RESEARCH ARTICLE

REVISED The effect of ingestion of red dragon fruit extract on levels of malondialdehyde and superoxide dismutase after strenuous exercise in rats (*Rattus norvegicus*) [version 2; peer review: 1 approved, 1 approved with reservations]

Gusbakti Rusip[1], Syafrudin Ilyas[2], I. Nyoman Ehrich Lister[1], Chrismis N. Ginting[3], Indra Mukti[4]

[1]Department of Physiology, Faculty of Medicine, University Prima Indonesia, Medan, Sumatra Utara, 20118, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University Sumatera Utara, Medan, Sumatra Utara, 20132, Indonesia
[3]Universitas Prima Indonesia, Medan, Sumatera Utara, Indonesia
[4]Department of Surgery, Universitas Prima Indonesia, Medan, Sumatera Utara, Indonesia

v2 **First published:** 18 Oct 2021, **10**:1061
https://doi.org/10.12688/f1000research.54254.1
Latest published: 11 Jul 2022, **10**:1061
https://doi.org/10.12688/f1000research.54254.2

Abstract

Background: Prolonged activation of skeletal muscles causes a decrease in the production of fatigue. Exercise with strenuous intensity causes an increase in Reactive Oxygen Species (ROS). An increase in free radicals causes oxidative stress resulting in damage to cell function to mitochondrial dysfunction, and fatigue. This study aimed to determine the antioxidant potential of red dragon fruit (RDF) to delay fatigue due to oxidative stress, which improves cell function in mitochondria.

Methods: 25 male rats *(Rattus norvegicus)* aged three months were divided into five groups: Group K1 was N.A. (No Activity) but drinking and eating; Group K2 performed strenuous exercise without RDF treatment; Groups 3, 4, and 5 (P1, P2 and P3, respectively) performed strenuous exercise and were treated with 75 mg kg^{-1}.bw, 150 mg kg^{-1}.bw, and 300 mg kg^{-1}.bw of RDF extract, respectively. The exercise for the rats involved intense swimming for 20 minutes every day, four days a week for 31 days. Malondialdehyde (MDA) was measured with the ELISA and histopathology for muscle soleus and lung tissue. SOD?

Results: Strenuous exercise followed by RDF extract ingestion was compared for fatigue in terms of duration and time; before (24.55±1.38 minute) and after (95.31±7.82 minute) and led to a significant difference of 39% ($p<0.01$). The study also compared MDA before and after RDF extract ingestion in the K2 vs. the P1 group ($p<0.05$). At the same time, P2 differed more significantly ($p<0.01$). This indicated a spread of free radicals and featured histopathological P3?

Open Peer Review

Approval Status ✓ ?

	1	2
version 2 (revision) 11 Jul 2022	✓ view	
version 1 18 Oct 2021	? view	? view

1. **Farzaneh Taghian**, University of Isfahan, Isfahan, Iran

2. **Ermita I. Ibrahim Ilyas**, Universitas Indonesia, Jakarta, Indonesia

Any reports and responses or comments on the article can be found at the end of the article.

damage of muscle cells. However, ingestion of RDF extract leads to improvement of soleus muscle cells; thus, repairs cell function, delaying fatigue.
Conclusion: This study confirmed that strenuous exercise, which causes an increase in ROS, intensifies free radicals with RDF extract ingestion and declines oxidative stress, repairing cell function and delaying fatigue.

Keywords
Red Dragon Fruit (RDF), Strenuous exercise, MDA, Improve function cell, Fatigue

Corresponding author: Gusbakti Rusip (gusrusip@gmail.com)

Author roles: Rusip G: Conceptualization, Data Curation, Formal Analysis, Methodology, Resources, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Ilyas S**: Formal Analysis, Methodology, Supervision, Validation; **Lister INE**: Funding Acquisition, Investigation, Resources; **Ginting CN**: Funding Acquisition, Investigation, Project Administration, Resources; **Mukti I**: Formal Analysis, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: We acknowledge the Faculty Medicine of University Prima Indonesia for their fund, facilities, and supports in this research.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Rusip G, Ilyas S, Lister INE *et al.* **The effect of ingestion of red dragon fruit extract on levels of malondialdehyde and superoxide dismutase after strenuous exercise in rats (*Rattus norvegicus*) [version 2; peer review: 1 approved, 1 approved with reservations]** F1000Research 2022, **10**:1061 https://doi.org/10.12688/f1000research.54254.2

First published: 18 Oct 2021, **10**:1061 https://doi.org/10.12688/f1000research.54254.1

REVISED **Amendments from Version 1**

There are several other enhancements

1. In the abstract, the conclusions are adjusted to the results of the study.
2. Methods, Study Design, and Histopathology are described in detail.
3. Results, explanation of the legend from Figure 1 and 2.
4. Conclusion explanation of the objectives and research results obtained.

Any further responses from the reviewers can be found at the end of the article

Introduction

Increased frequency, intensity, and duration of regular physical exercise improves performance and delays fatigue in daily work.[1,2] All living things, except those that are anaerobic, require oxygen to produce energy efficiently. Oxygen is an essential component of cellular metabolism. Exercise causes an increase in oxygen consumption by 10-12 times in the body, causing oxidative damage to the lipids of various tissues.[3–7] Moderate to high-intensity exercise can result in an increase in reactive oxygen species (ROS), free radicals in the body, which is characterized by an increase in malondialdehyde (MDA), and a decrease in superoxide dismutase (SOD) which is an endogenous antioxidant to suppress excess of free radicals. Several studies showed that reactive oxygen species (ROS) formed due to tissue hypoxia during muscle contraction have an adaptive physiological role during physical exercise. Oxidative stress is an imbalance between free radicals and antioxidants. Endogenous antioxidants cannot neutralize free radicals if they are formed excessively.[8,9] Oxidative stress causes damage to muscle cells and lungs, known as oxidative damage. It is the breakdown of biomolecules that make up cells due to reactions with free radicals.[10] Strenuous physical exercise will increase the growth of the free radicals found in muscle and liver tissue 2 to 3 times in experimental animals, which also will increase ROS. As a defense action, the body will be countered by the endogenous antioxidant system,[11] which is known as oxidative stress. This can be seen based on the ability of antioxidants in the tissue to neutralize ROS,[12] particularly the antioxidants produced by the body known as endogenous antioxidants that come from outside or exogenous antioxidants. These antioxidants come from food, such as fruit. Red dragon fruit (RDF) has been proven to protect the tissue from damage caused by ROS in the body.[8,9,13] This study aimed to examine effect of strenuous exercise on changes malondialdehyde and superoxide dismutase and by ingestion red dragon fruit extract improves MDA, SOD, muscle and lung tissue delay fatigue in rats (*Rattus norvegicus*).

