# The Effects of Red Fruit *(Pandanus Conoideus Lam)* Supplementation on Total Antioxidant Capacity and Creatine Kinase in Rats after Maximal Physical Activity

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Abstract: Maximal physical activity can produce an imbalance between ROS and antioxidants and thus may cause oxidative stress and muscle damage which is possibly related to fatigue and tissue injury. Red fruit oil (RFO) contains high beta-carotene and tocopherol as antioxidants which could overcome oxidative stress condition. This study investigated the effect of RFO on total antioxidant capacity (TAC) and creatine kinase (CK) after maximal physical activity. Forty 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 RFO (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 TAC, CK levels and time of swimming to exhaustion were measured in all groups. The results showed that the obtained CK level decreased significantly ($P<0.05$), TAC and time of swimming increased significantly ($P< 0.05$) in the intervention groups. The results suggest that red fruit oil can obviously reduce CK level, increased TAC and endurance; it can also delay fatigue which is induced by maximal physical activity in the rats.



## 1 INTRODUCTION

Skeletal muscle is a highly specialized tissue with excellent plasticity in response to external stimuli such as exercise and training. The repetitive muscle contractions conducted during endurance training lead to a variety of phenotypic and physiological responses. These responses include activation of mitochondrial biogenesis, fiber type transformation and angiogenesis. Together, they increase the muscle's capacity of aerobic metabolism and its resistance to fatigue. High muscle activity also involves a strong increase in reactive oxygen species (ROS) production (Steinbacher, and Eckl, 2015). 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 via a process called oxidation (Powers et al., 2011). 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 (Broome1 et al., 2018). Malondialdehyde (MDA) is one of the results of lipid peroxidation induced by free radicals during maximum physical exercise or high-intensity endurance training (Yan and Hao, 2016; Lamou et al., 2016), so MDA is a general indicator used to determine the number of free radicals and indirectly assess the body's oxidant capacity (Teng and Wu, 2017).

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 (Thirumalai et al., 2011; Bulduk et al., 2011), damages on the muscle tissue which is thought to be involved in the process of fatigue, causing muscle pain (Wan et al., 2017), with indicators of increased levels of creatine kinase and lactate dehydrogenase (Callegari et al., 2017), changes in the value of hematocrit, erythrocytes,



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leukocytes (Senturk et al., 2004), decreased hemoglobin levels and morphological changes in the cells of erythrocytes (Senturk et al., 2005), which in turn can affect performance. It is known, creatine kinase (CK) is one indicator of the occurrence of damage from muscle cells (Nogueira et al., 2019).

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) (Ighodaro et al., 2018). 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 (Hao, 2014). 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 (Teng and Wu, 2017; Xua et al., 2017; Kalpana, 2012).

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 (Budi, 2005; Alamsyah, 2005). 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 total antioxidant capacity (TAC) and creatine kinase (CK) after 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.

## 2 MATERIALS AND METHODS

### 2.1 Tools

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, parchament, spatula, thermometer, air pump and ruler.

### 2.2 Animal

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.

### 2.3 Materials

Red fruit oil was taken from Papua, Indonesia. Commercial assay kits for the detection of total antioxidant capacity (TAC) and creatine kinase (CK) were bought from Shanghai Korain Biotech Co., Ltd (Shanghai, China). All other chemicals used were of analytical grade and purchased from local suppliers.

### 2.4 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 anti-fatigue effects. Blood samples were collected immediately after the exhaustive exercise, and then TAC; CK levels were measured using spectrophotometry.

## 2.5 Biochemical Assay

Blood sample (3ml) 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, total antioxidant capacity (TAC) and creatine kinase (CK). TAC and CK were analyzed by using a creatine kinase and total antioxidant capacity assay kit according to the manufacturer's instruction.

## 2.6 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.

# 3 RESULTS AND DISCUSSION

## 3.1 Result

### 3.1.1 Effect of Red Fruit Oil on Creatine Kinase Level

Based on the results of the analysis, it was found that the mean level of creatine kinase (CK) in group I, II, and III were 227.84±2.26, 203.77±1.94, 166.74±1.56; 129.29±1.62 U/L, respectively. The normality and homogenous tests showed that the data were normally and homogeneously distributed ($p>0.01$). Meaning analysis using One Way ANOVA test showed that the mean CK levels in the four groups were significantly different ($p<0.01$).

