Supplementation of *Garcinia mangostana* Linn and *Vasconcellea pubescens* A.DC Extract Reduced Exercise-induced Oxidative Stress in Rats

Heru Sasongko1*, Sugiyarto2, Renita Wahyu Nur Hidayati1, Bayu Anggoro Saputro1
1Department of Pharmacy, Universitas Sebelas Maret
2Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret

**ABSTRACT**

The objective of this study was to see how supplementation with *Garcinia mangostana* extract (GME) and *Vasconcellea pubescens* extract (VPE) affected exercise performance and oxidative stress in intensely exercised rats. Twenty-five male rats were used in the study. Rats were divided into five groups: normal, stress oxidative control, combination GME:VPE (50:50), combination GME:VPE (75:25), and combination GME:VPE (25:75) groups. The antioxidant activity of a single extract from the literature was used to make the percentage comparison between extracts. The chronic exercise was done by swimming at 10 m/day for two weeks. Rats in the acute exercise groups were treated by swimming on the pail containing water. Blood samples were collected from the orbital sinus to determine hematological parameters. Liver tissue samples for the analysis of malondialdehyde (MDA) and glutathione (GPx) markers. Data analysis was performed statistically using One Way ANOVA. The combination of GME and VPE was shown to be effective in reducing oxidative stress and increasing MDA and GPx enzyme activity. The administration of both extracts also showed changes in the hematological profile.

**Key words:** exercise; hematology; malondialdehyde; total flavonoid.

**INTRODUCTION**

Oxidative stress is defined as a disruption in the balance of reactive oxygen species production and antioxidant defenses (Betteridge, 2000). Free radicals derived from oxygen are classified as Reactive Oxygen Species (ROS), including superoxide radicals (O2-), hydroxyl radicals (OH-), and hydrogen peroxide radicals (H2O2). Enzymes that play a role in increasing the production of superoxide ions include the mitochondrial electron transport chain, NAD(P)H Oxidase, and Xanthine Oxidase. In the body, ROS is constantly being produced and eliminated, as long as the cell still has an endogenous defense against the oxidant. It is shown that low levels of ROS play a role in the physiology of normal cell signaling, or it is important to maintain homeostasis. Uncontrolled ROS production or failure ROS can be caused by oxidative stress, and several pathological abnormalities (Rush et al., 2005).

Oxidative stress causes oxidative failure to fats, proteins, and DNA (Asmat et al., 2016). ROS can cause a lipid peroxidation process that triggers various diseases agents, such as cancer, inflammation, atherosclerosis, and the aging process (Chen et al., 2018). Uncontrolled physical exercise is one of the factors producing oxidative stress (Ribeiro-Samora et al., 2017). Oxidative stress conditions also affect the hematological profile (Abdel-Moneim et al., 2019a), which is thought to be due to the release of inflammatory mediators (Boskabady and Farhadi, 2008). Aerobic exercise can increase oxygen consumption 10-20 times in the body and 100-200 times in skeletal muscle (Revan and Erol, 2011). Several recent investigations have found that NADPH oxidase enzymes in the skeletal play a signaling role in physiological responses to exercise and that they, together with xanthine oxidase enzymes, may contribute to the relative increase in whole-body oxidation (Jackson et al., 2016).

Antioxidants are compounds that can inhibit the process of free radical oxidation by donating an electron. In the body, antioxidants act as a defense mechanism against free radicals, some of them are naturally present in the body, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione-S-transferase (GST) (Sakr and Al-Amoudi, 2012; Sasongko et al., 2018). However, the increase of free radical formation due to external influences can make the defense system less effective so it requires the intake of antioxidants from the outside. Non-essential antioxidants such as α-tocopherol, β-carotene, vitamin C (Grażyna et al., 2017; Olale et al., 2019) and flavonoids can be obtained from various types of vegetables and fruits (Jiménez-Aguilar and Grusak, 2017).

*Corresponding author : Heru Sasongko  
Email : heru_sasongko@staff.uns.ac.id*
Mangosteen (Garcinia mangostana L.) and mountain papaya (Vasconcellea pubescens A.DC.) are some types of plants that are often found in Indonesia (Sasongko et al., 2019). Mountain papaya is a typical plant of the Dieng plateau which has been known to contain flavonoid compounds that can be used as exogenous antioxidants. Mountain papaya extracts have IC50 of 0.983 - 1.2945 mg / 100 mL (Laily et al., 2012). Mangosteen rind contains various secondary metabolites such as xanthone, mangostin, tannin, and flavonoids (Im et al., 2017). These compounds are thought to play a role in determining the amount of antioxidants activity (Hassan et al., 2015). Innovations in oral antioxidants from natural products have been developed strongly in the future (Thomford et al., 2018). The use of a single extract is often considered to lack a strong effect (Sasongko et al., 2020). In natural products, extract mixes are used rather than separate components. At an equivalent dosage, crude plant extracts have been shown to have more activity than separated components (Rasoanaivo et al., 2011). In this study, a combination of mountain papaya and Garcinia mangostana peel extract is investigated thought to have a synergistic effect as an antioxidant. Although not all combinations of substances have a synergistic impact.

