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Giving Banana 'Raja' Peel Extract (Musades Paradisiaca Sapientium) After Moderate Intensity Training Reduces MDA Levels In Rats

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ABSTRACT
Exercise that is done regularly, measured and continuously has a positive impact in reducing levels of malondialdehyde (MDA). MDA is an end product of fat peroxidation that can be used as a biological biomaker to describe the degree of oxidative stress. This study aims to analyze the effect of Raja Banana peel extract (mussa paradisiaca sapientum) on reducing MDA levels in Rattus Norvegicus Strain Wistar rats after moderate and high intensity exercise. The study design was the randomized posttest-only group design, 24 tails, male Rattus Norvegicus Strain Wistar, 8 weeks, 160±20 grams and randomly divided into 4 groups. K_1 (n=6, moderate intensity exercise, treadmill with a speed of 14-16 m/min for 15-30 minutes), K_2 (n=6, moderate intensity exercise + giving Raja Banana peel extract, treadmill with a speed of 14-16 m/min for 15-30 minutes), K_3 (n=6, high intensity exercise, treadmill at a speed of 22-25 m/min for 10-20 minutes) and K_4 (n=6, high intensity exercise + giving Raja Banana peel extract, treadmill with a speed of 22-25 m/min for 10-20 minutes). The training interventions were conducted at 17.00-21.00 p.m with a frequency of 7 times/week for 8 weeks. The blood was drawn 12 hours after the last exercise intervention. MDA levels were measured using the Thiobarbituric Acid Reactive substance (TBARs) method. Data analysis techniques was using ANOVA test and LSD post hoc test with the Statistical Package for Social Science (SPSS). Results obtained mean MDA levels at K_1 (291.518±5.551) ng/mL, K_2 (255.037±4.851) ng/mL, K_3 (317.074±7.006) ng/mL and K_4 (274.666±4.919) ng/mL ($p = 0.000$). Based on the results of the study, it is concluded that giving Raja Banana peel extract after moderate and high intensity exercise reduced MDA levels in rats.

Keywords: Banana peel extract, MDA levels, moderate intensity exercise, high intensity exercise.

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BACKGROUND

Exercise is often associated with increased-oxidative stress (Algul *et al*., 2018; Huang *et al*., 2015; Moflehi *et al*., 2012). Oxidative stress is an imbalance between free radical production and antioxidants (Arsana *et al*., 2013; Otani, 2011; Giustarini *et al*., 2009). Oxidative stress conditions are characterized by increased production of Reactive Oxygen Species (ROS) (Esgalhado *et al*., 2015). Excessive production of ROS and oxidative stress contribute to the development of cellular macromolecular oxidation such as lipids, proteins and DNA which can cause pathogenesis of various degenerative and chronic diseases (Spector, 2000). However, this is not well understood.

Exercise can cause an increase in ROS production and oxidative stress (Sachdev and Davies, 2008). Increased ROS production and free oxidative stress depend on the intensity and dose of exercise that performed (Mrakic-Sposta *et al*., 2015). High intensity exercise increases ROS production and oxidative stress more than moderate intensity exercise (Vezzoli *et al*., 2014; Finaud *et al*., 2007; Bailey *et al*., 2007). Increased ROS production and oxidative stress in the body correlate with pathogenesis of various degenerative diseases such as hypertension, atherosclerosis, chronic kidney disease (CKD), heart failure (Esgalhado *et al*., 2015), diabetes mellitus (Bloomer *et al*., 2006), stroke (Hairrudin and Helianti, 2009), coronary heart disease, aging, cancer (Rosahdi *et al*., 2013), insulin resistance and metabolic syndrome (Otani, 2011; Giustarini *et al*., 2009). Therefore, to reduce the risk of illnesses that caused by the effects of increased oxidative stress, it is necessary to have a specific strategy to reduce oxidative stress. Oxidative stress is an imbalance between free radical production and antioxidants (Sandhiutami *et al*., 2017; Arsana *et al*., 2013). Methods that can be used to maintain the balance of free radical production with antioxidants through giving Raja Banana peel extract (*mussa paradisiaca sapientum*) after exercise.

