioxidant enzyme activity in diabetic rats (Coskun et al., 2005). Mangiferin is a xanthone that is found in high levels in several parts of the mango. It is also found in Arabica coffee leaves and is thought to be found in Robusta coffee leaves (Chen, 2019). Mangiferin confers its antioxidant effects by having a high iron-chelating ability (Imran et al., 2017). The administration of mangiferin at 50 and 100 mg.kg\(^{-1}\) reduces MDA levels in plasma and cardiac tissues and increases the level of SOD in cardiac tissues (Arozal et al., 2015). In previous study, mangiferin at the dose of 10 mg.dL\(^{-1}\) and 20 mg.dL\(^{-1}\) in diabetic rats increased antioxidant defense mechanism, such as SOD and catalase (Muruganandan et al., 2002) Mangiferin at 40 mg per kg per day significantly reduced blood glucose levels and increased plasma insulin levels and antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase. Mangiferin from *Salacia chinensis* prevents oxidative stress and protects pancreatic β-cells in rats with streptozotocin-induced diabetes (Sellamuthu et al., 2013).

### Table 4 Total Antioxidant Status Before and After Treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre Mean ±SD</th>
<th>Post Mean ±SD</th>
<th>Δ Mean ±SD</th>
<th>P</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 (n=6)</td>
<td>2.25 ±0.17</td>
<td>2.06 ±0.90</td>
<td>–0.19 ±0.11</td>
<td>0.010(*)</td>
<td>0.000(**)</td>
</tr>
<tr>
<td>K2 (n=6)</td>
<td>0.29</td>
<td>0.22</td>
<td>–0.14</td>
<td>0.063(*)</td>
<td></td>
</tr>
<tr>
<td>X1 (n=6)</td>
<td>(0.15 – 0.44)</td>
<td>(0.15 – 0.29)</td>
<td>(–0.15 – 0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X2 (n=6)</td>
<td>0.26 ±0.10</td>
<td>0.66 ±0.15</td>
<td>0.44</td>
<td>0.027(*)</td>
<td></td>
</tr>
<tr>
<td>X3 (n=6)</td>
<td>0.26 ±0.10</td>
<td>1.31 ±0.22</td>
<td>0.74</td>
<td>0.027(*)</td>
<td></td>
</tr>
<tr>
<td>X4 (n=6)</td>
<td>0.26 ±0.10</td>
<td>1.37 ±0.20</td>
<td>1.10 ±1.18</td>
<td>0.000(*)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The rats were classified into the following groups: normal control group (K1); metabolic syndrome control group without treatment (K2); mangiferin treatment group (X1); brewed Robusta coffee leaves 0.09 g/200 BW group (X2); brewed Robusta coffee leaves 0.18 g/200 BW group (X3); brewed Robusta coffee leaves 0.36 g/200 BW group (X4).

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