Methods

Animal experiment

Animal models, particularly rodents, are widely used in biological sciences, and the findings of animal research are traditionally projected to human response similar to physiological stimuli.[14] This article was reported in line with the ARRIVE guidelines. The study was a randomized post-test-only control group approved by the Animal Research Ethics Committee, Department of Biology - Faculty of Mathematics and Science, Universitas Sumatera Utara (approval number 0005/KEPH-FMIPA/2020).

In this study, we used 25 three-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 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 were 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 randomised post-test for the control group. Simple random sampling was used to categorise the laboratory rats into five groups as follows: group K1 with no activity and no RDF; group K2 subjected to strenuous exercise without RDF (Red Dragon Fruit); and groups P1, P2, and P3 subjected to strenuous exercise and treated with 75, 150, and 300 mg kg^{-1} body weight of RDF 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 RDF. The RDF powder was stored in a container with 96% ethanol (ratio of 1:7, fruit powder: ethanol) and then soaked for 3 d. The RDF was macerated using a rotary evaporator at 45 °C until the extract thickened. The macerated RDF was extracted using

96% ethanol. The remaining extract was then evaporated in a water bath until a thick extract was obtained. Next, 100 mg RDF 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

The strenuous exercise given to all rats involved a morning swim between 08 – 09 AM for 20 minutes a day three times a week for four weeks.[15] The rats were treated with RDF extract every day for four weeks respectively at half an hour before the strenuous exercise. All rats completed the strenuous exercise test. At the end of the study, the results were obtained in the fourth week of exercise testing until the maximum exercise was swimming until almost drowning.

Outcomes

One of the biomarkers of oxidative stress is a high level of malondialdehyde (MDA) and decreased SOD activity due to excessive lipid peroxidation processes in cells. One way to control excessive oxidative stress is by consuming antioxidants from food (exogenous antioxidants); one source of exogenous antioxidants is RDF, which consists of Group P1 treated with 75 mg kg^{-1}.bw; Group P2 with 150 mg kg^{-1}.bw; and Group P3 with 300 mg kg^{-1}.bw of RDF extract.

The consumption of RDF extract suppresses the increase in free radicals due to strenuous exercise. It increases SOD, an endogenous antioxidant, so oxidative stress does not occur, and repair mitochondrial cell function has fatigue delaying effect.

Analysis of blood

All of the rats completed the strenuous exercise course. They experienced maximal physical activity, i.e., swimming, until they almost drowned. Blood for MDA and SOD was taken consecutively was assessed with enzyme-linked immune sorbent assay (ELISA) method and spectrophotometry with a wavelength of 450 nm. The assessment was done by using mouse malondialdehyde ELISA kit (Brand Bioassay TL, catalogue: EO625Mo). The SOD kit (Brand Bioassay TL, catalogue: EO168Ra) rat super oxidase dismutase ELISA kit was determined using the equation obtained from the standard curve.[16–18]

Histopathological study muscle tissue, and lung organs

Muscle soleus and lung tissue samples were collected by performing a biopsy to determine the degree of muscle damage based on haematoxylin and eosin (H&E) staining. The soleus muscle tissues of the rats were collected and fixed with 10% formalin for 24 h. The muscle and lung tissues were embedded in paraffin, sectioned to a 4 μm thickness, and stained via H&E staining. The stained sections were then examined under a light microscope (400× magnification) with 10 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.

Statistical analysis

Normality was assessed with Shapiro-Wilk test ($p>0.05$). Data Analysis was done by one-way analysis of variance (ANOVA) to indicate the effect of treatments for each group. The data were analysed with SPSS version 25 software and presented in tabulated and graphical forms as means and standard deviation. Significant differences were determined at $p<0.05$. The Post Hoc Bonferroni test was conducted after the significant results were obtained.

Ethical approval

The animal subjects' research was performed according to the ethical standards by the Animal Research Ethics Committees/AREC, Faculty Mathematics and Natural Sciences Universitas Sumatera Utara, Indonesia (approval number 0005/KEPH-FMIPA/2021).

Results

During consumption of the antioxidant RDF extract, all rats were accustomed to reducing stress-related disorders and seemed to be in good condition. No rats were poisoned, and there were no deaths in the experiment period.

A normality test indicated that the data are normally distributed (Table 1).

Strenuous exercise followed by RDF extract ingestion was compared for fatigue in terms of duration and time; before (24.55 ± 1.38 minute) and after (95.31 ± 7.82 minute) and led to a significant difference of 39% ($p<0.01$).

Table 1. Normality for test MDA and SOD, with Shapiro-Wilk test, p>0.05.

Parameter	Group	Normality test	
		Statistic	p-value
Malondialdehyde (MDA)	K1	0.791	0.068
	K+	0.969	0.867
	P1	0.853	0.204
	P2	0.790	0.068
	P3	0.959	0.804
Superoxide dismutase (SOD)	K1	0.892	0.369
	K+	0.968	0.864
	P1	0.906	0.446
	P2	0.806	0.090
	P3	0.862	0.236

Table 2. Comparison of the MDA level results before and after treatment with RDF extract and the results of the One-Way ANOVA test.

Groups	MDA level (µg/dL)	p-value
K1	0.4191 ± 0.2080^{bc}	p<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}	

Notation. bc, c: p>0.05.
Notation. ab, ab: p>0.05.
Notation. c vs ab, ab, a: p<0.05.

The effects of strenuous exercise on MDA before and after RDF extract treatment

The results of One-Way ANOVA test for groups K2, P1, P2, and P3 showed significant differences (Table 2). It is known that the measurement of MDA levels is a marker for assessing the increase in free radical production in rats treated with physical activity.

MDA expression (Table 2 and Figure 1) was decreased after treatment with RDF extract (0.4191 vs 0.5471 vs 0.3120 vs 0.3159 vs 0.2531 µg/dL). The P3 group had the lowest score compared to the other groups. This study showed a significant reduction between groups.

The study results compared the MDA of rats after ingestion of RDF extract, which was tested with the Post Hoc test - Bonferroni. In the K2 vs. P1 group, there was a significant difference of p<0.05, the K2 vs. P2 group had a significant difference of p<0.05, and the K2 vs. P3 group had an increased significant difference of p<0.01.

The effects of strenuous exercise on SOD before and after RDF extract treatment

The free radicals in the body are balanced with endogenous defense mechanisms, and the body will produce antioxidants with an anti-free radical effect. In this study, the K2 group performed physical activity and SOD levels were 0.4632±0.2449 ng/mL. There was an increase in SOD levels in the K1 group (0.8647±0.1744 ng/mL) that did not perform physical activity. The increase in SOD continued with RDF extract treatment in groups K1 (1.3499±0.1359 ng/mL), P2 (1.9370±0.0236 ng/mL) and P3 (1.9521±0.0239 ng/mL). The three groups were given RDF treatment and showed significant differences (p<0.05), analysed with the One-Way ANOVA test (Table 3 and Figure 2).

