The Influence of Red Fruit Oil on Creatin Kinase Level at Maximum Physical Activity

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Abstract. Heavy physical activities can cause the oxidative stress which resulting in muscle damage with an indicator of elevated levels of Creatin Kinase (CK) enzyme. The oxidative stress can be prevented or reduced by antioxidant supplementation. One of natural resources which contain antioxidant is Red Fruit (Pandanus conoideus) Oil (RFO). This study aims to see the effect of Red Fruit Oil on Creatin Kinase (CK) level at maximum physical activity. This study is an experimental research by using the design of randomized control group pretest-posttest. This study was using 24 male mice divided into four groups, the control group was given aquadest, the treatment groups P1, P2, and P3 were given the RFO orally of 0.15 ml/kgBW, 0.3 ml/kgBW, and 0.6 ml/kgBW, respectively, for a month. The level of CK was checked for all groups at the beginning of study and after the maximum physical activity. The obtained data were then tested statistically by using t-test and ANOVA. The result shows the RFO supplementation during exercise decreased the CK level in P1, P2, and P3 groups with p<0.05, and the higher RFO dosage resulted in decreased CK level at p<0.05. The conclusion of this study is the Red Fruit Oil could decrease the level of CK at maximum physical activity.

1. Introduction
Severe physical activity may result in oxidative stress resulting from the production of free radicals exceeding the amount of antioxidants in the body [1-2]. Under conditions of oxidative stress, free radicals will cause cell membrane lipid peroxidation [3]. Malondialdehyde (MDA) is one result of lipid peroxidation, so MDA is a common indicator used to determine the amount of free radicals and indirectly assess the body's oxidant capacity [4-5].

The results showed that maximal physical activity could lead to lower antioxidant levels of superoxide dismutase (SOD), Catalase (CAT), Glutathid Peroxidase (GPx) and glutathione-S-transferase in gastrocnemius [6]. The results of research conducted by Mofleh et al (2013) reported with increased intensity of exercise can lead to increased levels of MDA and creatin kinese (CK). It is known, CK is one indicator of the occurrence of damage from muscle cells (Koch, 2014). CK enzyme levels increase after high intensity exercise caused by damage to sarcolemma due to continuous movement in high intensity. Sarkolema damage causes the release of CK enzymes from muscle cells to the circulatory system so that this can be used as an indicator of muscle damage (Tortora 2009). Increased index of muscle damage associated with decreased performance (White et al. 2008). Muscle damage occurs when the muscles receive excessive physical stimuli. In exercise and exercise, muscle damage is caused by physical trauma, resulting in tearing, bruising, or rupture of muscle fibers, and miofilament disorders (Nosaka 2007).
In the body there is a mechanism of antioxidants or anti-radical free endogenically. The free radicals formed will be neutralized by the elaboration of the defense system between antioxidant enzymes and a number of non-enzyme antioxidants [2,8]. Research results reported that administration of antioxidants derived from natural or synthetic sources from outside the body is needed to neutralize the free radicals that are formed during physical activity especially heavy physical activity [9-12]. The results of a study conducted by Poulab et al (2015) showed that vitamin C supplementation for four weeks significantly decreased serum CK after eccentric exercise. The results of research conducted by Zoppi et al (2009) reported that supplementation of vitamin C and E in soccer players during undergoing exercise program can reduce the occurrence of lipid peroxidation and muscle damage but can not improve performance.

One natural source that is known to contain antioxidants such as beta carotene, tocopherol and fatty acids is a red fruit (Pandanus conoideus Lam) found in Papua [13-14]. As an antioxidant RFO is believed its ability to prevent chronic diseases such as cardiovascular, atherosclerosis, and cancer. In addition, Papuans believe that red fruit can increase stamina, but it still needs to be scientifically proven.

The results of research conducted by Sinaga (2015) reported that red fruit oil can increase the amount of erythrocytes, hematocrit and hemoglobin and decrease the number of rat leukocytes during the maximum physical activity. In his study also reported that the use of red fruit oil can decrease MDA and increase the resistance of mice when performing maximum physical activity [15]. The results of this study is very interesting so it needs to be examined further about the effects of red fruit oil in humans especially on CK levels in maximum physical activity.

The targeted finding/innovation is the discovery of one of the antioxidants to prevent or reduce free radicals due to physical activity so as to improve athlete performance and athletic health.

The results of the study are expected to contribute to the development of science and technology, especially as a basis for further research and development of phytopharmaca for improving public health, especially for athlete's health.

The results of this study can be applied to athletes during an exercise program or at the time of the competition so as to support development programs in the field of sports achievements and health. In terms of science and technology development, this research is one form of disciplinary contribution other than the discipline of sports to support the performance of athletes.

2. Methods
The research will be conducted at Animal Laboratory Unit of Biology Division, Faculty of Natural Sciences State University of Medan for 6 weeks. The examination of CK levels was conducted at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra.

Implementation of Research
The number of male rats used in the study were 24 tails. Selected healthy male rats and performed adaptation for 7 days. Then the rats were divided into 4 groups randomly. Each group consists of 6 rats. P0 (control group) was given 2ml water, group P2, P3 and P4 were given MBM respectively 0.15ml, 0.3ml and 0.6ml / day using Gavage syringes. Next all the rats were made swimming exercises for a month. In the first week for 15 minutes / day, Sunday II 20 minutes, Sunday III 25 minutes and week IV 30 minutes. After 30 days, all rats were ordered to perform maximum physical activity by means of rats forced swimming in a container with no way out. Further measurements of CK levels and duration of swimming mice (endurance).

