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Effects of Red-Fleshed Pitaya (*Selenicereus polyrhizus*) Ingestion after Strenuous Exercise on Creatine Kinase and Mitochondrial Function in Rat Muscle Cells

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Abstract

BACKGROUND: Free radicals formed during strenuous exercise through an increase in reactive oxygen species induce damage to tissues (e.g., muscle and liver) and cause oxidative damage to cells, resulting in mitochondrial dysfunction.

AIM: As an effective method to repair mitochondrial muscle cell function, this study investigated the effects of red-fleshed pitaya (RFP) ingestion on creatine kinase (CK), which is a biomarker for muscle tissue damage, and malondialdehyde (MDA) levels during strenuous exercise.

METHODS: This study involved 25 3-month-old male rats with an average weight of 200 g. The RFP extract was obtained through ethanol extraction and concentrated using an air-drying method. Rats were randomly allocated into five groups as follows: Two control groups (K1 [no-exercise, no RFP] and K2 [exercise, no RFP]) and three test groups (P1, P2, and P3; subjected to exercise and treated with 75, 150, and 300 mg kg^{-1} body weight of RFP, respectively). The exercise was in the form of swimming for 20 min 3 times/week for 31 days. CK and MDA were measured through an enzyme-linked immunosorbent assay, and histopathological examinations were performed through hematoxylin and eosin staining of rat muscles.

RESULTS: The MDA levels after the ingestion of RFP extracts were compared between the K2 group and the P1, P2, and P3 groups. The results showed significant differences ($p < 0.05$ for P1 and P2, and $p < 0.01$ for P3), indicating the production of free radicals and CK, with features of damaged muscle cells based on histopathology. Ingestion of the RFP extract led to improvements in soleus muscle cells, resulting in cell function repair.

CONCLUSION: Levels of MDA and CK increased during exercise, which caused significant muscle damage. However, after treatment with the RFP extract, the levels of both markers decreased. Thus, strenuous exercise causes an increase in reactive oxygen species, resulting in increased free radical levels. RFP ingestion decreased oxidative stress levels, thus repairing mitochondrial cell function.

Introduction

Strenuous exercise that activates skeletal muscle increases metabolism and the consumption of oxygen 100–200-fold [1], [2]. The increase in oxygen requirements, especially for muscle contraction, generates free radicals, which play a role in ischemia-reperfusion injury. Free radicals formed during physical exercise induce damage to tissues such as the blood and liver [3], [4]. Creatine kinase (CK) activity also increases during strenuous exercise, leading to muscle cell damage [5], [6]. Therefore, CK activity is a potential biomarker of muscle tissue damage, although it depends on pathological and physiological conditions [7], [8].

Several studies have shown that reactive oxygen species (ROS) produced by tissue hypoxia during muscle contraction play an adaptive physiological role during physical exercise. ROS are formed in low to moderate amounts during moderate-intensity activities to increase endogenous antioxidant levels for oxidative stress prevention in the body [9], [10]. Red-fleshed pitaya (RFP) protects tissues from damage caused by ROS in the body [9], [10], [11]. This research aimed to determine the effect of pre-exercise RFP ingestion on CK levels after strenuous exercise and on the mitochondrial function of muscle cells.

Materials and Methods

In this study, we used 25 3-month-old male rats with an average weight of 200 g. The rats were obtained from the Animal House Unit of the Biology Laboratory,

Universitas Sumatera Utara, Indonesia. All rats were maintained in groups in the experimental animal cages in the laboratory. The cage (30 cm × 20 cm × 10 cm) was made of plastic and covered with fine wire mesh. The cage base was covered with rice husks with a thickness of 0.5–1 cm, which was replaced every day during the study. The room light was controlled to deliver a 12 h light/12 h dark cycle, the temperature was set to 25–27°C, and the humidity of the room was adjusted to a normal range of 35–50%. The rats were fed standard rat pellets and given tap water *ad libitum*.