Histopathologic changes in muscle and lung tissue

The histopathological examination were observed under a microscope. It was seen that in group K1 changes in muscle and lung tissue did not occur. Ingroup K2 the changes were very significant, and many inflammatory cells and necrosis were observed in both the muscles and lungs. In contrast to P1, the P2 and P3 groups showed a decrease in inflammatory

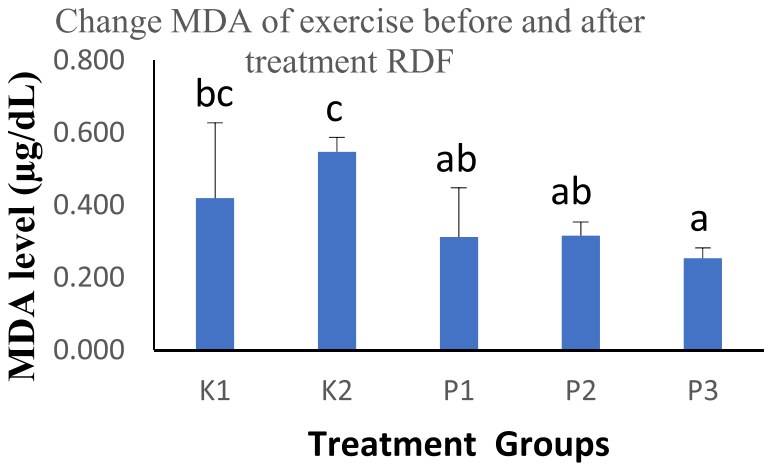

Figure 1. Graph changes of MDA levels (µg/dL) before and after treatment RDF extract; Mean SD. Note: The different notation letters on the bar graph are significantly different ($p<0.05$). Legend of figure: a bc = notation to see the difference is real or not, if a means the difference is not real. If a b = significant difference ($p<0.05$).

Table 3. Comparison of the results of the SOD level before and after treatment RDF extract.

Groups	SOD level (ng/mL)	p-value
K1	0.8647 ± 0.1744^{b}	$p<0.05$
K2	0.4632 ± 0.2449^{a}	
P1	1.3499 ± 0.1359^{c}	
P2	1.9370 ± 0.0236^{d}	
P3	1.9521 ± 0.0239^{d}	

Notation. a, b, c, d: $p<0.05$.
Notation. d, d: $p>0.05$.

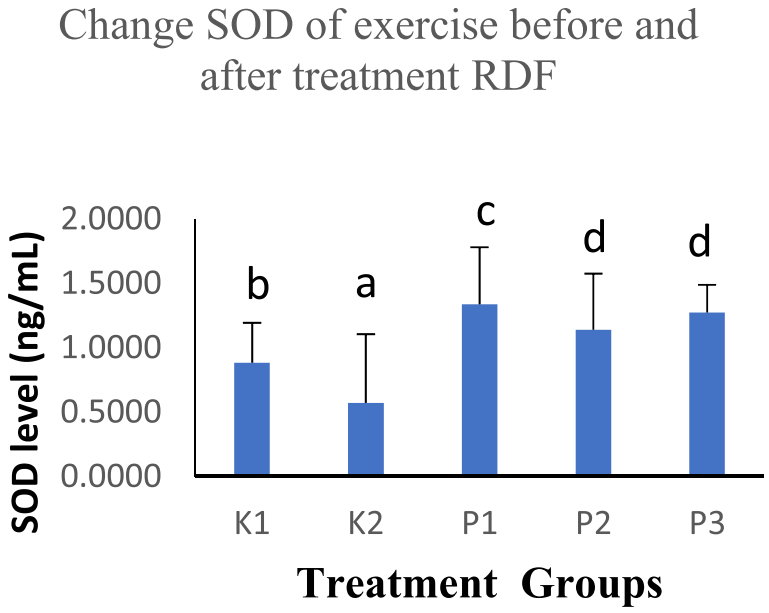

Figure 2. Graph changes of SOD levels (ng/mL) before and after treatment RDF extract. Note: The different notation letters on the bar graph are significantly different ($p<0.05$). Legend, explanation of a,b,c,d.d

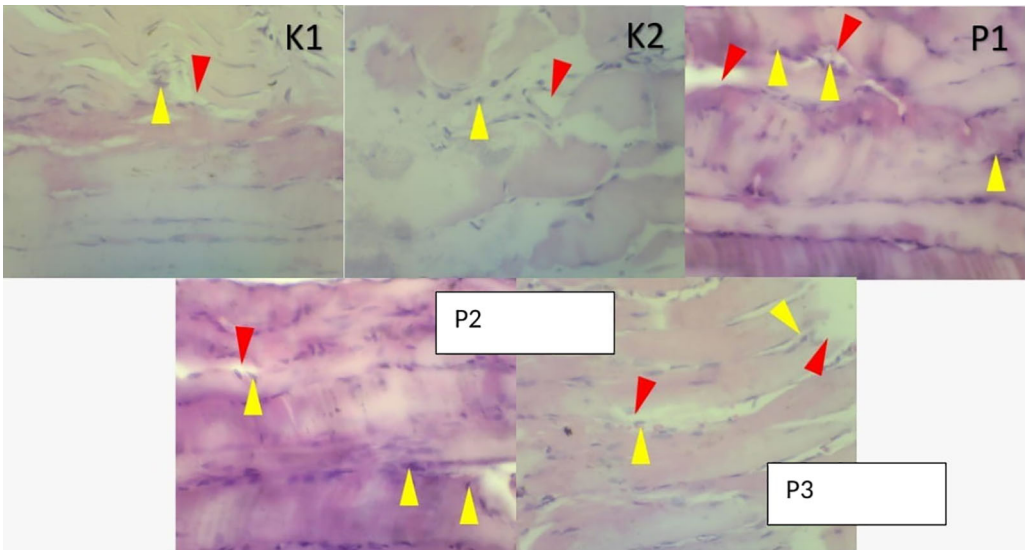

Figure 3. A picture of changes in muscle soleus cells before and after treatment RDF extract (antioxidant exogen). Arrow yellow: inflammatory cells, Arrow red: necrosis.