Raja Banana peel extract contains flavonoids and saponins which are effective as scavenger hydroxyl radicals (*OH) and peroxyl radicals (ROO*) (Lee *et al*., 2004). Flavonoids (flavonoids-OH) are reported to act as scavenger peroxyl radicals (ROO*) that will be regenerated into ROOH and act as scavenger hydroxyl radicals (*OH) and regenerated into hydrogen peroxide (H2O2). Peroxil radical and hydroxyl radical regeneration compounds are more stable, while phenoxyl radicals formed (flavonoids-O*) become less reactive to carry out the propagation reaction. Phenoxyl radical compounds become inactive due to increased hydroxyl reactivity of flavonoid compounds (Nijveldt *et al*., 2001). Flavonoid functions as anti-inflammatory, because flavonoids can inhibit the formation of proinflammatory cytokines such as Tumor Necrosis Factor alpha (TNF-α), interleukin-6 (IL-6), Interleukin 1 beta (IL-1β) and interferon-γ (IFN-γ) (IFF-α), interleukin-6 (IL-6), Interleukin 1 beta (IL-1β) and interferon-γ (IFN-γ) (IFN-γ) (Akhlaghi, 2009). Flavonoids can function as chelating agents of Cu and Fe metals which act as catalysts in the Fenton reaction. This reaction includes the reaction of changing hydrogen peroxide to *OH. This chelating process will reduce the catalytic activity of Cu and Fe metals, thereby reducing the formation of radicals *OH will automatically reduce the damage process of Deoxyribonucleic Acid (DNA), reduce the process of fat peroxidation (Akhlaghi, 2009) and reduce oxidative stress in terms of decreased levels malondialdehyde (MDA). MDA is an end product of fat peroxidation that can be used as a biological biomaker to describe the degree of oxidative stress (Bhale *et al*., 2014). Based on the background above, this study aims to analyze the effect of Raja Banana peel extract (*mussa paradisiaca sapientum*) on reducing MDA levels in *Rattus Norvegicus Strain Wistar* after moderate and high intensity exercise.

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METHOD

The study design was the randomized posttest-only group design, 24 tails, male Rattus Norvegicus StrainWistar, 8 weeks, 160±20 grams and randomly divided into 4 groups. K_1 (n=6, moderate intensity exercise, treadmill with a speed of 14-16 m/min for 15-30 minutes), K_2 (n=6, moderate intensity exercise + giving Raja Banana peel extract, treadmill with a speed of 14-16 m/min for 15-30 minutes), K_3 (n=6, high intensity exercise, treadmill at a speed of 22-25 m/min for 10-20 minutes) and K_4 (n=6, high intensity exercise + giving Raja Banana peel extract, treadmill with a speed of 22-25 m/min for 10-20 minutes). All the procedures of the present study were approved by Ethical Committee of Faculty of Medicine, Brawijaya University number 302/EC/KEPK/FKUA/2019.

Moderate intensity exercise was done by rats running on a treadmill with a speed of 14-16 m/min for 15-30 minutes, while high intensity training with a speed of 22-25 m/min for 15-30 minutes (Pranoto *et al*., 2020; Kim *et al*., 2013). The intervention was carried out at 17.00-20.00 a.m with a frequency of 7 times/week for 8 weeks. Giving Raja Banana peel extract was done 12 hours after moderate and high intensity exercise with a dose of 80 mg/kg body weight of experimental animals. Giving Raja Banana peel extract was done by `sonde`. The blood was drawn from the left ventricle of experimental animals as much as 3 ml. It was carried out 12 hours after the last exercise. MDA levels were measured using the Thiobarbituric Acid Reactive substance (TBARs) method (Esgalhado *et al*., 2015).

Statistical analysis was using Statistical Packet For Social Science (SPSS) software. The normality test uses the Shapiro-Wilk test. After the data is normally distributed, homogeneity tests are done using the Levene test. After the data were normally distributed and homogeneous, the ANOVA test was performed and continued with the post hoc LSD test with a significance level (p<0.01).

RESULT

The results of the descriptive analysis of the mean MDA levels can be seen in Table 1.

Table 1.The Average of MDA Levels in Each Group

Group	n	Mean±SD(ng/mL)	F	ANOVA *P-Value*
K_1	6	291.518±5.551	130.046	0.000
K_2	6	255.037±4.851		
K_3	6	317.074±7.006		
K_4	6	274.666±4.919		

Based on Table 1 shows that the average MDA levels in K_2 is lower compared to K_1, K_3 and K_4. Based on the ANOVA test, there is a significant difference in the mean MDA level (p=0.000). Based on the post hoc LSD test, there are significant differences in MDA levels between K_2 and K_1 (p=0.000), K_2 with K_3 (p=0.000), K_2 with K_4 (p=0.000), K_1 with K_3 (p=0.000), K_1 with K_4 (p=0.000), K_3 with K_4 (p=0.000).