Study design

We used an *in vitro* experimental method with a true experimental design and a randomized post-test for the control group. Simple random sampling was used to categorize the laboratory rats into five groups as follows: Group K1 with no activity and no RFP; group K2 subjected to strenuous exercise without RFP; and groups P1, P2, and P3 subjected to strenuous exercise and treated with 75, 150, and 300 mg kg^{-1} body weight of RFP extract, respectively. In the fruit market, it is easy to find RFP fruit, acquired from farmers in Indonesia, was peeled, washed, cut into small pieces, and then dried in a drying cabinet. Next, the fruit was blended using a blender, and the extract was obtained by the maceration method with 96% ethanol, which was distilled by 10 times the weight of RFP. The RFP powder was stored in a container with 96% ethanol (ratio of 1:7, fruit powder: ethanol) and then soaked for 3 d. The RFP was macerated using a rotary evaporator at 45°C until the extract thickened. The macerated RFP was extracted using 96% ethanol. The remaining extract was then evaporated in a water bath until a thick extract was obtained. Next, 100 mg RFP extract was weighed and crushed using a pestle and mortar. Subsequently, carboxymethylcellulose Na solution (0.5% w/v) was slowly added until a homogeneous extract was obtained, and the resulting volume was 10 mL. This final RFP extract was administered to the rats at appropriate dosages; specifically, rats weighing 200 g were fed 1.5, 3.0, or 6.0 mL of the RFP extract suspension, which corresponded to doses of 75, 150, or 300 mg kg^{-1} body weight, respectively.

Experimental procedures

Strenuous exercise involved a morning swim between 8:00 and 9:00 am for 20 min, 3 times per week for 4 weeks. The rats were treated with RFP 30 min before the heavy physical exercise.

Analysis of blood samples

All rats performed strenuous exercise until they reached their maximum effort (i.e., swimming until they almost drowned). At this time, blood samples were sequentially taken to analyze malondialdehyde (MDA) and CK using the enzyme-linked immune sorbent assay (ELISA) method with spectrophotometry at a wavelength of 450 nm. The mouse malondialdehyde ELISA kit (Brand Bioassay TL, catalogue: EO625Mo) was used to analyze the MDA levels. The CK kit (Rat Creatine Kinase ELISA, Bioenzy Brand, catalogue: BZ- 08183841-EB) was used for CK analysis.

Histopathological study

Muscle tissue samples were collected by performing a biopsy to determine the degree of muscle damage based on hematoxylin and eosin (H&E) staining. The soleus muscle tissues of the rats were collected and fixed with 10% formalin for 24 h. The muscle tissues were embedded in paraffin, sectioned to a 4 μm thickness, and stained through H&E staining. The stained sections were then examined under a light microscope (400× magnification) with ten fields of view to determine the degree of damage concerning inflammatory cells and necrosis. The examination was conducted by a pathologist who was blinded to the applied treatment [12], [13].

Data analysis

Experimental data were analyzed using SPSS 25 for Windows. The Shapiro–Wilk test ($p > 0.05$) was used to determine the normality of the data. If the data were normally distributed, then parametric analysis was performed; otherwise, non-parametric analysis was performed. The effect of each treatment was determined using one-way analysis of variance (ANOVA). When a statistically significant result was obtained, the procedure was followed by Fisher's least significant difference test, which is a two-step test for pairwise comparisons of several treatment groups, or the Bonferroni multiple comparison test, which is a designed adjustment to prevent data from incorrectly appearing as statistically significant. The results are presented as the mean ± SD.

Ethical approval

According to the ethical standards, animal research was performed with the approval of the Animal Research Ethics Committee (AREC), Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Indonesia (approval number 0005/KEPH-FMIPA/2021).