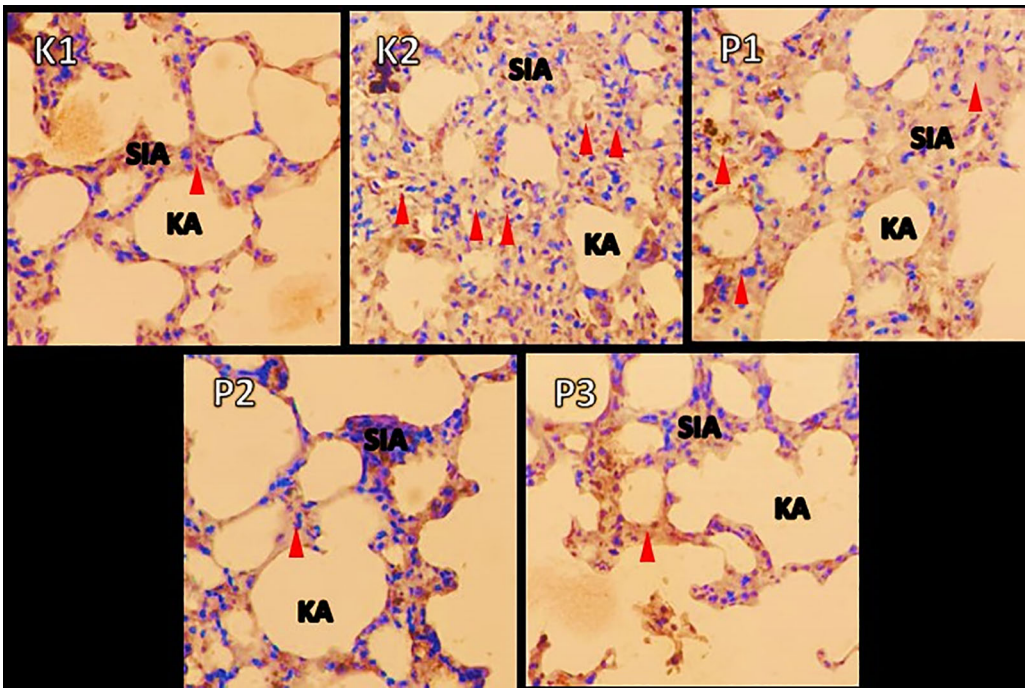

Figure 4. A picture of changes in the lung organs in the rat before and after treatment RDF extract (antioxidant exogen). Note: Red arrow = inflammatory cells; SIA = interalveolar septum, KA = alveolar sac.

cells. In addition, in these two groups compared to P1, the lungs in the intra-alveolar and the alveolar sacs were dilated, and tissue repair was shown by the hyalinization process. Results showed changes in free radicals that could damage tissue in the positive control group K2. In contrast, the histopathological features of the P1, P2, P3 groups showed lung tissue and muscle cell repair, after being given RDF (Figures 3 and 4).

Discussion

Free radicals in the skeletal muscles cause muscle fatigue. The free radicals significantly reduce muscle strength, contributing to muscle fatigue during prolonged training.[7,19,20] The role of oxidants in muscle fatigue has been

investigated in various animal models in vitro and situ during exercise. Oxidants are detectable in muscle at low levels during rest and at higher levels during contractions. RNS depress force production but do not appear to cause fatigue of healthy muscle. In contrast, muscle-derived ROS contribute to fatigue because loss of function can be delayed by ROS-specific antioxidants.[21–23] A study showed that exogenous antioxidants derived from food to capture ROS slowed down muscle fatigue, and enzymatic and nonenzymatic antioxidants delayed muscle fatigue during contraction. In the study, the subject characteristics have been standardized in accordance with WHO, adjusted to the provisions of the criteria[24–26] in the Research Guideline for Evaluating the Safety and Efficacy of Herbal Medicines.

In skeletal muscles, antioxidants are enzymatic (e.g., Glutathione peroxidase (GPx) and catalase) and nonenzymatic (for example, GSH, uric acid, bilirubin, vitamin E, vitamin C, etc.) function as an integrated antioxidant complex that acts to capture ROS.[27,28] These intracellular antioxidants are usually present in cells, cytoplasm, and organelles (for example, mitochondria) whose role is to protect muscle fibres from damage caused by ROS.[27,29–31] Endogenous free radicals are formed as a normal response to the chain reaction of respiration in the body. The free radicals in the body are balanced by an endogenous defense system mechanism,[32] in which the body produces antioxidants that have an anti-free radical effect. One of the endogenous antioxidants is SOD, which is the body's first line of defense against ROS activation.[8] When the level of ROS rises beyond the endogenous defense capacity, oxidative instability, known as oxidative stress, occurs.[9,29] Oxidative stress conditions due to free radicals will cause lipid peroxidation of cell membranes and damage cell membrane organization. One of the biomarkers of oxidative stress is a high level of MDA[33] and decreased SOD activity due to excessive lipid peroxidation processes in cells.[9] One way to control excessive oxidative stress is by consuming antioxidants from food (exogenous antioxidants).[34] One source of exogenous antioxidants is RDF that can be found in Indonesia.

In this study, the endogenous antioxidants in the body were superoxide dismutase (SOD), and they are unable to neutralize free radicals. This condition results in an imbalance of free radicals and antioxidants, leading to oxidative damage, as reported in previous studies.[35] Unstabilized oxidative stress produces free radicals, which can damage muscle tissue and lungs and cause impaired cell function, which is involved in muscle fatigue. RDF treatment can increase SOD significantly ($p<0.05$) and function as a good source of several natural antioxidants, such as betalain, polyphenols, and ascorbic acid, as evidenced in previous studies.[36,37] During strenuous exercise, the increase in ROS formation during contractile activity is directly related to increased oxygen consumption. This condition results in a 50 or 100 fold increase in mitochondrial activity in the formation of superoxide in skeletal muscle during aerobic contraction.[38,39] An increase in oxidative stress, as observed, leads to an increase in lipid peroxidation accompanied by a decline in SOD level activity, as the antioxidants are given depending on the dose affect the increase in SOD levels.[28] This improvement in oxidative status suggests that the natural antioxidants in the extract with high doses were responsible for delaying fatigue in this study, as reported in previous studies.[40,41] In this study, it was found that the higher the dose given, the greater the SOD, as shown in group P3 that was on treatment so that this SOD level could neutralize free radicals. The SOD enzyme is the first defense system against free radicals. Thus, moderate-intensity regular exercise has been shown to increase antioxidant defenses by increasing the activity of endogenous antioxidant enzymes, such as SOD, glutathione peroxidase, and catalase.[42,43] These enzymes can suppress or inhibit the formation of free radicals by breaking the chain reaction so that the product is more stable. This process is known as the antioxidant chain-breaking reaction.

RDF is rich in antioxidants, such as phenol and flavonoid compounds. Phenolic compounds that function as antioxidants neutralize free radicals and peroxide radicals to inhibit lipid oxidation effectively. Flavonoids are exogenous antioxidants that are beneficial in preventing cell damage due to oxidative stress. Its role is to donate hydrogen ions to neutralize the toxic effects from free radicals due to exercise. RDF consumption can also increase the VO2max value.[44]

The relationship between the provision of antioxidants after treatment with RDF extract is that the administration of exogenous antioxidants helps suppress the spread of free radicals in the body because antioxidants can come from within the body (endogenous) or come from outside the body (exogenous), simultaneously suppressing free radicals due to exercise.