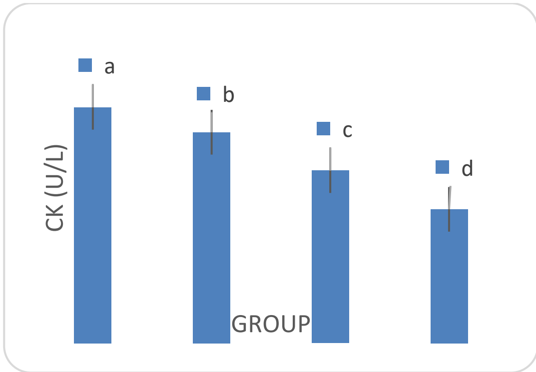


Figure 1: Effect of Red Fruit Oil on Creatine Kinase (CK) levels in serum of rats. Different letters indicate significant difference at $p < 0.05$ by one-way ANOVA

As shown in Fig. 1, the creatine kinase (CK) level of the II, III, and IV groups were significantly lower than that of the I group ($p < 0.01$). The level decreased level of creatine kinase was 10.57, 26.81 and 43.25% respectively.

### 3.1.2 Effect of Red Fruit Oil on Total Antioxidant Capacity Level

Based on the results of the analysis, it was found that the mean level of total antioxidant capacity (TAC) in group I, II, III and IV were 1.54±0.04, 2.34±0.02, 2.64±0.03; 3.25±0.03 U/ml, respectively. The normality and homogenous tests showed that the data were normally and homogeneously distributed ($p>0.05$).

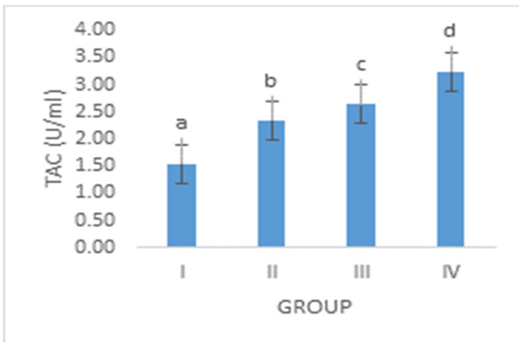


Figure 2: Effect of Red Fruit Oil on Total Antioxidant Capacity (TAC) levels in serum of rats

Meaning analysis using One Way ANOVA test showed that the mean scores of TAC in the four groups were significantly different ($p < 0.05$). As shown in Fig. 2, the total antioxidant capacity (TAC) levels of the group II, III, and IV were significantly higher than that of the group I ($p < 0.01$). The increased Total Antioxidant Capacity (TAC) levels were 34.02, 109.30 and 100.17% respectively.

#### 3.1.3 Effect of Red Fruit Oil on Swimming Time to Exhaustion of Rats

As shown in Fig. 3, the exhaustive swimming times in the group II, III, and IV (65.83 ±1.47, 76.50 ±1.05, and 107.5 ± 1.87 min, respectively) were significantly higher than that in the group I (44.00 ± 1.41 min) ($p < 0.01$). 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.

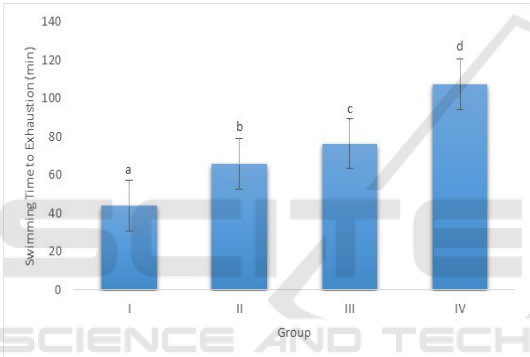


Figure 3: Effects of red fruit oil on swimming time to exhaustion of rats. Different letters indicate significant difference at $p < 0.05$ by one-way ANOVA

### 3.2 Discussion

It is known that physical activity can increase the production of various types of free radicals which can damage cell membranes, skeletal muscle performance, macromolecule and cellular function impairment (Simioni et al., 2018). Free radicals formed in the body will be neutralized by the body's defense systems such as antioxidants enzymes such as catalase (CAT), superoxid dismutase (SOD), glutathione peroxidase (GPx), and a number of non-enzyme antioxidants such as vitamin A, E and C, glutathione, ubiquinone, and flavonoids (Rao et al., 2011). When the production of free radicals exceeds cellular defense antioxidants, oxidative stress will occur (Kawamura and Muraoka, 2018). Oxidative stress has been identified as one of the factors leading to fatigue (Theofilidis et al., 2018).