Results

The characteristics of the rats are presented in Table 1. Age and weight were similar among the control

Table 1: Rat characteristics (*n*=25)

Treatment group	Body weight (g)	Age (weeks)
K1	222 ± 22.9	12.87 ± 0.84
K2	218 ± 19.9	12.88 ± 0.84
P1	239 ± 33.4	13.25 ± 0.89
P2	220 ± 33.7	13.25 ± 0.89
P3	235 ± 26.9	12.50 ± 0.76
P	0.61	0.69

(K1 and K2) and test (P1, P2, and P3) groups. Body weight was measured before treatment.

Malondialdehyde levels serve as a marker for assessing the increase in free radical production in rats subjected to physical activity. The one-way ANOVA revealed significant differences in MDA levels among K2, P1, P2, and P3 groups (Table 2). The MDA levels in the control K2 group (with RFP) were higher than those in the control K1 group (no RFP). In addition, compared to those in the K2 group, there was a significant decrease in the MDA levels in the P1 and P2 groups ($p < 0.05$), as well as in the P3 group ($p < 0.01$). These results show that the administration of exogenous antioxidants suppresses the production of free radicals.

Based on the one-way ANOVA, CK levels in the control group K2 were higher than those in the untreated group K1 (Table 3). Furthermore, compared to those in the K2 group, there was a decrease in the CK levels in the P1 and P2 groups ($p < 0.05$) and in the P3 group ($p < 0.01$). In addition, there was a significant difference in the CK levels between P2 and P3 groups ($p < 0.001$).

The histopathological examination showed the changes in the levels of free radicals that could damage tissues in the K2 group, whereas the histopathological features of groups P1, P2, and P3 showed muscle cell repair, as observed in Figure 1 (yellow markings).

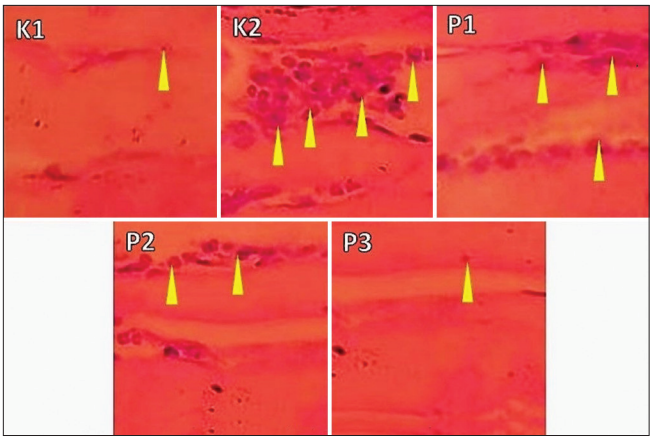

Figure 1: Effects of red-fleshed pitaya extract on soleus muscle cells. Yellow arrows represent inflammatory cells.

Table 2: Effects of red-fleshed pitaya extract treatment on malondialdehyde levels and results of one-way ANOVA

Group	MDA level (μg/dL; mean ± SD)	*P*
K1	0.4191 ± 0.2080^{bc}	< 0.05
K2	0.5471 ± 0.0399^{c}	
P1	0.3120 ± 0.1357^{ab}	
P2	0.3159 ± 0.0377^{ab}	
P3	0.2531 ± 0.0284^{a}	

Different letters indicate statistically significant differences ($P < 0.05$). MDA: Malondialdehyde, SD: Standard deviation.

Discussion

Creatine kinase level as a biomarker for muscle damage

The increase in the CK levels after strenuous exercise is mainly caused by hypoxia (a reduced level of oxygen in muscles). Under hypoxic conditions, the energy is produced anaerobically [14]. Rats that were subjected to strenuous exercise showed an increase in CK levels, which, in turn, disrupted the muscle cell membrane. If the mechanical strain is more significant than the ability of the muscle to resist it, the contractile apparatus, myofibrils, plasma membrane, and sarcoplasmic reticulum will be disrupted [15], disrupting the intracellular calcium homeostasis and contractile function.