Anthocyanin is one type of flavonoid widely found in dragon fruit,[45] which is able to improve mitochondrial function by influencing free radicals. Anthocyanins can suppress the occurrence of lipid peroxidation as an inflammatory response due to free radicals, thereby suppressing the production of MDA.[46]

An increase in the free radicals in the body causes an imbalance between oxidants and antioxidants. This condition leads to oxidative stress. The earliest known and widely studied cell or tissue mechanism is lipid peroxidation. RDF extract contains anthocyanin pigments which function as antioxidants.[18,47,48] Anthocyanins can play a role in inhibiting free radicals that occur due to strenuous exercise. This study examined the provision of RDF extract comprising anthocyanins, one of the types contained in flavonoids, which provides a response to inflammation in the muscles and lung tissue.

The presence of anthocyanins repairs damaged tissue so that physiological mitochondrial function returns, as anthocyanins can suppress the occurrence of lipid peroxidation and suppress MDA production so that MDA levels decrease.[49,50] Anthocyanins can quickly bind metal ions to form a stable anthocyanin-metal complex. This means that anthocyanins bind to the transitioned ion metal to prevent highly toxic and reactive hydroxyl reactions. In the end, anthocyanins can suppress lipid peroxidation and suppress MDA production to reduce MDA levels.

Conclusions

Strenuous exercise causes an increase in ROS, resulting in increased free radical levels, leading to oxidative stress to occur. Ingesting RDF extracts suppresses the increase. The group that was given RDF doses of 150 mg, and 300 mg performed better than the group with a dose of 75 mg in responding to oxidative stress with strenuous exercise. RDF extract dose resulted in decreased oxidative stress, repaired muscle and lung tissue.

Data availability

Underlying data

Figshare: Datasets, https://doi.org/10.6084/m9.figshare.15074544.v5.[51] This project contains the following underlying data:

- MDA RAT.xls (MDA levels for all groups)
- SOD RAT 23 Maret 21.xls (SOD levels for all groups)
- Table HEnew.docx (scoring for microscopy results)

Reporting guidelines

Figshare: ARRIVE checklist for 'The effect of ingestion of Red dragon fruit extract on levels of malondialdehyde and superoxide dismutase after strenuous exercise in rats (*Rattus norvegicus*)', https://doi.org/10.6084/m9.figshare.15074544.v5.[51]

References

1. Gruet M, Temesi J, Rupp T, *et al.*: **Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue.** *Neurosci.* 2013; **231**: 384–399.
PubMed Abstract | Publisher Full Text
2. Taylor JL, Amann M, Duchateau J, *et al.*: **Neural Contributions to Muscle Fatigue: From the Brain to the Muscle and Back Again.** 2016; **48**: 2294–2306.
PubMed Abstract | Publisher Full Text | Free Full Text
3. Dillard CJ, Litov RE, Savin WM, *et al.*: **Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation.** *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 1978; **45**(6): 927–932.
PubMed Abstract | Publisher Full Text
4. Viña J, Gomez-Cabrera MC, Lloret A, *et al.*: **Free radicals in exhaustive physical exercise: Mechanism of production, and protection by antioxidants.** *IUBMB Life.* 2000; **50**(4-5): 271–277.
Publisher Full Text
5. Dekkers JC, Van Doornen LJP, Kemper HCG: **The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage.** *Sports Med.* 1996; **21**(3): 213–238.
PubMed Abstract | Publisher Full Text
6. Halliwell B, Gutteridge JM: **Celullar respons to oxidative stress: adaptation damage repair, senescence and death**. Halliwell B, Gutteridge JM, editors. *Free Radicals in Biology and Medicine.* Oxford University Press; 5th ed. 2015; 254–338.
Publisher Full Text
7. Moopanar TR, Allen DG: **Reactive oxygen species reduce myofibrillar Ca2+ sensitivity in fatiguing mouse skeletal muscle at 37°C.** *J. Physiol.* 2005; **564**(1): 189–199.
PubMed Abstract | Publisher Full Text | Free Full Text
8. Gomez-Cabrera M, Domenech E, Viña J: **Moderate exercise is an antioxidant: Upregulation of antioxidant genes by training.** *Free Radic. Biol. Med.* 2008; **44**: 126–131.
PubMed Abstract | Publisher Full Text
9. Berzosa C, Cebrián I, Fuentes-Broto L, *et al.*: **Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men.** *J. Biomed. Biotechnol.* 2011; **2011**: 1–7.
Publisher Full Text
10. Kregel KC, Zhang HJ: **An integrated view of oxidative stress in aging: Basic mechanisms, functional effects, and pathological considerations.** *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2007; **292**(1): R18–R36.
Publisher Full Text
11. Atsumi T, Iwakura I, Kashiwagi Y, *et al.*: **Free radical scavenging activity in the nonenzymatic fraction of human showing saliva: A Simple D P P H assay the effect of Physical exercise.** *Antioxid. Redox Signal.* 1999; **1**(4): 537–546.
PubMed Abstract | Publisher Full Text
12. Wilson DO, Johnson P: **Exercise modulates antioxidant enzyme gene expression in rat myocardium and liver.** *J. Appl. Physiol.* 2000; **88**: 1791–1796.
PubMed Abstract | Publisher Full Text
13. Rimbach G, Hohler D, Fischer A, *et al.*: **Methods to assess free radicals and oxidative stress in biological system.** *ArchAnimNutr.* 1999; **52**(August 2013): 203–222.
Publisher Full Text
14. Goutianos G, Tzioura A, Kyparos A, *et al.*: **The rat adequately reflects human responses to exercise in blood biochemical profile: A comparative study.** *Physiol. Rep.* 2015; **3**(2): e12293.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Schwiebert R, Ang J, Chang C: *The Laboratory Mouse - Breeding.* Singapore: NUS; 2007; Schwiebert, R., Ang, J. & Chang, C. (2007) The Laboratory Mouse - Breeding. Singapore, NUS, Singapore, NUS.

