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Effects of Red Fruit Oil on Exercise Endurance and Oxidative Stress in Rats

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***Abstract*—This study investigated the effect of red fruit oil on biomarkers of oxidative stress in maximal physical activity. Fourty rats were divided into 4 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, glutathione peroxidase levels and time of swimming to exhaustion were measured in all groups. The results showed that MDA level obtained was decreasing significantly (P<0.05), glutathione peroxidase and time of swimming was increasing significantly (P< 0.05) in the intervention groups. The results suggest that red fruit oil can obviously reduce MDA level, increased glutathione peroxidase and endurance and delay fatigue induced by maximal physical activity in the rat.**

Keywords—red fruit oil, malondialdehyde, antioxidant

I. Introduction

Physical activity if done routinely and regularly is very beneficial for health because it can reduce the risk of cardiovascular disease, diabetes, osteoporosis and also some cancers [1]. Aerobic exercise is very useful for improving the quality of life, strength of the cardiopulmonary system, musculoskeletal function and also work efficiency [2]. Besides giving a positive impact on the body, physical exercise also has a negative impact. Maximum physical activity can cause oxidative stress which causes an imbalance between the production of free radicals with endogenous antioxidants in the body. Free radicals that are formed will react with cell membranes, proteins, DNA and damage cells through oxidation reactions [3, 4]. The free radical reaction that is formed will react with the mitochondrial membrane and damage it thereby reducing 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].

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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], 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 research reports indicate that the administration of natural antioxidants can neutralize the production of free radicals during physical activity, especially strenuous physical activity [17-20].

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]. It is known, Tocopherol and beta-carotene are potential antioxidants to prevent degenerative and chronic diseases such as cardiovascular disease, atherosclerosis and also 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 of this study are expected to contribute to improving the performance and health of athletes while undergoing an exercise program.

II. Materials and Methods

A. Tools

The tools used in this research were laboratsory 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, parchament, spatula, thermometer, air pump and ruler.

B. Animals

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 to 30^{0}C, relative air humidity 45 to 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.

C. Materials

Red fruit oil was taken from Papua, Indonesia. Commercial assay kits for the detection of malondialdehyde (MDA) and glutathione peroxidase (GPx) were bought from Shanghai Korain Biotech Co., Ltd (Shanghai, China). All other chemicals used were of analytical grade and purchased from local suppliers.

D. Experimental Design

This study used 40 healthy male rats. The rats were divided into four groups randomly consisted of ten 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 the maximal activity by putting the rats in water with no exit. 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 seconds. 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

E. Biochemical Assay

Blood samples (3ml) was collected into a plain tube. Then the serum was separated by centrifugation at 2500 rpm for 15 minutes. The serum obtained is used to measure biochemical parameters, namely, malondialdehyde (MDA) and Glutathione Peroxidase (GPx). MDA and GPx were analyzed by using a malondialdehyde and total antioxidant capacity assay kit according to the manufacturer's instruction.

F. Statistical Analysis

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 Tukey test to determine the differences value between treatment groups. Based on the significance value, $p<0.05$ is considered statistically significant.

III. Result And Discussion

A. Experimental Animal Characteristic

The average weight of rats in the research can be seen 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	204,33	204,66	203,50	204,66	0,201
After	225,33	225,83	225,16	226,00	0,753
Δ	21,00	21,16	21,66	21,33	0,719

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.

B. Effect of Red Fruit Oil on malondialdehyde (MDA) level

Based on the results of the analysis it was found that the mean level of malondialdehyde (MDA) in group I, II, and III were 12.32±0.44, 5.20±0.79, 3.72±0.37; 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 ($p<0.05$).

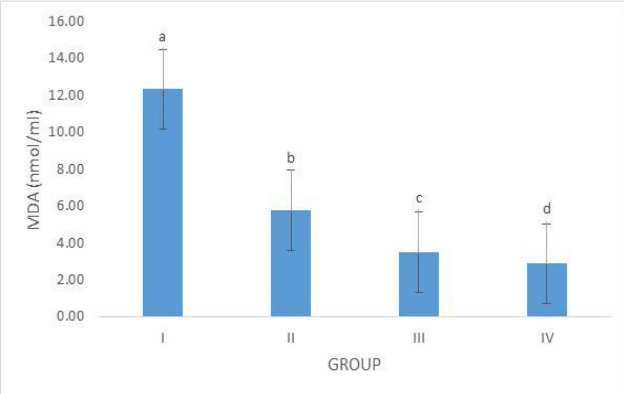

Fig 1. Effect of Red Fruit Oil on malondialdehyde levels in serum of rats. Data are the mean±SD of 6 animal in each group. I, control group, II, 0.15ml/kgbw of RFO, III, 0.30 ml/kgbw of RFO, IV, 0.60 ml/kgbw of RFO. Different letters indicate significant difference at $p < 0.05$ by one-way ANOVA

Oxidative stress caused by strenuous physical activity is one of the factors causing fatigue [23]. Oxidative stress will produce recative oxygen species (ROS) where this species is a very reactive molecule. The reaction of ROS with the mitochondrial membrane causes lipid peroxidation and damages the mitochondrial membrane, thereby affecting the process of adenosine triphosphate (ATP) formation [24]. Malondialdehyde (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 [4-6].

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 [4-6].

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 [26-27]. 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 [28].

C. Effect of Red Fruit Oil on Glutathione Peroxidase Level

Based on the results of the analysis it was found that the mean level of glutathione peroxidase (GPx) level in group I, II, and III were 35.33±0.66, 57.33±1.43, 66.47±1.13; 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 Fig. 2, the glutathione peroxidase (GPx) level of the II, III, and IV groups were significantly higher than that of the I group ($p < 0.05$). Glutathione peroxidase (GPx) level increased were 62.27, 88.14 and 148.85% respectively.