In this study, the administration of red fruit oil in mice who got training for one month could increase total capacity antioxidants, time of swimming and reduce creatine kinase levels when the rats carried out maximum physical activity. 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 (Budi, 2005; Roreng et al., 2014). Carotenoids (e.g. β-carotenes) lipid-soluble antioxidants located primarily in biological membranes, could 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 (Dhankhar et al., 2012). A 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 (Rohman et al., 2010). 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 increased as the dosage of red fruit oil is risen (Sandhiutami et al., 2012).

This study also found that the administration of red fruit oil could increase total antioxidant capacity (TAC) and reduce creatine kinase levels. The increased total antioxidant capacity (TAC) and reduced creatine kinase levels 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) (Roreng et al., 2014). The increased antioxidant total antioxidant capacity (TAC) is supported by the results of research conducted by several researchers. Derami and Roohi (2019) reported that the administration of omega-3 fatty acid for in nonathlete young males after four weeks of endurance exercise could increase total antioxidant activity (Derami and Roohi, 2019). Omega 3 is a rich source of antioxidants, such as marine carotenoids (for example astaxanthin and fucoxanthin), vitamins A and E, and phospholipids containing long-chain n-3 e polyunsaturated fatty acids (PUFAs) (Gammone et al., 2019). Poulab et al. reported the effect of a four-week acute vitamin C supplementation on the markers of oxidative stress and inflammation following eccentric exercise in active men can significantly increase total antioxidant capacity (0.19 mm/l) and reduce creatine kinase levels (Poulab et al., 2015). Taghiyar et al. reported the results of his research that supplements of vitamins C and E play a role in reducing the marker of muscle damage in aerobic exercise characterized by the reduction of creatine kinase levels (Taghiyar et al., 2013). Dehghan et al. reported that additional use of regular training and cinnamon bark extract (CBE) supplementation increase TAC and protect healthy male rats against oxidative damage induced by exhaustive exercise (Dehghan et al., 2015).

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 support this result, namely because of the high antioxidant content in red fruit oil. Antioxidants in red fruit oil were expected to prevent lipid oxidation in cellular membrane especially in erythrocyte cells. Some research showed that physical activities are able to induce the formation of oxidized lipid and generate the oxidative stress condition. Oxidized lipids can be the cause of erythrocyte cell damage and thus cause the "sport anemia" (Marjan Wouthuyzen-Bakker and Sander van Assen, 2015; Sinaga, 2017) and muscle tissues damage (Sinaga and Purba, 2018). 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. The increased swimming time due to administration of antioxidants in all treatment groups compared to the control group was supported by the results of research conducted by several researchers. Xianchu et al. reported that treatment of grape seed proanthocyanidin extract (GSPE) at a dose of 50 and 100 mg/kg/day of body weight significantly relieved exhaustive exercise-induced fatigue, which was indicated by the increasing forced swimming time. In addition, the treatment of GSPE significantly improved the creatine phosphokinase and lactic dehydrogenase, as well as lactic acid level in exhaustive swimming. For underlying mechanisms, treatment of GSPE had anti-fatigue effects by promoting antioxidant ability and resisting oxidative effect, as represented by increased total antioxidative capability levels, enhanced superoxide dismutase and catalase activities, and ameliorated malondialdehyde levels (Xianchu et al., 2018). A study about the effect of antioxidant on the endurance has been conducted and it reported that vitamin C was also able to increase endurance in the rat model (Ozaslan et al., 2004). Lamou et al. reported that the leaf aqueous extract of *M. Oleifera* possesses anti-fatigue properties. It improved the swimming ability of rats by delaying the accumulation of blood lactate and blood urea nitrogen, by increasing the mobilization and the use of body fats and by slowing the depletion of glycogen stores. The anti-fatigue potential may be expressed through mechanisms that involve the antioxidant activity of the extract (Lamou et al., 2016). Xu and Wang reported that flavonoids from Lotus (Nelumbo nuficera Gaertn) leaf (FFL) can extend the exhaustive swimming time of the mice, as well as increase the superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, but decrease the malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels. These results indicated that FLL possessed protective effects against exhaustive swimming exercise-induced oxidative stress (Xu and Wang, 2018). 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 (Bing and Wang, 2010). A similar result was also reported by Miao et al using corn peptide (Miao et al., 2010).

## 4 CONCLUSIONS

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 that involve the following protects exercise-induced oxidative stress by increasing the levels of TAC, as well as decreasing the CK levels of rats.

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