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Effects of Red Fruit (*Pandanus Conoideus Lam*) Oil on Exercise Endurance and Oxidative Stress in Rats at Maximal Physical Activity

Fajar Apollo Sinaga[1]*, Pangondian Hotliber Purba[1], Rika Nailuvar Sinaga, Ramlan Silaban[2]

[1]*Department of Sports Sciences, Faculty of Sports Sciences, Universitas Negeri Medan, Medan, North Sumatra, Indonesia;* [2]*Departement of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan, North Sumatera, Indonesia*

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***Correspondence:** Fajar Apollo Sinaga, Department of Sports Sciences, Faculty of Sports Sciences, Universitas Negeri Medan, Medan, North Sumatra, Indonesia. E-mail: sinaga_fajar@yahoo.com
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Abstract

BACKGROUND: Living cells continuously produce free radicals and reactive oxygen species (ROS) as a part of metabolic processes. These free radicals are neutralized by an antioxidant defense system. Maximal physical activity can produce an imbalance between ROS and antioxidants and thus may cause oxidative stress, which is possibly related to fatigue and tissue injury. Red fruit oil contains high beta-carotene and tocopherol as antioxidants which could overcome oxidative stress condition.

AIM: The aim of this study was to determine the effect of red fruit oil on exercise endurance and oxidative stress in rats at a maximal physical activity.

METHODS: Twenty-four male rats were divided into four groups. The control group (I) was administered with 1.5 ml distilled water, intervention groups (II), (III), and (IV) were administered with different doses of red fruit oil (0.15 ml/kgBW, 0.3 ml/kgBW, and 0.6 ml/kgBW, respectively). All groups were trained to swim for 4 weeks and then were forced to swim without a load until being exhausted. The malondialdehyde (MDA), glutathione peroxidase (GPx) levels, and time of swimming to exhaustion were measured in all groups.

RESULT: The results showed that MDA level obtained was decreasing significantly ($p < 0.05$), GPx and time of swimming were increasing significantly ($p < 0.05$) in the intervention groups.

CONCLUSION: The results suggest that red fruit oil can obviously reduce MDA level, increased GPx, and endurance and delay fatigue induced by maximal physical activity in the rat.

Introduction

Physical activity and exercise have significant positive effects in preventing or alleviating mental illness, including depressive symptoms, and anxiety or stress-related disease [1]. Aerobic exercise can provide human health benefits by improving cardiorespiratory fitness which can increase the quality of life, work efficiency, musculoskeletal function, and cardiopulmonary system strength [2]. Besides giving a positive impact on the body, physical exercise also has a negative impact. Maximum physical activity, as well as exhaustive exercise, can elevate oxidative stress, leading to an imbalance between the body's oxidation system and antioxidant enzymes. Hence, accumulation of free radicals such as reactive oxygen species (ROS) can cause damage to many parts of the cells such as proteins, DNA, and cell membranes by stealing their electrons through a process called oxidation [3], [4]. The release of ROS could result in lipid peroxidation in the mitochondrial membrane. Damaged mitochondria were found to reduce cellular respiration and adenosine triphosphate (ATP) generation; they are also among the primary causes of fatigue [5]. Malondialdehyde (MDA) is one of the results of lipid peroxidation induced by free radicals during maximum physical exercise or high-intensity endurance training [6], [7], so MDA is a general indicator used to determine the number of free radicals and indirectly assess the body's oxidant capacity [8].

Several studies claim that oxidative stress can lead to a decrease in the amount of antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx), and glutathione s-transferase (GST) [9], [10], damages on the muscle tissue which is thought to be involved in the process of fatigue, causing muscle pain [11], changes in the value of hematocrit, erythrocytes, leukocytes [12], and decreased hemoglobin levels and morphological changes in the cells of erythrocytes [13], which in turn can affect performance. It is known, creatine kinase (CK) is one indicator of the occurrence of damage from muscle cells [14].