Table 3: Effect of red-fleshed pitaya treatment on creatine kinase and results of one-way analysis of variance

Group	CK (ng/mL; mean ± SD)	*P*
K1	3.4510 ± 0.4983	< 0.05
K2	3.6710 ± 0.4145	
P1	3.2592 ± 0.3358	
P2	3.9049 ± 0.0593	
P3	2.8972 ± 0.1587	

CK: Creatine kinase, SD: Standard deviation.

Muscle damage and increased ROS after exercise enhance an acute-phase local inflammatory response characterized by the release of inflammatory cytokines such as interleukin (IL)-6 [16] from various cell types [17] and stimulating the recruitment of neutrophils and monocytes to areas of inflammation to repair the damaged tissue [18]. Immune cell mobilization and activation during exercise are mediated by stress hormones such as cortisol [19]. This ROS-mediated disruption of cellular homeostasis can result in muscle injury, pain, fatigue, and consequently decreased physical performance [20]. Figure 1 shows that ROS produced by phagocytic cells might have an essential role in muscle regeneration, and efforts to prevent post-exercise production through interventions with the antioxidant RFP extract can impair the recovery process by inhibiting the removal of degraded tissue proteins and the regeneration of muscle fibers. A previous study reported a similar decrease in cytochrome c oxidase activity (40%) associated with the breakdown of specific unsaturated phospholipids at the mitochondrial membrane surface in rat skeletal muscle after ischemia and reperfusion [21].

Changes in creatine kinase levels from muscle cells due to strenuous exercise

Most oxygen is utilized in the mitochondria to produce adenosine triphosphate (ATP). During oxidative phosphorylation, superoxide and hydroxyl radicals are produced by the univalent reduction of oxygen and their leakage out of the electron transfer chain [22]. Thus, intensive exercise can cause a substantial attack by

oxygen radicals on the skeletal muscle tissue, resulting in lipid peroxidation of the cell membranes. In severe cases, a free radical attack might lead to cell necrosis and inflammatory processes. Lipid peroxidation causes an increase in membrane permeability, resulting in the loss of cytosolic proteins [23]. In addition, increased membrane permeability induced by the lipid peroxidation of unsaturated fatty acids during strenuous exercise triggers CK efflux from muscle cells. In this study, the higher levels of MDA and CK in the group subjected to exercise (K2), relative to those in the no-exercise group (K1), indicated muscle damage related to oxidative stress. Groups P1, P2, and P3 showed improved mitochondrial cell function, marked by a decrease in MDA and CK levels compared to those in the K1 and K2 groups.

Role of antioxidants in decreasing malondialdehyde and creatine kinase levels

RFP is a membrane-bound, lipid-soluble antioxidant that quenches singlet oxygen and stabilizes superoxide anion and hydroxyl radicals [24]. In this study, the administration of the RFP extract suppressed the increase in MDA and CK levels during exercise compared to the levels in the control groups (K1 and K2). Therefore, the RFP extract served as an active antioxidant throughout the exercise period. Marzatico et al. showed that an imbalance in the cellular concentrations of antioxidants and peroxides increases lipid peroxidation, which leads to CK efflux [25]. Similar increases observed in serum CK activity on exercise in groups K1 (control/no-exercise; 3.4510 ± 0.4983 ng/mL) and K2 (control/exercise; 3.6710 ± 0.4145 ng/mL) indicated that antioxidants affect sarcolemma integrity, which supports a similar response of thiobarbituric acid reactive substances to exercise with or without RFP treatment [26].

Conclusion

An increase in CK levels might indicate a more efficient mechanism of ATP re-synthesis that results in muscle damage. This study showed increased levels of MDA and CK during exercise and a decrease in the levels of both enzymes after RFP extract treatment. This result indicates a significant association between higher ATP-phosphocreatine activity and muscle efficiency, in that oxidative stress significantly causes muscle damage. The findings of this study suggest that increased oxygen consumption is not the only mechanism associated with oxidative stress-induced muscle damage. The antioxidant RFP extract suppressed muscle injury caused by strenuous exercise. The evidence thus suggests that RFP decreases the exercise-induced generation of ROS by suppressing the activation of critical cellular signals involved in cellular adaptation in the mitochondria during exercise and physical activity.