METHODS

Materials

Mountain papaya fruits from Dieng plateau Wonosobo and mangosteen peel from Tawangmangu Karanganyar were used as samples test in this study. To confirm the validity of the plant, its sample was determined at the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Wistar rats were obtained from the Faculty of Medicine, Universitas Muhamadiyah Surakarta with the number 1809/A.1/KEPK-FKUMS/1/ 2019. Twenty-five male rats were acclimatized for 7 days. Rats were divided into normal group, control group, and three combination extract doses. Based on a single successive dosage, the combinations of VPE and GME extracts were (60 mg/kg : 300 mg/kg), (120 mg/kg : 200 mg/kg), and (180 mg/kg : 100 mg/kg). The chronic exercise was done by swimming at 10 min/day for two weeks. Rats in the acute exercise groups were treated by swimming on the pail containing water (Arsana et al., 2013). Blood samples were collected from the orbital sinus to determine hematological parameters (Mai et al., 2017). The rats were then euthanized, and their livers were taken for evaluation of oxidative stress.

Measurement of hematological parameters

Blood taken from the rats was then given anticoagulants for analysis of the hematological profile. Measurement of hematologic parameters consisted of erythrocyte (RBC), hemoglobin (HGB), neutrophil (NEU), leukocyte (WBC), and lymphocyte (LYM) followed by Guder (Guder et al., 2014)

Malondialdehyde (MDA) and glutathione peroxidase (GPx) measurement

Five hundred milligrams of the liver were taken and added in 5 mL of 0.15 M Tris-HCl (pH 7.4) solution then homogenized by shaking it until 10% b/v homogenate was obtained. The thiobarbituric acid reactive substances (TBARS) technique was used to measure malondialdehyde using a UV-Vis spectrophotometer (Nurrochmad et al., 2013). Ellman’s method of measuring
glutathione peroxidase levels is known as glutathione peroxidase level measurement (Ellman, 1959).

**Statistical analysis**

The stress oxidative and hematological data were collected to a one-way analysis of variance (ANOVA), supported by a Tukey HSD test post hoc to analyze the significant differences between groups at p<0.05. Subsequently, all data were proved as mean±standard deviation (SD).

**RESULT AND DISCUSSION**

**Levels of MDA and GPx**

The results showed that average MDA and GPx levels differ significantly (p<0.05) between treatments, as shown in Figures 2 and Figure 3. The results showed an increase in MDA levels in the control group, while the treated group also experienced an increase compared with normal rats. When compared to the control group, the levels of MDA in all extract combination groups were significantly different (p<0.05). The group of rats given a combination dose of GME and VPE with a ratio of 75:25 and 50:50 showed the same and significantly different reduction in MDA when compared to a dose of 25:75.

Increased levels of MDA in the control group due to the production of free radicals caused by physical activity exceeds the body’s antioxidant cellular defense resulting in oxidative stress (Jackson et al., 2016; Revan and Erol, 2011). As is known Malondialdehyde (MDA) is one of the results of lipid peroxidation caused by free radicals during maximum physical exercise or endurance training with high intensity (Tsikas, 2017). Glutathione peroxidase levels showed that the control rat group significantly decrease when compared to all other groups (p<0.05). The group of GME and VPE rats with a ratio of 75:25 and 50:50 showed no significant difference when compared with GPx levels of the normal group (p>0.05). This study shows that the group with a higher amount of GME produces a relatively better antioxidant effect. The findings of this study match with Chang et al (2020), a hypothesis that GME intake can reduce hepatic and muscle MDA levels. Exhaustive exercise resulted in higher MDA levels, according to this study. Endurance and high-intensity or exhausting activities result in oxidative stress and inflammation, which causes muscle fatigue and pain (Zheng et al., 2012). MDA levels in the liver have also been found to be higher after prolonged or severe exercise in previous investigations (Chang et al., 2020). After prolonged or severe exercise, xanthine oxidase (XO)-derived ROS, as well as mitochondrial-derived ROS, are major sources of ROS (Hellsten et al., 1997). The oxidation of hypoxanthine to xanthine is mediated by the xanthine oxidase (XO); the subsequent oxidation of xanthine to uric acid creates hydrogen peroxide, which is then removed by GPx and CAT by water conversion (Viña et al., 2000).