Naturally, the body has a defense mechanism against ROS by an endogenous antioxidant system which consists of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) [15]. This enzyme plays an important role as a first-line protection against the harmful effects of ROS generated by various sources. However, when the production of ROS is excessive, the function of endogenous antioxidant will be limited. Therefore, the supplementation of exogenous antioxidant from diet becomes important to protect cells against the deleterious effect of ROS [16]. The results of several studies reported that the administration of antioxidants derived from natural or synthetic products from outside the body is required to neutralize the free radicals formed during physical activity, especially strenuous physical activity [17], [18], [19, [20].

One of the known natural sources of antioxidants is red fruit (*Pandanus conoideus Lam*) grown in Papua. Research on the content of active compounds in red fruit oil which has medicinal properties has been carried out and was originally intended to reveal its nutritional content. Red fruit oil contains beneficial nutrients or high levels of active compounds, including beta-carotene, tocopherol, and fatty acids such as oleic acid, linoleic acid, linolenic acid, and decanoic acid. [21], [22]. Tocopherol and beta-carotene are active antioxidants believed to be potential on its ability to prevent degenerative and chronic diseases such as cardiovascular disease, atherosclerosis, and cancer. In addition, the Papuan people believe that red fruit can improve physical performance, but it still needs to be proven scientifically.

The purpose of this study was to determine the antioxidant effect of red fruit oil on exercise endurance and oxidative stress in rats at a maximal physical activity. The results are expected to contribute to the development of science and technology, especially as a basis for further research and development phytopharmaca for the improvement of public health, especially for the health of athletes. The results could be applied to athletes during training programs or during the competition so as to support program development, especially the development in the field of sports achievement and health. In terms of the development of science and technology, this research is a form of contribution to disciplines other than sports disciplines to support the athlete's performance.

Materials and Methods

The tools used in this research were laboratory glassware, vortex (Thermo), test tube (Iwaki), Beckman coulter (Beckman), link Dako epitope retrieval (Dako), tissue processor (Leica), spectrophotometer (Shimadzu), analytical balance (Boeco), syringe for oral feeding, flask 10 ml, stopwatch, hairdryer, animal box, syringe 1 ml, funnel, pipette, parchment, spatula, thermometer, air pump, and ruler.

Male rats of Wistar strain weighing 200–220 g were obtained from the Animal House Faculty of Pharmacy, University of Sumatera, Utara. They were placed in plastic cages in a room under standard laboratory conditions (temperature 20–30°C, relative air humidity 45–55%, and 12/12 h light/dark cycle). The rats were fed with a basal diet and water *ad libitum*. All animal experiments conducted during the present study got prior permission from Institutional Animal Ethics Committee, Department of Biology, Faculty of Mathematics and Science, University of Sumatera Utara. Red fruit oil was taken from Papua, Indonesia. Commercial assay kits for the detection of MDA and GPx were bought from Shanghai Korain Biotech Co., Ltd (Shanghai, China). All other chemicals used were of analytical grade and purchased from local suppliers.

This study used 24 healthy male rats. The rats were divided into four groups randomly consisted of six rats in each group. The control group (I) was administered with 1.5 ml of distilled water, intervention groups (II), (III), and (IV) were administered with different doses of red fruit oil (0.15 ml/kgBW, 0.3 ml/kgBW, and 0.6 ml/kgBW, respectively), per day using gavage spuit, for 28 days. The rats were trained to swim for a month, 30 min/day in the 1st week, 35 min/day in the 2nd week, 40 min/day in the 3rd week, and 45 min/day in the 4th week. After 28 days, the rats were forced to perform maximum activity by putting the mice in the water without coming out until the rats were exhausted. The apparatus used was an acrylic plastic pool (60, 50, and 50 cm in length, width, and height, respectively) filled with fresh water, which was maintained at 25 ± 0.5°C at a depth of 40 cm. Exhaustion was determined by observing the loss of coordinated movements and failure to return to the surface within 10 s. The exhaustive swimming time was used as an indicator of exercise endurance and antifatigue effects. Blood samples were collected immediately after the exhaustive exercise, and then MDA; GPx levels were measured using spectrophotometry.

