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Research Article

Potential of The Red Dragon Fruit (*Hylocereus polyrhizus*) as an Antioxidant in Strenuous Exercise¹Novita Sari Harahap, ¹Nurhayati Simatupang and ²Suprayitno

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Abstract

Background and Objective: The increase of oxidative stress has negatively impacted several constituent components of cell membrane which include lipid peroxidation to form malondialdehyde (MDA), also related to liver function in which will release alkaline phosphatase enzymes. The purpose to know the protective of red dragon fruit (RDF) extract in strenuous exercise indicate the increase of MDA and alkaline phosphatase. **Materials and Methods:** The subject are 24 white male rats aged 3 months, weight of 200 g. They are grouped into 3 behavioral groups of 8 each. The strenuous exercise without red dragon fruit extract (SE-RDF) group: given treatment of strenuous exercise without the extract from RDF, the strenuous exercise with red dragon fruit extract 100 mg kg⁻¹ b.wt., (SE+RDF 100) group: a treated with strenuous exercise and being given 100 mg kg⁻¹ b.wt., of RDF extract and the strenuous exercise with red dragon fruit extract 200 mg kg⁻¹ b.wt., (SE+RDF 200) group: given a dosage of 200 mg kg⁻¹ b.wt. **Results:** The group SE+RDF 200 has the lowest malondialdehyde is 0.48 nmol mL⁻¹ and alkaline phosphatase is 116.38 U L⁻¹. The highest malondialdehyde is 0.78 nmol mL⁻¹ and the highest alkaline phosphatase is 191.13 U L⁻¹ is found at the group SE-RDF. **Conclusion:** The groups with strenuous exercise and being given 200 mg kg⁻¹ b.wt., are better in responding to oxidative stress by having lower malondialdehyde and alkaline phosphatase compared to the other group.

Key words: Exercise, antioxidant, malondialdehyde, alkaline phosphatase oxidative stress, red dragon fruit, strenuous exercise

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In a strenuous exercise, there is an increase of oxygen consumption and oxidative metabolism. Those cause electron leakage from mitochondria transportation system which results in the forming of free radical. The body has a limited amount of antioxidant. During a heavy workout, the balance between free radical production and antioxidant defence mechanism is disturbed. This is what being called as oxidation stress^{1,2}. Oxidative stress has a big potential in damaging cells which is known as oxidative damages. It is the broken of biomolecules as cells constituents due to the reaction with free radicals³.

The increase of oxidative stress has negatively impacted several constituent components of cell membrane which include lipid peroxidation to form malondialdehyde (MDA), protein, carbohydrate and DNA damages. In previous research, oxidative stress induced by swimming and fasting can increase MDA level in rat's liver due to lipid peroxidation in the body because of free radicals. Oxidative damages due to free radicals imply to various pathology condition such as cell, tissue and organ like liver, kidneys and heart damages either in human or animals^{4,5}. The intensity of exercise related to liver function in which liver will release enzymes to the blood when there are damages in SGOT, SGPT and alkaline phosphatase enzymes⁶. Those enzymes are involved in liver metabolic, therefore the damages risk in liver cell membranes are higher in a strenuous exercise^{7,8}. The research done to a healthy subject who did intensive weightlifting shows a change in liver function test⁹.

Red dragon fruit (RDF) (*Hylocereus polyrhizus*) contains antioxidant like tocopherol, beta-carotene and anthocyanin¹⁰. They are useful in reducing oxidative stress⁸ which damages hepar. Several research have shown that red dragon fruit extract has the ability of being antioxidant^{11,12}. The aims to know the protective of RDF extract in response to oxidative stress due to strenuous exercise indicated by the increase of MDA and alkaline phosphatase level in the blood.

MATERIALS AND METHODS

Study area: This research project was conducted from Maret 2018 to April, 2018 in Pharmacy Laboratory, Faculty of Pharmacy, University of North Sumatra.

Ethical clearance approval: The research was only done after the recommendation approval by Animal Research Ethics Committees/AREC, Universitas Sumatera Utara, No. 0011/KEPH-FMIPA/2018.