DISCUSSION

This study aims to analyse giving Raja Banana skin extract (*mussa paradisiaca sapientum*) to reduce MDA levels in *Rattus Norvegicus Strain Wistar* rats after moderate and high intensity exercise. Based on the results of the study showed that the average level of MDA in K_2 is lower compared to K_1, K_3 and K_4. Based on the ANOVA test, there is a significant difference in the mean MDA level (p=0.000). These results are in line with the

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results of research conducted by Sandhiutami *et al*. (2017) concluded that papaya seed ethanol extract containing flavonoids (antioxidants) significantly reduced MDA levels in rats. Likewise in research conducted by Yuliati *et al*. (2019) concluded that administration of macassar fruit extract (*brucei javanica*) significantly reduced MDA levels in rats. The decrease in MDA levels in K_2 is probably caused by giving Raja Banana peel extract (*mussa paradisiaca sapientum*). The content contained in peel extracts, such as flavonoids and saponins, are effective as scavenger hydroxyl radicals (*OH) and peroxyl radicals (ROO*) (Lee *et al*., 2004). Flavonoids (flavonoids-OH) are reported to act as scavenger peroxyl radicals (ROO*) that will be regenerated into ROOH and act as scavenger hydroxyl radicals (*OH) and regenerated into hydrogen peroxide (H2O2). Peroxil radical and hydroxyl radical regeneration compounds are more stable, while phenoxyl radicals formed (flavonoids-O*) become less reactive to carry out the propagation reaction. Phenoxyl radical compounds become inactive due to increased hydroxyl reactivity of flavonoid compounds (Nijveldt *et al*., 2001). Flavonoid functions as anti-inflammatory, because flavonoids can inhibit the formation of proinflammatory cytokines such as Tumor Necrosis Factor alpha (TNF-α), interleukin-6 (IL-6), Interleukin 1 beta (IL-1β) and interferon-γ (IFN-γ) (IFF-α), interleukin-6 (IL-6), Interleukin 1 beta (IL-1β) and interferon-γ (IFN-γ) (IFN-γ) Akhlaghi, 2009). Flavonoids can function as chelating agents of Cu and Fe metals which act as catalysts in the Fenton reaction. This reaction includes the reaction of changing hydrogen peroxide to *OH. This chelating process will reduce the catalytic activity of Cu and Fe metals, thereby reducing the formation of *OH radicals and will automatically reduce the process of deoxyribonucleic acid (DNA) damage, reduce the process of fat peroxidation (Akhlaghi, 2009) and reduce oxidative stress reviewed from decreased levels MDA.

Exercise can cause an increase in free radical production (Sachdev and Davies, 2008). Increased free radical production depends on the intensity and dose of exercise that is done (Mrakic-Sposta *et al*., 2015). High-intensity exercise increases free radical production more than moderate-intensity exercise (Vezzoli *et al*. 2014; Finaud *et al*., 2007; Bailey *et al*., 2007). Based on the results of the study showed that the average level of MDA in high intensity exercise is higher than moderate intensity exercise. These results are in line with the results of research conducted by Moflehi *et al*. (2012) concluded that MDA levels in high intensity exercise were higher than moderate intensity exercise. High levels of MDA in high intensity exercise are likely because during exercise the oxygen demand increases 10 to 20 times and the oxygen to the muscles increases 100 to 200 times (Candrawati, 2013; Sen, 1995). When oxidative phosphorylation is in the mitochondria, oxygen is reduced by the mitochondrial electron transport system for the formation of adenosine triphosphate (ATP) and water (H2O) (Mrakic-Sposta *et al*., 2015). In oxidative phosphorylation (electron transport) as much as 2-5% of the total oxygen demand can be converted into free radicals so as to produce ROS (Anita, 2014; Arsana *et al*., 2013). In addition, a training that done with heavy intensity and wrong dosage can increase the production of reactive oxygen species (ROS), reduce antioxidants and increase oxidative stress. Increased ROS production and decreased antioxidant production can increase lipid peroxidation which has an impact on increasing MDA production (Pingitore, 2015).

CONCLUSION

Based on the results of the study it is concluded that giving Raja Banana peel extract after moderate and high intensity exercise reduces MDA levels in rats. Giving Raja Banana peel extract at a dose of 80 mg/kg body weight post moderate intensity exercise

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performed 7 times/week for 8 weeks is more effective in reducing MDA levels compared to moderate intensity exercise and high intensity exercise without giving Raja Banana peel extract.

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