Blood sample (3 ml) was collected into a plain tube and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters, namely, MDA and GPx. MDA and GPx were analyzed using a MDA and total antioxidant capacity assay kit according to the manufacturer's instruction.

Data of research were tested for homogeneity and normality to determine the type of statistics to be used. Data were analyzed using one-way ANOVA test to determine the mean difference between treatments using SPPS 25.0 program. If there is a significant difference, further proceed with the Turkey test to determine the differences value between treatment groups. Based on the significance value, $p < 0.05$ is considered statistically significant.

Results

Experimental animal characteristic

The average weight of rats in the research is shown in Table 1.

Table 1: The average weight of rats (g) before and after the administration of red fruit oil

Body weight (g)	Group				p*
	I	II	III	IV	
Before treatment	204.33	204.66	203.50	204.66	0.201
After treatment	225.33	225.83	225.16	226.00	0.753

Normality and homogeneity tests data show that the average weight of rats before and after the administration of red fruit oil is normal and homogenous ($p > 0.05$). Based on statistical test with one-way ANOVA test, there is no significant difference in weight of rats before and after treatment intervention ($p > 0.05$). This result shows that the use of experimental animals in the research has met the ethical standard for animal welfare. Following the 3-R principle; replacement, reduction, and refinement, in the animal welfare ethical standard is mandatory.

Effect of red fruit oil on MDA level

Based on the results of the analysis, it was found that the mean level of MDA in Groups I, II, and III was 12.32 ± 0.44, 5.20 ± 0.79, 3.72 ± 0.37; and 1.84 ± 0.26 nmol/L, respectively. The normality and homogenous tests showed that the data were normally and homogeneously distributed ($p > 0.05$). Meaning analysis using one-way ANOVA test showed that the mean urea levels in the four groups were significantly different ($p < 0.05$).

As shown in Figure 1, the MDA level of the II, III, and IV groups was significantly lower than that of the I group ($p < 0.05$). MDA level decreased was 57.74, 69.79, and 85.05%, respectively.

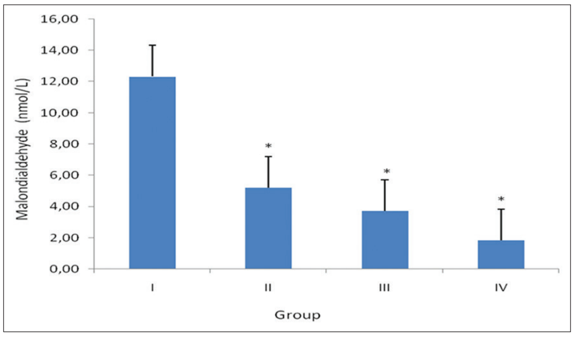

Figure 1: Effect of red fruit oil on malondialdehyde levels in serum of rats.

Effect of red fruit oil on GPx level

Based on the results of the analysis, it was found that the mean level of GPx level in Groups I, II, and III was 35.33 ± 0.66, 57.33 ± 1.43, 66.47 ± 1.13; and 87.92 ± 2.05 U/L, respectively. The normality and homogenous tests showed that the data were normally and homogeneously distributed ($p > 0.05$). Meaning analysis using one-way ANOVA test showed that the mean urea levels in the four groups were significantly different ($p < 0.05$). As shown in Figure 2, the GPx level of the II, III, and IV groups was significantly higher than that of the I group ($p < 0.05$). GPx level increased was 62.27, 88.14, and 148.85%, respectively.