16. Stathopulos PB, Rumfeldt JAO, Karbassi F, *et al.*: **Calorimetric analysis of thermodynamic stability and aggregation for Apo and Holo amyotrophic lateral sclerosis-associated Gly-93 mutants of superoxide dismutase.** *J. Biol. Chem.* 2006; **281**(10): 6184–6193.
Publisher Full Text

17. Sentman ML, Granström M, Jakobson H, *et al.*: **Phenotypes of mice lacking extracellular superoxide dismutase and copper- and zinc-containing superoxide dismutase.** *J. Biol. Chem.* 2006; **281**(11): 6904–6909.
Publisher Full Text

18. Jaafar RA, Abdul Rahman ARB, Mahmod NZC, *et al.*: **Proximate analysis of dragon fruit (hylecereus polyhizus).** *Am. J. Appl. Sci.* 2009; **6**(7): 1341–1346.
Publisher Full Text

19. Vasilaki A, Mansouri A, Van Remmen H, *et al.*: **Free radical generation by skeletal muscle of adult and old mice: effect of contractile activity.** *Aging Cell.* November 2005; **5**: 109–117.
PubMed Abstract | Publisher Full Text

20. Richardson RS, Noyszewski EA, Kendrick KF, *et al.*: **Myoglobin O_2 desaturation during exercise: Evidence of limited O_2 transport.** *J. Clin. Invest.* 1995; **96**(4): 1916–1926.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Ferreira LF, Reid MB: **Muscle-derived ROS and thiol regulation in muscle fatigue.** *J. Appl. Physiol.* 2008; **104**(3): 853–860.
PubMed Abstract | Publisher Full Text

22. Kozlov AV, Szalay L, Umar F, *et al.*: **Skeletal muscles, heart, and lung are the main sources of oxygen radicals in old rats.** *Biochim. Biophys. Acta Mol. Basis Dis.* 2005; **1740**(3): 382–389.
PubMed Abstract | Publisher Full Text

23. Reid MB, Haack KE, Franchek KM, *et al.*: **Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro.** *J. Appl. Physiol.* 1992; **73**(5): 1797–1804.
PubMed Abstract | Publisher Full Text

24. WHO: *Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicine.* 1993.

25. WHO: *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine World Health Organization.* Geneva: WHO; 2000.

26. WHO: *WHO Guidelines on Good Manufacturing Practice (GMP) for Herbal Medicine.* 2007.
Reference Source

27. Powers SK, Ji LL, Kavazis AN, *et al.*: **Reactive oxygen species: Impact on skeletal muscle.** *Compr. Physiol.* 2011; **1**(April): 941–969.
Publisher Full Text

28. Kayode OT, Kayode AAA, Nwonuma CO: **Alcoholic bitters modulates sex hormones and some biochemical parameters of testicular function in male wistar rats [version 1; peer review: 1 approved, 1 approved with reservations].** *F1000Res.* 2018; **7**: 1–9.
Publisher Full Text

29. Powers SK, Jackson MJ: **Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production.** *Physiol. Rev.* 2008; **88**(4): 1243–1276.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Kurepa J, Shull TE, Smalle JA: **Quercetin feeding protects plants against oxidative stress.** *F1000Res.* 2016; **5**(May): 2430.
Publisher Full Text

31. Hubert SM, Athrey G: **Energy metabolism and sources of oxidative stress in wooden breast - a review [version 1; peer review: 1 approved, 1 approved with reservations].** *F1000Res.* 2020; **9**(319): 1–15.
Publisher Full Text

32. Novita Sari H, 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): 2232–2235.
PubMed Abstract | Publisher Full Text | Free Full Text

33. Tsikas D: **Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges.** *Anal. Biochem.* 2017; **524**: 13–30.
PubMed Abstract | Publisher Full Text

34. Reid MB: **Redox modulation of skeletal muscle contraction: what we know and what we don't.** *J. Appl. Physiol.* 2001; **90**(Feb): 724–731.
PubMed Abstract | Publisher Full Text

35. Caramori G, Papi A: **Oxidants and asthma.** *Thorax.* 2004; **59**(2): 170–173.
PubMed Abstract | Publisher Full Text | Free Full Text

36. Machha A, Achike FI, Mustafa AM, *et al.*: **Quercetin, a flavonoid antioxidant, modulates endothelium-derived nitric oxide bioavailability in diabetic rat aortas.** *Nitric Oxide Biol. Chem.* 2007; **16**(4): 442–447.
PubMed Abstract | Publisher Full Text

37. Woo KK, Ngou FH, Ngo LS, *et al.*: **Stability of betalain pigment from red dragon fruit (Hylocereus polyrhizus).** *Am. J. Food Technol.* 2011; **6**(2): 140–148.
Publisher Full Text

38. Karazis A, Powers S: **Impact of exercise, reactive oxygen and reactive nitrogen species on tumor growth.** *Exerc. Energy Balanc. Cancer.* 2013; 1–239.
Publisher Full Text

39. Kang SW, Rhee SG, Chang TS, *et al.*: **2-Cys peroxiredoxin function in intracellular signal transduction: Therapeutic implications.** *Trends Mol. Med.* 2005; **11**(12): 571–578.
PubMed Abstract | Publisher Full Text | Free Full Text

40. Anand Swarup KRL, Sattar MA, Abdullah NA, *et al.*: **Effect of dragon fruit extract on oxidative stress and aortic stiffness in streptozotocin-induced diabetes in rats.** *Pharm. Res.* 2010; **2**(1): 31–35.
PubMed Abstract | Publisher Full Text | Free Full Text

41. Sumardika IW, dan Jawi IM: **Department of Pharmacology, Medical School, Udayana University.** *J Ilm Kedokt Med.* 2014; **36**(September): 19–24.
Reference Source

42. Gusbakti R, Mukti SS: **Effects of moderate intensity exercise on glutathione peroxidase activity and VO2 max in elderly women.** *Maced. J. Med. Sci.* 2020; **8**((A)(25)): 230–233.
Publisher Full Text

43. Miyata M, Kasai H, Kawai K, *et al.*: **Changes of urinary 8-hydroxydeoxyguanosine levels during a two-day ultramarathon race period in Japanese non-professional runners.** *Int. J. Sports Med.* 2008; **29**: 27–33.
Publisher Full Text

44. Prakoso LO, Yusmaini H, Thadeus MS, *et al.*: **Perbedaan efek ekstrak buah naga merah (Hylocereus polyrhizus) dan ekstrak buah naga putih (Hylocereus undatus) terhadap kadar kolesterol total tikus putih (Rattus norvegicus).** *J Gizi dan Pangan.* 2017; **12**(3): 195–202.
Publisher Full Text

45. Jamilah B, Shu CE, Kharidah M, *et al.*: **Physico-chemical characteristics of red pitaya (hylocereus polyrhizus) peel.** *Int. Food Res. J.* 2011; **18**(1): 279–286.
Reference Source

46. Miguel MG: **Antioxidant activity of medicinal and aromatic plants. A review.** *Flavour.* 2009; **25**(November): 291–312.
Publisher Full Text

47. Stintzing FC, Carle R: **Functional properties of anthocyanins and betalains in plants, food, and in human nutrition.** *Trends Food Sci. Technol.* 2004; **15**(1): 19–38.
Publisher Full Text

48. Wu LC, Hsu HW, Chen YC, *et al.*: **Antioxidant and antiproliferative activities of red pitaya.** *Food Chem.* 2006; **95**(2): 319–327.
Publisher Full Text