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References

1. Santalla A, Naranjo J, Terrados N. Muscle efficiency improves over time in world-class cyclists. Med Sci Sports Exerc. 2009;41(5):1096-101. https://doi.org/10.1249/MSS.0b013e318191c802
   PMid:19346977
2. Cooper CE, Vollaard NB, Choueiri T, Wilson MT. Exercise, free radicals and oxidative stress. Introduction: Free radicals and oxidative stress. Biochem Soc Trans. 2002;30(7):280-5.
   PMid:12023865
3. Simioni C, Zauli G, Martelli AM, Vitale M, Gonelli A, Neri LM, *et al.* Oxidative stress: Role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget. 2018;9(24):17181-98. https://doi.org/10.18632/oncotarget.24729
   PMid:29682215
4. Margonis K, Fatouros LG, Jamurtas AZ, Nikolaidis MG, Douroudos L, Chatzinikolaou A, *et al.* Oxidative stress biomarkers responses to physical overtraining: Implications for diagnosis. Free Radic Biol Med. 2007;43(6):901-10. https://doi.org/10.1016/j.freeradbiomed.2007.05.022
   PMid:17697935
5. Banfi G, Colombini A, Lombardi G, Lubkowska A. Metabolic markers in sports medicine. Adv Clin Chem. 2012;56:1-54. https://doi.org/10.1016/B978-0-12-394317-0.00015-7
   PMid:22397027
6. Mougios V. Reference intervals for serum creatine kinase in athletes. Br J Sports Med. 2007;41(10):674-8. https://doi.org/10.1136/bjsm.2006.034041
   PMid:17526622
7. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull. 2007;81-2:209-30. https://doi.org/10.1093/bmb/ldm014
   PMid:17569697
8. Coelho DB, Morandi RF, De Melo MA, Silami-Garcia E. Creatine kinase kinetics in professional soccer players during a competitive season. Rev Bras Cineantropom Desempenho Hum. 2011;13(3):189-94. https://doi.org/10.5007/1980-0037.2011v13n3p189
9. Gomez-Cabrera M, Domenech E, Viña J, Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training. Free Radic Biol Med. 2008;44(2):126-31. https://doi.