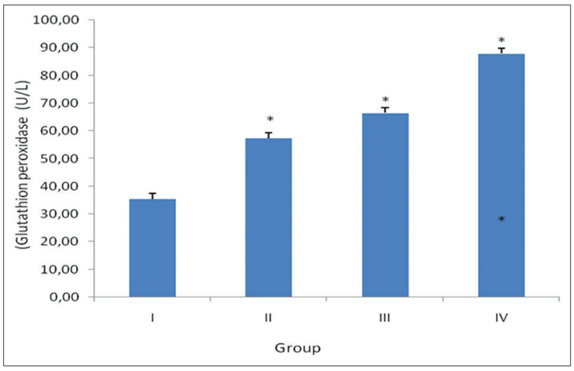

Figure 2: Effect of red fruit oil on glutathione peroxidase levels in serum of rats

Effect of red fruit oil on swimming time to exhaustion of rats

As shown in Figure 3, the exhaustive swimming times in the II, III, and IV groups (65.83 ± 1.47, 76.50 ± 1.05, and 107.5 ± 1.87 min, respectively) were significantly higher than that in the I group (44.00 ± 1.41 min) ($p < 0.05$). Swimming time increased was 49.61, 73.86, and 144.31%, respectively. These results suggest that red fruit oil has anti-fatigue activity and could enhance exercise endurance.

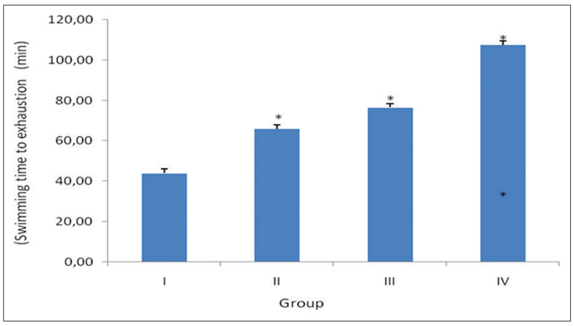

Figure 3: Effects of red fruit oil on swimming time to exhaustion of rats

Discussion

Oxidative stress has been identified as one of the factors leading to fatigue [23]. High levels of oxidative stress lead to an excessive generation of reactive oxygen species (ROS). These species are highly reactive molecules that cause lipid peroxidation in the membrane structure and damage the cellular structure. The release of ROS could result in lipid peroxidation in the mitochondrial membrane. Damaged mitochondria were found to reduce cellular respiration and adenosine triphosphate (ATP) generation; they are also among the primary causes of fatigue [24]. MDA is the main product in lipid cells and the MDA content in tissue is a common in the number of free radicals [25]. During strenuous exercise or high-intensity endurance exercise, of oxygen free radicals increased heavily, while the MDA is one of the main products peroxidation induced by the free radicals. Therefore, determining the MDA content in tissue can evaluate the degree of lipid peroxidation and indirectly body's antioxidant capacity [4], [5], [6].

The result showed the increasing level of MDA in the control group after treatment. The increasing level of MDA in the control group was due to the high free radicals production in maximal physical activity condition. The higher level of MDA compare to the level of defending cellular antioxidant will generate the oxidative stress condition. As mentioned above, the MDA is one of the oxidized lipid product formed by free radical during maximal physical and high-intensity endurance exercises [4], [5], [6].

Statistically, the MDA levels in all treatment groups were remaining low after treatment compared with the control group. This result was due to the antioxidants in red fruit oil that neutralize or scavenge the free radicals. Red fruit oil contains beneficial nutrients or bioactive compounds at high levels, such as beta-carotene, tocopherol, as well as fatty acids [21], [22]. Carotenoids (e.g., β-carotenes) lipid-soluble antioxidants located primarily in biological membranes could can reduce lipid peroxidation; studies show that astaxanthin, a member of the carotenoid family, and a dark-red pigment found in the marine world of algae and aquatic animals such as salmon, red sea bream, as well as in birds such as flamingo and quail, have potential health-promoting effects in the exercise-induced fatigue [26]. The mechanism of action of tocopherols in red fruit oil can easily contribute hydrogen atoms to the hydroxyl group from the ring structure to free radicals so that free radicals become unreactive. By donating hydrogen, tocopherol itself becomes radical, but is more stable because unpaired electrons in the oxygen atom experience delocalization into the aromatic ring structure.

