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Red Dragon Fruit (*Hylocereus Polyrhizus*) Extract Decreases Lactic Acid Level and Creatine Kinase Activity in Rats Receiving Heavy Physical Exercise

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Abstract

BACKGROUND: Heavy physical exercise causes relative hypoxia. In hypoxic condition, the cell's energy comes from anaerobic metabolism that produces lactic acid. An increment of oxygen need leads to ischemia-reperfusion, triggers free radical formation and damages muscles. Creatine kinase (CK) is a marker of muscle tissue damage. Red dragon fruit (RDF) has potential as antioxidant to reduce free radical formation.

AIM: This study aims to determine RDF extract potential to reduce the lactic acid level and CK activity after heavy physical exercise.

METHODS: A total of 32 male rats (Rattus Norvegicus) were randomly divided into 4 groups: group NORDF, treated heavy physical exercise and distilled water; group RDF100, treated heavy physical exercise and at 100 mg/kg BW RDF extract; group RDF200, treated heavy physical exercise and at 200 mg/kg BW RDF extract and group RDF300, treated heavy physical exercise and at 300 mg/kg BW RDF extract. The rats swam for 20 minutes, 3 times a week for 3 weeks.

RESULTS: RDF300 group showed lower lactic acid level and CK activity as compared to that of NORDF ($p = 0.00$) and RDF100 ($p = 0.00$) groups, but RDF300 are not significantly different for lactic acid ($p = 0.45$) and for CK ($p = 0.68$).

CONCLUSION: Red dragon fruit extract has potential in lowering lactic acid level and CK activity in male rats receiving heavy physical exercise.

Introduction

Regular and measured exercises by the formulation increase physical condition, fitness and performance, and decrease the injury risk [1]. Physical exercises need energy and oxygen supply, especially during the heavy one. Heavy physical exercises result in relative hypoxia, reduction of oxygen supply to tissues. In hypoxic condition, energy source comes from anaerobic metabolism with low energy but high lactic acid level. Lactic acid accumulation in myocytes disturbs muscle performance [2].

Heavy physical exercises increase metabolism and oxygen consumption by 100 – 200 folds [3], [4]. The increase in oxygen need, especially by muscles contraction, results in ischemic reperfusion and free radicals [5]. Free radicals formed during physical exercise induce tissues damage such as blood, liver and other tissues [6], [7].

Creatine kinase (CK) activity increases during heavy physical exercise due to muscle cells damage [8], [9]. Therefore, CK activity has potential as a biomarker for muscle tissues damage, although its activity depends on pathological and physiological conditions [10], [11]. CK activity in athletes who had

had different exercises intensity and frequency changes [8], [11], [12].

Curcuma zedoaria extract of 750 mg/d lowers lactic acid level in athletes [13]. Red dragon fruit (RDF) (*Hylocereus polyrhizus*) is a unique and useful fruit for its natural antioxidant potential [14], [15].

This study aims to determine the effect of red dragon fruit on lactic acid level and CK activity of rats after heavy physical exercise.

Methods

This experimental study used post-test and control group design. A total of 32 male rats, 3-4 months old and 180-200 gr, were acclimatised for a week in animal cages. Rats were accessed with food and water *ad libitum* in a room with 12 h light/dark cycle (lights on at 7:00 A.M.). Room temperature and humidity were set at natural condition. Ethical approval was obtained from the ethics committee of the Faculty of Mathematics and Sciences, Universitas Sumatera Utara.

Rats were randomly divided into 4 treatment groups, i.e. (i) group NORDF, treated heavy physical exercise and distilled water, (ii) group RDF100, treated heavy physical exercise and at 100 mg/kg BW RDF extract, (iii) group RDF200, treated heavy physical exercise and at 200 mg/kg BW RDF extract and (iv) group RDF300, treated heavy physical exercise and at 300 mg/kg BW RDF extract. Macerated RDF was extracted using 96% of ethanol.

Before the treatment, the maximum physical activity was carried out by calming the rats until they were almost drowned, and the results showed that the swimming resistance of rats was 30-35 minutes. To determine the length of time the heavy physical exercise of rats is 75% of the maximum endurance swimming of rats is an average of 20 minutes. All rats had heavy physical exercise in form of swimming for 20 min, 3 times a week for 3 weeks. Rats were treated with RDF extract every day for 3 weeks respectively at half an hour before they had heavy physical exercise.

Two days after the rats have completed heavy physical exercise course, all rats had maximal physical activity, i.e. swimming till they almost drown, soon the rat blood sample was taken for CK and lactic acid examination. Blood was examined for lactic acid level and CK activity. Accutrend® device (by Roche Diagnostics USA) with dipstick method measured lactic acid level. CK activity was measured using the enzymatic kinetic method (Randox Laboratories). Data were analysed using Anova test and post hoc Tukey test in SPSS program 23.

Results

The characteristics of the rats are described in Table 1 below. NORDF, RDF100, RDF200 and RDF300 groups were similar in age and weight. Body Weight was measured before treatment.

Table 1: Data of Rats Characteristic (n = 32)

Rats characteristics	NORDF	RDF100	RDF200	RDF300	p
Weight (gr)	197.48 ± 13.59	196.43 ± 15.64	196.71 ± 13.70	198.56 ± 12.82	0.61
Age (week)	12.88 ± 0.84	13.25 ± 0.89	13.25 ± 0.89	12.50 ± 0.76	0.89

Based on Table 1, it shows that NORDF, RDF100, RDF200 and RDF300 groups were similar in age and weight. Bodyweight was measured before treatment.