Animal maintenance: In this study, 24 of true breeding Sprague Dawley white male rats aged between 3-4 months with average weight of 200 g were used. The animals were made adapted to the environment in the cage for 1 week.

Red dragon fruit extract (RDF): RDF is washed and dried in a drying cabinet. After drying the dragon fruit is pureed using a blender. Red dragon fruit extract is made by maceration method using 96% ethanol which has been distilled as much as 10 times the weight of dragon fruit. Maceration is done for 3 days then filtered and the filtrate is collected. maceration liquid is concentrated using a rotary evaporator until the extract is almost thick. The almost thick extract was evaporated in a water bath until a thick extract was obtained for 2 week.

Experimental design: The research used experimental study method with experimental randomized post test group design only. Animal were randomly, they are grouped into 3 behavioural groups of 8 each. The strenuous exercise without red dragon fruit extract (SE-RDF) group, given treatment of strenuous exercise without the extract from red dragon fruit; the strenuous exercise with red dragon fruit extract 100 mg kg⁻¹ b.wt., (SE+RDF 100) group : treated with strenuous exercise and being given 100 mg kg⁻¹ b.wt., of red dragon fruit extract and the Strenuous Exercise with Red Dragon Fruit Extract 200 mg kg⁻¹ b.wt., (SE+RDF 200) group treated with strenuous exercise and being given 200 mg kg⁻¹ b.wt., of red dragon fruit extract. Oxidative stress is built by strenuous exercise, in which white rats swam 20 min/day for 3 times a week in 3 weeks. RDF extract was given in solution carboxymethyl cellulose (CMC) Natrium 0,5% as much as 1 mL per day for 3 weeks by using a feeding tube. Methanol maceration method was used for the extraction process and the extract was made concentrate with air-drying technique. After 3 weeks all the rats were given a maximum physical activity where they were asked to swim as hard as they could until they were about to drown, usually around 40-50 min. Rats were then execution through anaesthesia using chloroform. Blood samples was taken from the heart and were collected in microtubes and serum was separated at room temperature and stored at -20°C until analysis.

Measurement of malondialdehyde (MDA) and alkaline phosphatase: Malondialdehyde (MDA) and alkaline phosphatase levels was assessed with enzyme-linked immune sorbent assay (ELISA) method and spectrophotometry with

wavelength of 450 nm. Mouse Malondialdehyde Elisa Kit, Brand Bioassay TL, catalog: EO625Mo for MDA levels analysis and mouse alkaline phosphatase elisa kit, Brand Bioassay TL, catalog: EO199Mo for alkaline phosphatase levels analysis.

Statistical analysis: Data was estimated by one-way analysis of variance (ANOVA) and were analyzed with SPSS version 22 software and presented in form of means and standard deviation, tabulated and graphical form.

RESULTS

The analysis of MDA level from the three groups is presented on Table 1. It showed that the group with oxidative stress and being treated with 200 mg kg⁻¹ b.wt., of red dragon fruit extract has the lowest MDA level (0.48 µg mL⁻¹). The highest MDA level at 0.78 µg mL⁻¹ is found at the group with oxidative stress and not being given any red dragon fruit extract. ANOVA test shows a significant difference (p<0.05) of MDA level in all treatments. Bonferroni correction test also shows that oxidative stress with 200 mg kg⁻¹ b.wt., of red dragon fruit extract given, reduce MDA level greater compared to other groups.

This same all with the group strenuous exercise and being given 100 mg kg⁻¹ b.wt., dose of red dragon fruit extract can decrease of MDA level (0.70 µg mL⁻¹) compared to the group that didn't receive any red dragon fruit extract. This shows that oxidative stress built up from swimming to fatigue gives a negative impact to rats with the increase of free radicals in the bodies.

The analysis of alkaline phosphatase level from the three groups is presented in Table 1. Data showed that the group with oxidative stress and being treated with 200 mg kg⁻¹ b.wt., of red-dragon fruit extract has the lowest alkaline phosphatase level at 116.38 U L⁻¹. The highest level is 191.13 U L⁻¹, found in the group with oxidative stress and not being given any red dragon fruit extract. ANOVA test shows a significant different in alkaline phosphatase level towards all treatment.