**Measurement of hematological parameters**

Table 1 shows the results of hematological profile measurements. Based on the hematological profile, it is known that the control group experienced an increase in WBC, LYM, and NEU values as well as a decrease in RBC and HGB values. When compared to the control group, the GME: VPE (75:25) group showed a significant difference in the LYM and NEU parameters. The GME: VPE (25:75) administration showed significant differences in all parameters besides the HGB value, and GME: VPE (50:50) dose showed no better change when compared to the control group. Administration of GME: VPE (75:25 and 25:75) showed a significant decrease when compared to the normal group. Oxidative stress can affect the increase of WBC, NEU, and decrease of RBC, HB, and HCT values (Abdel-Moneim et al., 2019a, 2019b). According to Sinaga and Zulaini's
that giving mangosteen rind extract showed a decrease of LYM and NEU levels in rats given acute oxidative stress. Exercise-induced IL-6 release was linked to neutrophil mobilization and oxidative activity priming (Boskabady and Farhadi, 2008). Furthermore, IL-6 and adiponectin concentrations are thought to be the most effective agents in correcting WBC count (Johannsen et al., 2012). Repeated exposure to stress, on the other hand, can cause changes in the release rhythm as

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>Control Stress Oxidative</th>
<th>GME: VPE (50:50)%</th>
<th>GME: VPE (75:25)%</th>
<th>GME: VPE (25:75)%</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^3/µL)</td>
<td>94.00 ± 6.08*</td>
<td>101.00 ± 21.7</td>
<td>172.33 ± 73.16</td>
<td>91.00 ± 6.08</td>
<td>81.33 ± 27.90*</td>
</tr>
<tr>
<td>RBC (10^3/µL)</td>
<td>779.33 ± 144.68*</td>
<td>626.67 ± 36.68</td>
<td>714.33 ± 64.22</td>
<td>619.67 ± 142.61*</td>
<td>744.33 ± 10.02*</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>12.50 ± 2.18</td>
<td>11.20 ± 0.87</td>
<td>13.20 ± 2.11</td>
<td>11.47 ± 3.23</td>
<td>12.53 ± 0.50</td>
</tr>
<tr>
<td>LYM (10^3/µL)</td>
<td>67.00 ± 19.31*</td>
<td>95.00 ± 19.29</td>
<td>108.50 ± 38.89</td>
<td>75.50 ± 23.33*</td>
<td>63.67 ± 22.68*</td>
</tr>
<tr>
<td>NEU (10^3/µL)</td>
<td>64.00 ± 26.00*</td>
<td>96.67 ± 11.2</td>
<td>100.00 ± 12.91</td>
<td>40.67 ± 10.80**</td>
<td>17.67 ± 10.43**</td>
</tr>
</tbody>
</table>

Symbols represent statistical significance: *p < 0.05, as compared to negative control group, #p < 0.05, as compared to normal group (n = 4/ group). WBC: White blood cell; RBC: Red blood cell; HGB: Hemoglobin; LYM: Lymphocyte; NEU: Neutrophile.
well as sensitivity to the hormone glucocorticoids (Duclos et al., 2003). The increase in these hormones after acute and chronic exercise can cause other cells and tissues (blood monocytes) to become less sensitive to hormones or their receptors (e Silva et al., 2010).

Exercise causes the body to use more oxygen, which raises the formation of reactive oxygen species (ROS) and affects both enzymatic and non-enzymatic antioxidant defense mechanisms in target tissues and blood (Davies et al., 1982, Husain, 2003). Increased oxygen intake, catecholamine levels, lactic acid generation, raised rate of Hb auto-oxidation, and hyperthermia can all lead to an increase in ROS production during and after exercise (Osorio et al., 2003). Antioxidant intake prevented oxidative damage caused by intensive exercise (Belviranlı et al., 2012). Mountain papaya and mangosteen have flavonoid compounds that can act as exogenous antioxidants (Im et al., 2017, Sasongko et al., 2019). However, few research has looked at the effects of combining different types of extracts as antioxidants, and not all combinations provide a synergistic effect. This study showed a synergistic activity of mountain papaya and mangosteen, however more research needs to be done. Increasing the dosage of extract does not always result in an equal impact. The current study found that acute strenuous exercise resulted in oxidative stress and decreased antioxidant enzyme activity.

CONCLUSION

The combination of GME and VPE was shown to be effective in reducing oxidative stress and increasing MDA and GPx enzyme activity. Increasing the dosage of extract does not always result in an equal impact. Administration of GME and VPE also showed changes in the hematological profile. To be more complete, additional study with a longer exercise period and other blood biochemical markers is required.

REFERENCES


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