Table 2: Lactic Acid Level and CK Activity In 4 Treatment Groups of Male Rats Treated with Heavy Exercise and Various Doses of RDF Extract

Variable	NORDF	RDF100	RDF200	RDF300	p
Lactic acid (mmol/l)	6.83 ± 0.43	5.19 ± 0.57	4.49 ± 0.51	4.08 ± 0.21	0.00*
Creatine kinase (IU/l)	578.37±64.01	307.67±70.77	183.85±47.23	142.26±79.53	0.00*

Based on Table 2 and Figure 1, red dragon fruit (Hylocereus polyrhizus) extract decreases lactic acid level in rats receiving heavy physical exercise. The lactic acid level in the RDF 300 group had a lower-trends than the other groups and found the highest lactic acid of the NORDF group. RDF extract in a dose of 300 mg/kg BW can decrease lactic acid significantly different compared at group RDF100 (p = 0.000) and group RDF200 given RDF extract in a dose 100 mg/kg BW (p = 0.000) but group RDF300 which in a dose 200 mg/kg BW of RDF extract are not significantly different (p = 0.476). It indicated that giving in a dose 300 mg/kg BW and 200 mg/kg BW of RDF extract were equally better in decreasing lactic acid than group NORDF, group without giving RDF extract.

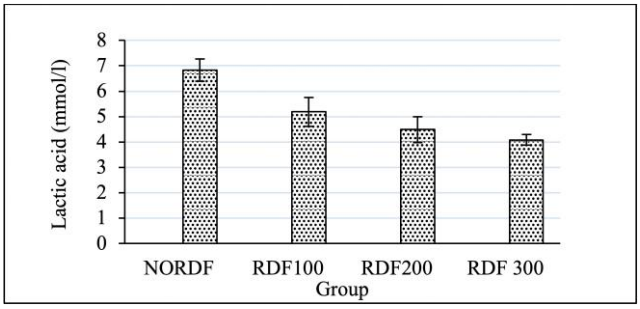

Figure 1: The Relationship Giving RDF and Lactic Acid Between Study Groups

Based on Table 2 and Figure 2, red dragon fruit (*Hylocereus polyrhizus*) extract decreases creatine kinase activity in rats receiving heavy physical exercise. CK activity in the RDF 300 group

had a lower trend than the other groups and found the highest CK activity in the NORDF group Administration of 300 mg/kg BW RDF extract (RDF300 group) decreased CK activity significantly as compared to that of RDF100 group (p = 0.00) and but not to RDF200 group (Table, Figure 4). It showed that administration of 300 mg/kg and 200 mg/kg of RDF extract were better than 100 mg/kg RDF extract in decreasing CK activity, as compared to that of NORDF and RDF100 groups.

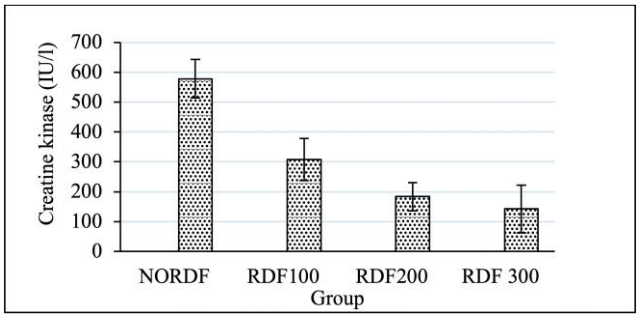

Figure 2: The Relationship Giving RDF and CK Between Study Groups

Discussion

The increment of the lactic acid level after having heavy physical exercise is mainly caused by hypoxia. Hypoxia is the decrease in the amount of oxygen in muscles. In hypoxic condition, energy is obtained from anaerobic metabolism [16], [17]. Rats which were treated for heavy physical activity increased the lactic acid level [18]. The increase in lactic acid level causes purine catabolism into xanthine and indicates acute muscle deoxygenation and ischemic-reperfusion. Purine catabolism and ischemic reperfusion activate xanthene oxidation leading to form free radicals and their accumulation [19]. Free radicals were formed during heavy physical exercise and were inhibited in the presence of adequate amount of antioxidant [20], [21]. The antioxidant in RDF extract indirectly decreased lactic acid level. This present study showed that lactic acid level after heavy physical exercise in NORDF group was 6.83 mmol/L, which is higher than RDF100 group (5.19 mmol/L), RDF 200 group (4.49 mmol/L) and RDF300 group (4.08 mmol/L).

In this study, CK activity after heavy physical exercise in NORDF was 5783.75 U/L, which is higher than that of RDF100 group (3076.75 U/L), RDF200 group (1838.50 U/L) and RDF300 (1422.63 U/L). RDF extract in a dose of 300 mg/kg and 200 mg/kg BW decreased CK activity in rats treated with heavy physical exercise. CK activity increases due to muscle tissues damage [22]. Long and high-intensity exercise results in higher metabolism and physical activity [10]. In mild to moderate physical exercise membrane permeability does not change. However, if the intensity increased to heavy physical exercise, the membrane permeability will surpass the muscle capacity limit and lead the CK to enter circulation [23]. Post-exercise recovery reduces CK activity since low-intensity physical activity lowers CK lymphatic transport and CK release from muscles, the intensity of physical activity relates to serum CK activity [24], [25]. The previous study showed that giving Changbai Mountain after a heavy activity can reduce lactic acid levels and creatine kinase [26]. The study showed an increase in lactic acid and CK after physical exercise [27]. The decrease in lactic acid levels and CK in this study was due to the antioxidant content found in red dragon fruit [28], [29], [30].

In conclusion, red dragon fruit extract decreases lactic acid levels and CK activity in rats treated with heavy physical exercise. A dose of 300 and 200 mg/kg were potential doses in reducing lactic acid and CK activity.

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