org/10.1016/j.freeradbiomed.2007.02.001
PMid:18191748

10. Berzosa C, Cebrián I, Fuentes-Broto L, Gómez-Trullén E, Piedrafita E, Martínez-Ballarín E, *et al.* Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. J Biomed Biotechnol. 2011;2011:540458. https://doi.org/10.1155/2011/540458
PMid:21436993
11. Rimbach G, Höhler D, Fischer A, Roy S, Virgili F, Pallauf J, *et al.* Methods to assess free radicals and oxidative stress in biological system. Arch Anim Nutr. 1999;52(3):203-22. https://doi.org/10.1080/17450399909386163
PMid:10553486
12. Abe K, Inage K, Sakuma Y, Orita S, Yamauchi K, Suzuki M, *et al.* Evaluation of histological changes in back muscle injuries in rats over time. Asian Spine J. 2017;11(1):88-92. https://doi.org/10.4184/asj.2017.11.1.88
PMid:28243375
13. Rizo-Roca D, Ríos-Kristjánsson JG, Núñez-Espinosa C, Ascensão A, Magalhães J, Torrella JR, *et al.* A semiquantitative scoring tool to evaluate eccentric exercise-induced muscle damage in trained rats. Eur J Histochem. 2015;59(4):2544. https://doi.org/10.4081/ejh.2015.2544
PMid:26708179
14. Davis JM, Murphy A, Carmichael MD, Zielinski MR, Groschwitz CM, Brown AS, *et al.* Curcumin effects on inflammation and performance recovery following eccentric exercise-induced muscle damage. Am J Physiol Regul Integr Comp Physiol. 2007;292(6):2168-73. https://doi.org/10.1152/ajpregu.00858.2006
PMid:17332159
15. Lin CH, Lin YA, Chen SL, Hsu MC, Hsu CC. American ginseng attenuates eccentric exercise-induced muscle damage via the modulation of lipid peroxidation and inflammatory adaptation in males. Nutrients. 2021;14(1):78. https://doi.org/10.3390/nu14010078
PMid:35010953
16. Fischer CP, Hiscock NJ, Penkowa M, Basu S, Vessby B, Kallner A, *et al.* Supplementation with Vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. J Physiol. 2004;558(2):633-45. https://doi.org/10.1113/jphysiol.2004.066779
PMid:15169848
17. Vassilakopoulos T, Karatza M, Katsaounou P, Kollintza A, Zakynthinos S, Roussos C. Antioxidants attenuate the plasma cytokine response to exercise in humans. J Appl Physiol. 2003;94(3):1025-32. https://doi.org/10.1152/japplphysiol.00735.2002
PMid:12571133
18. Mastaloudis A, Morrow JD, Hopkin DW, Devaral S, Traber MG. Antioxidant supplementation prevents exercises-induced lipid peroxidation, but not inflammation, in ultramarathon runners. Free Radic Biol Med. 2004;36(10):1329-41. https://doi.org/10.1016/j.freeradbiomed.2004.02.069
PMid:15110397
19. Peake JM, Suzuki K, Coombes JS, The influence of antioxidant supplementation on markers of inflammation and the relationship to oxidative stress after exercise. J Nutr Biochem. 2007;18(6):357-71. https://doi.org/10.1016/j.jnutbio.2006.10.005
PMid:17156994
20. Avery NG, Kaiser JL, Sharman MJ, Scheett TP, Barnes DM, Gómez AL, *et al.* Effects of Vitamin E supplementation on recovery from repeated bouts of resistance exercise. J Strength Cond Res. 2003;17(4):801-9. https://doi.org/10.1519/1533-4287(2003)017<0801:EOVESO>2.0.CO;2
PMid:14636105
21. Paradies G, Petrosillo G, Paradies V, Ruggiero FM. Oxidative stress, mitochondrial bioenergetics, and cardiolipin in aging. Free Radic Biol Med. 2010;48(10):1286-95. https://doi.org/10.1016/j.freeradbiomed.2010.02.020
PMid:20176101
22. Sachdev S, Davies KJ. Production, detection, and adaptive responses to free radicals in exercise. Free Radic Biol Med. 2008;44(2):215-23. https://doi.org/10.1016/j.freeradbiomed.2007.07.019
PMid:18191757
23. Fernández-Lázaro D, Fernandez-Lazaro CI, Mielgo-Ayuso J, Navascués LJ, Martínez AC, Seco-Calvo J, *et al.* The Role of selenium mineral trace element in exercise: Antioxidant defense system, muscle performance, hormone response, and athletic performance. A systematic review. Nutrients. 2020;12(6):1790. https://doi.org/10.3390/nu12061790
PMid:32560188
24. Thirupathi A, Wang M, Lin JK, Fekete G, Biro I, Baker JS, *et al.* Effect of different exercise modalities on oxidative stress: A systematic review. Biomed Res Int. 2021;2021:1-10. https://doi.org/10.1155/2021/1947928
PMid:33628774
25. Marzatico F, Pansarasa O, Bertorelli L, Somenzini L, Valle GD. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. J Sports Med Phys Fitness. 1997;37(4):235-9.
PMid:9509820
26. Harahap NS, Amelia R. Red dragon fruit (*Hylocereus polyrhizus*) extract decreases lactic acid level and creatine kinase activity in rats receiving heavy physical exercise. Open Access Maced J Med Sci. 2019;7(14):2232-5. https://doi.10.3889/oamjms.2019.626
PMid:31592070