3. Results
Data of CK levels before treatment and after treatment in each group are shown in Table 1.

| Level | Treatment | P0 | P1 | P2 | P3 | F | p |
|-------|-----------|----|----|----|----|---|---|
| CK    | Before    | 128,93 | 128,67 | 128,33 | 128,40 | 0,505 | 0,683 |
|       | After     | 226,82 | 203,51 | 166,89 | 129,71 | 2792,344 | 0,000 |
| t dependent | 0,000 | 0,000 | 0,000 | 0,000 |
Data of CK levels both before treatment and after treatment in each group were tested for normality were tested for normality by using Shapiro-Wilk test and homogeneity with Levene's test. The result shows that all data are normal and homogeneous distribution (p > 0.05), so parametric test, One Way Anova test and t-paired test.

Analysis of significance with One Way Anova test of CK content before administration of MBM showed that F = 0.505 and p = 0.683. This means that the four groups of CK average are not significantly different (p > 0.05). Analysis of significance with One Way Anova test of CK level after MBM administration during one month of training program showed that F = 2792.344 and p = 0.000. This means that the four groups of mean CK differed significantly (p < 0.05).

Based on t-paired test, it was found that there was an increase of CK levels in the control group (P0), whereas in group P1, P2 and P3 decreased significantly (P < 0.05).

Figure 1. Level of Control Group CK and Treatment Group Before and After Performing Maximum Physical Activity

4. Discussion

Decrease Creatin Kinase (CK) Content After Red Fruit Oil Based on the analysis result, it was found that the mean of Creatin Kinase (CK) of control group was 128.93, mean of MBM group dose 0.15 ml/day was 128.67 dose 0.3 ml/day was 128.33 and the dose of 0.6 ml/day was 128.40. Analysis of significance with One Way Anova test showed that the mean Creatin Kinase (CK) content in all four groups before treatment was not different (p > 0.05). Furthermore, after treatment, based on the analysis result showed that the average Creatin Kinase (CK) of control group was 226.82, the mean of MBM group dose 0.15 ml/day was 203.51, dose 0.3 ml/day is 166.89, and the dose of 0.6ml/day is 129.71. Based on the results of the analysis it was found that after treatment between the four groups the results differed significantly (p < 0.05).

In normal circumstances, free radicals form slowly, then neutralized by the antioxidants present in the body. But if the rate of free radical formation is greatly increased because triggered by excessive exercise, the amount of free radical will exceed the ability of the body's defense system and can not be neutralized by antioxidants in the body. This can result in damage to the cell membranes, muscles, bones and tissues [16].

The increase in Creatin Kinase (CK) levels after being given maximum physical training, can cause physical stress. For that it takes a recovery period that is the time it takes the body to return to its original state from the state of the training activity [17]. Increased oxygen consumption by the body during heavy exercise may increase ten to twenty times or more. Under high stress, in muscle fibers there is an increased use of oxygen above the normal requirement. This extraordinary increase in oxygen can trigger the release of free radicals in the mitochondria, resulting in increase CK use in the
body. This causes the number of CK in the body to increase. While in the non-contracting muscles, the amount of oxygen will decrease which causes the ischemic process. With antioxidant supplementation it is expected that CK count will decrease [16].

In this study, administration of red fruit oil can decrease CK levels. The decrease in CK levels is due to red fruit oils rich in natural antioxidants such as 11,500 ppm carotenoids, beta carotene 694,80 ppm, total tocoferol 11,200 ppm and α-tocopherol 495.50 ppm [14].

As one source of natural antioxidants, antioxidant activity of Red Fruit Oil has been investigated. In a study conducted by Rohman et al (2010) reported that red fruit oil had antioxidant activity on in vitro assay with IC50 value of 451.51 μg / ml. In in vivo testing, red fruit oil dose 0.15 ml / kg BW; 0.3 ml / kg BW and 0.6 ml / kg BW may decrease MDA levels in the blood [15,19]. Malondialdehyde is one result of lipid peroxidation [4,5,15], so MDA is a common indicator used to determine the amount of free radicals and indirectly assess the oxidant capacity of the body.

The results of this study were supported by the results of a study conducted by Sandhiutami (2012) who examined the determination of tocopherol content in red fruit oil in blood in male wistar rats subjected to maximum physical activity load. In the study, measurements of tocopherol content in the blood of rats fed red fruit oil with different doses (0.15ml / kgBW, 0.3ml / kg / BW and 0.6ml kg / BW). The results of measurements show that red fruit oil in rats may increase levels of tocopherol in rat blood after maximum physical activity [19].

The results of a study conducted by Armiyanti et al (2009) who examined the effect of red fruit oil with various concentrations on endothelial cells exposed to serum of severe malaria patients and normal neutrophils concluded red fruit oil can decrease oxidative stress characterized by decreased production of Reactive Oxygen Intermediates (ROI) in the treatment group with red fruit oil concentration 2.8%, 5.7%, and 11.3%. In the treatment group treated with red fruit oil the concentrations showed higher concentrations of less endothelial ROI cell production, even at the highest concentration (11.3%) of ROI production near zero [20].

5. Conclusion
Red Fruit Oil supplementation during exercise program can increase the level of Creatin Kinase at maximal physical activity

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