DISCUSSION

MDA as the indicator of free radicals in the body and the final product of lipid peroxidation. It is used as an indirect parameter to show that there are oxidation and lipid peroxidation damages in rats which are given oxidative stress. Free radicals can be produced either through endogenic or exogenic process. Endogenous free radicals are formed through normal metabolic reactions in the body which involved oxidation-reduction reactions¹³. Free radicals produced throughout normal metabolic process is the endogenous source from cellular process that can cause cellular damages. Free radicals react with constituent components of cell membranes which results in the interference and damaged of cells¹⁴.

This study aligns with the relationship between MDA level and antioxidant defense system in subjects who do swimming exercise for 2-2.5 km/day with a frequency of 4 times in a week. It shows that MDA level after the training is significantly higher compared to before the exercise¹⁵. High antioxidant activity will be supported by the amount of bioactivity compounds contained in the extract. Bioactive compounds with the potential to be antioxidant in red-fleshed pitaya are vitamin C, E and B, carotenoids, flavonoid and polyphenol¹⁶. MDA level is lower in the groups of rats with oxidative stress and being given extract of red dragon fruit than the group without red dragon fruit extract. This shows that red dragon fruit extract as antioxidant can prevent lipid peroxidation by free radicals which reduced the production of MDA¹⁷.

Oxidative stress built up from swimming to fatigue gives a negative impact to rats with the increase of alkaline phosphatase level that indicates damages in liver tissues due to oxidative stress¹⁸. Liver cell contains various of enzymes, some of them are important in diagnosing damages in liver functions. The enzymes activity can be measured in the blood to indicate any liver failures^{19,20}. In normal condition, the enzymes stay in liver cells however in abnormal conditions they are released to the blood circulation^{21,22}. The other research found the response of liver enzymes such as alkaline

Table 1: Effect of red dragon fruit and strenuous exercise on malondialdehyde and alkaline phosphatase

Parameters	SE-RDF group	SE+RDF 100 group	SE+RDF 200 group
Malondialdehyde (µg mL ⁻¹)	0.78±0.04	0.70±0.03	0.48±0.08 ^a
Alkaline phosphatase (U L ⁻¹)	191.13±13.93	151.00±14.03	116.38±9.87 ^b

Data are shown as Mean±SD, SE-RDF: Oxidative stress without red dragon fruit group, SE+RDF 100: Strenuous exercise with 100mg kg⁻¹ b.wt., red dragon fruit group, SE+RDF 200: Strenuous exercise with 200 mg kg⁻¹ b.wt., red dragon fruit group, ^ap<0.05, SE+RDF 200 vs. SE-RDF, ^bp<0.05, SE+RDF 200 vs. SE-RDF

phosphatase in elite soccer players who do high intensity training show a significant rise of SGOT ($p = 0.006$) and alkaline phosphatase (0.001)^{23,24}.

CONCLUSION

The oxidative stress built up from swimming to fatigue gives a negative impact to rats with the increase of malondialdehyde and alkaline phosphatase level that indicates damages in liver tissues due to oxidative stress. The groups with oxidative stress and being given 200 mg kg⁻¹ b.wt., and 100 mg kg⁻¹ b.wt., of red dragon fruit are better in responding to oxidative stress by having lower malondialdehyde and alkaline phosphatase levels compared to the other group. Antioxidant in the red dragon fruit extract can prevent the increase of malondialdehyde and alkaline phosphatase at rats with oxidative stress condition.

SIGNIFICANCE STATEMENT

This study discovered the red dragon fruit (*Hylocereus polyrhizus*) has potential as a good antioxidant that can be beneficial for decrease oxidative stress markers due to strenuous exercise such as malondialdehyde and alkaline phosphatase compared without giving red dragon fruit (*Hylocereus polyrhizus*). This research provide scientific proof of the features of the red dragon fruit (*Hylocereus polyrhizus*) is an antioxidant if given when strenuous exercise is useful to improve performance.

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