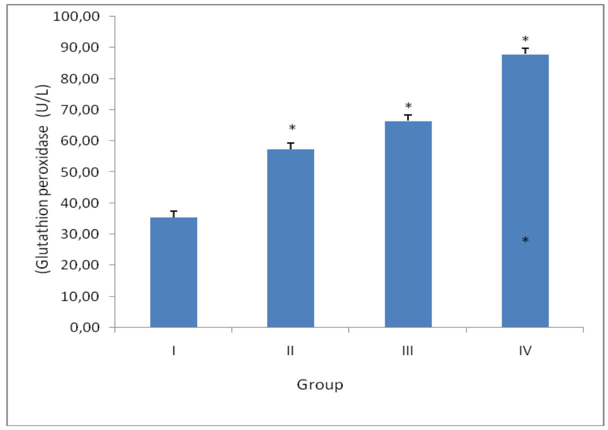

Fig 2. Effect of Red Fruit Oil on Glutathion Peroxidase (GPx) levels in serum of rats. Data are the mean±SD of 6 animal in each group. I, control group, II, 0.15ml/kgbw of RFO, III, 0.30 ml/kgbw of RFO, IV, 0.60 ml/kgbw of RFO. *$P<0.05$ compared with the control (C) group

Results of research conducted by Rohman et al. reported that red fruit has antioxidant activity that can be used as free radical scavengers. Rohman *et al*. reported in vitro study showed that the red fruit oil exhibited antioxidant activity with IC50 of 451.51 μg/ml. In vivo study, red fruit oil with a dosage of 0.15, 0.3, and 0.6 mg/kg BW exhibited the ability to lower the blood MDA level [29]. The result of this research is in line with the research conducted by Sandhiutami et al. which studied the level of tocopherol after red fruit oil supplementation on male Wistar rats at the maximal activity. They found that the level of tocopherol was increasing as the dosage of red fruit oil is risen [30].

In this study also found that the administration of red fruit oil can increase glutathione peroxidase (GPx) levels. The increasing glutathione peroxidase (GPx) level was due to the high antioxidant content in red fruit oil such as carotenoids (11.500 ppm), β-carotene (694.80 ppm), tocopherols (11.200 ppm), and α-tocopherol (495.50 ppm) [27]. Increased antioxidant glutathione peroxidase is supported by the results of research conducted by several researchers. Jin and Wei reported that the administration of tartary buckwheat extract for 28 days in rats could increase glutathione peroxidase activity. Tartary buckwheat extract has been known has higher antioxidant activities, and it has been reported as strong antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation [31]. Jin and Wei. Reported than 20(S)-Rg3 has anti-fatigue effects by enhancing the exhaustive exercise time of mice, and its antifatigue mechanisms involves the following factors: first, 20(S)-Rg3 eliminates the accumulation of metabolites by reducing BLA and SUN levels; second, 20(S)-Rg3 activates energy metabolism by increasing the glycogen levels in liver and muscle; third, 20(S)-Rg3 protects exercise-induced oxidative stress by increasing the levels of SOD, GPx, and CAT, as well as decreasing the MDA levels in liver and muscle of mice [32].

D. Effect of Red Fruit Oil on Swimming Time to Exhaustion

As shown in Fig. 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 were 49.61, 73.86 and 144.31% respectively. These results suggest that red fruit oil have anti-fatigue activity and could enhance exercise endurance.

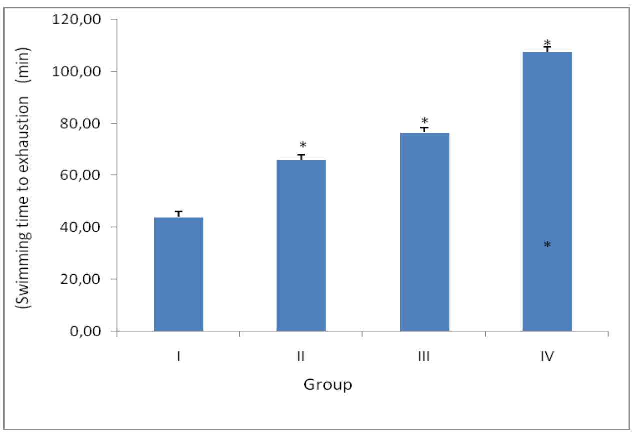

Fig 3. Effects of red fruit oil on swimming time to exhaustionof rats. Data are the mean±SD of 6 animal in each group. I, control group, II, 0.15ml/kgbw of RFO, III, 0.30 ml/kgbw of RFO, IV, 0.60 ml/kgbw of RFO. *P<0.05 compared with the control (C) group.

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 researche 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" [33], and muscle tissues damage [34]. The damage of muscle and blood cells are 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 [35]. Lamou et al. reported the leaf aqueous extract of *M. Oleifera* possesses antifatigue properties. It improved the swimming ability of rats by delaying the accumulation of blood lactate and blood urea nitrogen, by increasing the mobilization and use of body fats and by slowing the depletion of glycogen stores. The antifatigue potential may be expressed through mechanisms that involve the antioxidant activity of the extract. Further studies are needed to determine the effect of the extract on chronic physical activity [7]. Bing and Wang reported that Ginkgo biloba extract was able to increase the activities of antioxidant enzymes in rat liver tissues, reduce the level of oxidized lipid generated by free radicals and increase endurance and healing processes after maximal physical activities [36]. A similar result was also reported by Miao et al using corn peptide [37].

IV. CONCLUSION

This study shows that Red Fruit Oil can enhance exercise endurance and possesses a protective effect against exhaustive swimming exercise induced oxidative stress in rats. The mechanism underlying these effects is based on a decrease in MDA and LDH levels in the serum rats.

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