49. Harahap NS, Lelo A, Purba A, *et al.*: **The effect of red-fleshed pitaya (Hylocereus polyrhizus) on heat shock protein 70 and cortisol expression in strenuous exercise induced rats [version 1; peer review: 1 approved with reservations].** *F1000Res.* 2019; **8**: 1–12.
Publisher Full Text

50. Singh G, Pachouri UC, Khaidem DC, *et al.*: **Mitochondrial DNA Damage and Diseases.** *F1000Res.* 2015; **4**: 176–177.
PubMed Abstract | Publisher Full Text | Free Full Text

51. Rusip G: **Datasets. figshare.** *Dataset.* 2021.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ?

Version 2

Reviewer Report 11 July 2022

https://doi.org/10.5256/f1000research.135241.r143819

✔ **Farzaneh Taghian**
Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran

This paper sounds good.
Best wishes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Sport and nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 07 June 2022

https://doi.org/10.5256/f1000research.57721.r137352

? **Ermita I. Ibrahim Ilyas**
Faculty of Medicine, Department of Medical Physiology, Universitas Indonesia, Jakarta, Indonesia

This study was quite informative. There are some suggestions to improve this article:

1. The title should be in accordance with the result of the study.

2. Abstract should be rewritten since there were information missed in the methods, results

and conclusion. Please refer to the main body of the manuscript.

3. The introduction also needs to be rewritten to make it clear, focused and concise to the aim of the study. Authors have to provide a comprehensive description

Methods:

- Study design has to explain the grouping of the rats with proper terms.
- Experimental procedures in the main manuscript were different than in the abstract.
- Outcome: authors mentioned about repair mitochondrial cell function has fatigue delaying effect. However, authors did not analyse or measure the mitochondria function and fatigue duration and time.

Results:

- Write complete legends of the figures including clear explanation of the sign.
- Explain how to determine the improvement of lung and muscle cells (histopathological change in muscle and lung tissue) in the rats supplemented by RDF.
- How to determine the fatigue? Authors mentions the fatigue delay with no data showing this.

Discussion:

- Rewrite to make it systematic, focused and clear to provide a comprehensive report.

Conclusion:

- My answer to the question, "Are the conclusions drawn adequately supported by the results?" is 'No' because the conclusion of the manuscript was not written adequately from the result of the study. My suggestion is that they have to write the conclusion based on all the parameters they analyzed.

Conclusion in the abstract:
"This study confirmed that strenuous exercise that causes an increase in ROS intensifies free radicals with RDF extract ingestion and declines stress oxidative, repairing cell function and delaying fatigue."

My suggestion for Conclusion in the abstract:
"This study confirmed that strenuous exercise causes an increase in ROS intensifies free radicals shown by MDA increase and SOD decrease, and with RDF extract ingestion MDA decrease and SOD increase showed RDF declines stress oxidative, and 300 mg kg^{-1}.bw of RDF extract showed better result compared to other doses. The decrease of oxidative stress, showed improvement of muscle and lung tissue injuries."

Conclusion in the main body of the manuscript:
"Strenuous exercise causes an increase in ROS, resulting in increased free radical levels, leading to oxidative stress to occur. Ingesting RDF extracts suppresses the increase. The group was given RDF doses of 150 mg, and 300 mg performed better than 75 mg in responding to oxidative stress with strenuous exercise. This condition results in decreased oxidative stress, repaired muscle and lung tissue, so that cell function returned physiologically, which delayed fatigue."

My suggestion for Conclusion in the main body of manuscript:
"Strenuous exercise causes an increase in ROS, resulting in increased free radical levels, leading to oxidative stress to occur shown by MDA increase and SOD decrease. Ingesting RDF extracts

suppresses the increase of oxidative stress. The group that was given RDF doses of 150 mg and 300 mg performed better than 75 mg in responding to oxidative stress. The decrease of oxidative stress, showed improvement of muscle and lung tissue injuries."

Note:

- The manuscript's language and grammar should undergo a thorough proofreading.
- Authors should rewrite the manuscript based on reviewer's suggestions.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Exercise Physiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 21 Jun 2022

Prof Gusbakti Rusip, University Prima Indonesia, Medan, Indonesia

It has been a great sense of delight to receive your very friendly and professional comments. We want to thank the reviewer for the time and willingness to assess the quality of our manuscript. In the new version, our manuscript suggested changes in all sections have been added according to the reviewer's recommendation.

Kindest regards,

Prof Dr Gusbakti Rusip, MD, Sp.KKLP, AIFM, Department Physiology, University Prima

Indonesia

1. Introduction

One source of natural antioxidants is red dragon fruit peel (*Hylocereus polyrhizus*). Red dragon fruit peel (1 mg/kg) is able to inhibit 83.48% of free radicals. Red dragon fruit peels has antioxidant activity (IC50 = 43,836 microgram/mL). The antioxidant of red dragon fruit peel is higher than its flesh. Red dragon fruit peel contains a natural antioxidant compound of phenolic or polyphenolic. It has antioxidant activity of flavones, flavonols, isoflavones, catechins and kalkon. Red dragon fruit peel also contains betacyanin. Betacyanin is part of the pigment betalains and has antioxidant properties (neutralizing free radicals). Betacyanin of red dragon fruit peel, including phenolic compounds. Phenolic derivatives or polyphenols (as antioxidants) stabilize free radicals by supplementing lack of electrons possessing free radicals and inhibiting the occurrence of chain reactions from free radical formation.

2. Methods and materials

In this study, we used 25 three-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 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 daily during the study. The room light was controlled to deliver a 12h light/12h dark cycle, the temperature was set to 25–27 °C, and the room's humidity was adjusted to a normal range of 35–50%. The rats were fed standard rat pellets and given tap water *ad libitum*.

3. Design study

We used an in vitro experimental method with an actual experimental design and a randomised post-test for the control group. Simple random sampling was used to categorise the laboratory rats into five groups as follows: group K1 with no activity and no RFP; group K2 subjected to strenuous exercise without RDF (Red Dragon Fruit); and groups P1, P2, and P3 subjected to strenuous exercise and treated with 75, 150, and 300 mg kg^{-1} body weight of RDF extract, respectively. In the fruit market, it is easy to find RFP fruit acquired from farmers in Indonesia, 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 ten times the weight of RDF. The RDF powder was stored in a container with 96% ethanol (ratio of 1:7, fruit powder: ethanol) and then soaked for 3 d. The RDF was macerated using a rotary evaporator at 45 °C until the extract thickened. The macerated RDF was extracted using 96% ethanol. The remaining extract was evaporated in a water bath until a thick extract was obtained. Next, 100 mg RDF 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, corresponding to doses of 75, 150, or 300 mg kg^{-1} body weight, respectively.

4. 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 analyse malondialdehyde (MDA) and SOD 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, catalog: EO625Mo) was used to analyse the MDA levels. The SOD kit (Brand Bioassay TL, catalog: EO168Ra) Rat super Oxidase Dismutase ELISA kit was determined using the equation obtained from the standard curve.