The results of the study reported that red fruit has antioxidant activity that can be used as a free radical scavenger. *In vitro* research shows that red fruit oil shows antioxidant activity with IC50 451.51 μg/ml. In the *in vivo* study, red fruit oil at doses of 0.15, 0.3, and 0.6 mg/kg BW showed the ability to reduce blood MDA levels [27]. The results of this study are in line with the results of other researchers who studied the level of tocopherol after supplementation of red fruit oil in male Wistar rats at maximum activity. They found that tocopherol levels increased when the dose of red fruit oil was increased [28].

In this study also found that the administration of red fruit oil can increase levels of GPx. Increased levels of GPx are caused by high antioxidant content in red fruit oils such as carotene (11,500 ppm), β-carotene (694.80 ppm), tocopherol (11,200 ppm), and α-tocopherol (495.50 ppm) [27]. The increase in the antioxidant GPx is supported by the results of research conducted by several researchers. The administration of tartari buckwheat extract for 28 days in rats was reported to increase GPx activity. Tartary buckwheat extract has been known to have higher antioxidant activity and has been reported as a powerful antioxidant, scavengers from various reactive oxygen species and lipid peroxidation inhibitors [29]. Administration of 20 (S)-Rg3 is reported to have anti-fatigue effects by increasing the complete exercise time of mice, and its anti-fatigue mechanism involves the following factors: First, 20 (S)-Rg3 eliminates the accumulation of metabolites by reducing the level of BLA and SUN; second, 20 (S)-Rg3 activates energy metabolism by increasing glycogen levels in the liver and muscles; and third, 20 (S)-Rg3 protects oxidative stress caused by exercise by increasing levels of SOD, GPx, and CAT, as well as reducing MDA levels in the liver and muscles of mice [30].

The results of this study showed that red fruit oil was able to elevate the rat endurance. This effect was indicated by the longer swimming time in all treatment groups compared to the control group. Statistical analysis showed that the higher red fruit oil dose resulted in a longer swimming time. Several theories are supporting this result, namely, because of the high antioxidant content in red fruit oil. Antioxidants in red fruit oil were considered to prevent lipid oxidation in cellular membrane especially in erythrocyte cells. Some researchers showed that physical activities were able to induce the formation of oxidized lipid and generate the oxidative stress condition. Oxidized lipids are able to cause erythrocyte cell damage and thus cause the "sport anemia" [31], and muscle tissues damage [32]. The damage of muscle and blood cells is considered to be involved in exhaustion processes or the disability to generate energy and therefore decrease the endurance. A study about the effect of antioxidant on the endurance has been conducted and reported that vitamin C was also able to increase endurance in the rat model [33]. Administration of M. Oleifera leaf extract was reported to have anti-fatigue properties. It enhances the swimming ability of rats by delaying the accumulation of blood lactate and blood urea nitrogen, by increasing the mobilization and use of body fat and by slowing depletion of glycogen deposits. Anti-fatigue potential can be expressed through a mechanism that involves the antioxidant activity of the extract. Further research is needed to determine the effect of the extract on chronic physical activity [7]. Ginkgo biloba extract was also reported to be able to increase the activity of antioxidant enzymes in rat liver tissue, reduce the level of oxidized lipids produced by free radicals, and increase endurance and healing processes after maximum physical activity [34]. Similar results were also reported by Miao *et al*. using corn peptides [35].

Conclusion

It can be concluded from the present study that red fruit oil has endurance and delay fatigue induced by maximal physical activity in rat, and its anti-fatigue mechanisms involve the following protects exercise-induced oxidative stress by increasing the levels of GPx, as well as decreasing the MDA levels of rats.

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