5. Histopathological study

   Muscle and lung tissue samples were collected by performing a biopsy to determine the degree of muscle damage based on haematoxylin and eosin (H&E) staining. The soleus muscle tissues of the rats were collected and fixed with 10% formalin for 24 h. The muscle and lung tissues were embedded in paraffin, sectioned to a 4 μm thickness, and stained via H&E staining. The stained sections were then examined under a light microscope (400' magnification) with 10 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.

Competing Interests: No competency interests

Author Response 21 Jun 2022

Prof Gusbakti Rusip, University Prima Indonesia, Medan, Indonesia

We would like to thank the reviewer for the time and willingness to assess the quality of our manuscript. In the new version, the suggested changes in all sections have been added according to the reviewers recommendation. It has been a great sense of delight to receive your very nice and professional comments.

Kindest regards,

Prof Dr Gusbakti Rusip, MD, Sp.KKLP,AIFM, Department Physiology, University Prima Indonesia

1. The title should be in accordance with the result of the study.
   We agree that we have adjusted the results of the study.

2. Abstract should be rewritten since there were information missed in the methods, results and conclusion. Please refer to the main body of the manuscript.

The manuscript has been revised and has been completed.

3. The introduction also needs to be rewritten to make it clear, focused and concise to the aim of the study. Authors have to provide a comprehensive description.
   The manuscript has been revised and has been completed.

Methods:

1. Study design has to explain the grouping of the rats with proper terms. Experimental procedures in the main manuscript were different than in the abstract.
   It has been revised according to the research objectives and has been perfected.

2. Outcome: authors mentioned about repair mitochondrial cell function has fatigue delaying effect. However, authors did not analyse or measure the mitochondria function and fatigue duration and time.
   Mitochondrial function analysis has not been analyzed, the research is in progress.

Results:

1. Write complete legends of the figures including clear explanation of the sign.
   We have fixed it in the revised article.

2. Explain how to determine the improvement of lung and muscle cells (histopathological change in muscle and lung tissue) in the rats supplemented by RDF.
   Monitoring the repair of muscle and lung cells seen from the number of inflammatory cells and necrosis then examined under a light microscope (400x magnification) with ten fields of view to ascertain the degree of damage with respect to inflammatory cells and necrosis seen in Fig share table HE.

3. How to determine the fatigue? Authors mentions the fatigue delay with no data showing this.
   The results of this study are that we write down the time of fatigue before and after giving Red Dragon Fruit after we have entered the data on the results of the comparison.

Discussion:

- Rewrite to make it systematic, focused and clear to provide a comprehensive report.
  We have systematically improved and completed several discussions for the perfection of the article.

Conclusion:

- My answer to the question, "Are the conclusions drawn adequately supported by the results?" is 'No' because the conclusion of the manuscript was not written adequately from the result of the study. My suggestion is that they have to write the conclusion based on all the parameters they analyzed.
  We have adjusted the conclusion to the topic, we found the results of our research.

Competing Interests: No competing interests were disclosed.

Reviewer Report 29 March 2022

https://doi.org/10.5256/f1000research.57721.r127975

?

Farzaneh Taghian
Department of Exercise Physiology, Faculty of Sport Sciences, University of Isfahan, Isfahan, Iran

The author evaluated the effect of ingestion of red dragon fruit extract on levels of malondialdehyde and superoxide dismutase after strenuous exercise in rats. I think this study was informative and practical.

However, there are some comments to improve this study:

- In the Introduction section explain red dragon fruit in detail.
- The grouping is not clear. Describe it. What is N, A, or RDF? Completely explain the grouping.
- Study design is not suitable. The author has to explain the standard condition of the rats and grouping.
- Write the commercial name of the kits, catalog number.
- The grammar and writing is poor
- Write the protocol of Hematoxylin Eosin (H&E) staining
- The quality of the image is poor.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Sport and nutrition

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Apr 2022

Prof Gusbakti Rusip, University Prima Indonesia, Medan, Indonesia

We would like to thank the reviewer for the time and willingness to assess the quality of our manuscript. In the new version of our manuscript, suggested changes in all sections have been added according to the reviewer's recommendation. It has been a great delight to receive your very nice and professional comments.

Kindest regards,

Prof Dr Gusbakti Rusip, MD, Sp.KKLP,AIFM, Department Physiology, University Prima Indonesia

1. Introduction: One source of natural antioxidants is red dragon fruit peel (*Hylocereus polyrhizus*). Red dragon fruit peel (1 mg/kg) can inhibit 83.48% of free radicals. Red dragon fruit peels has antioxidant activity (IC50 = 43,836 microgram/mL). The antioxidants of red dragon fruit peel are higher than in its flesh. Red dragon fruit peel contains natural antioxidant compounds in phenolic or polyphenolic. It has antioxidant activity of flavones, flavonols, isoflavones, catechins and kalkon. Red dragon fruit peel also contains betacyanin. Betacyanin is part of the pigment betalains and has antioxidant properties (neutralizing free radicals). Betacyanin of red dragon fruit peel including phenolic compounds. Phenolic derivatives or polyphenols (as antioxidants) stabilize free radicals by supplementing lack of electrons possessing free radicals and inhibiting the occurrence of chain reactions from free radical formation.

2. Methods and Materials: In this study, we used 25 three-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 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 room's humidity was adjusted to a normal range of 35–50%. The rats were fed standard rat pellets and given tap water *ad libitum*.

3. Design study: We used an in vitro experimental method with a true experimental design and a randomised post-test for the control group. Simple random sampling was used to categorise the laboratory rats into five groups as follows: group K1 with

no activity and no RDF; group K2 subjected to strenuous exercise without RDF (Red Dragon Fruit); and groups P1, P2, and P3 subjected to strenuous exercise and treated with 75, 150, and 300 mg kg^{-1} body weight of RDF extract, respectively. It is easy to find RDF in the fruit market, acquired from farmers in Indonesia, 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 RDF. The RDF powder was stored in a container with 96% ethanol (ratio of 1:7, fruit powder: ethanol) and then soaked for 3 d. The RDF was macerated using a rotary evaporator at 45 °C until the extract thickened. The macerated RDF was extracted using 96% ethanol. The remaining extract was then evaporated in a water bath until a thick extract was obtained. Next, 100 mg RDF 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 RDF 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 RDF extract suspension, which corresponded to doses of 75, 150, or 300 mg kg^{-1} body weight, respectively.

4. Analysis of blood samples: 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 analyse malondialdehyde (MDA) and SOD 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, catalog: EO625Mo) was used to analyse the MDA levels. The SOD kit (Brand Bioassay TL, catalog: EO168Ra) Rat super Oxidase Dimutase ELISA kit was determined using the equation obtained from the standard curve.

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

Competing Interests: no competing interests