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]

Novita Sari Harahap 1, Aznan Lelo 2, Ambrosius Purba 3, Awaluddin Sibuea 4, Rina Amelia 5, Zulaini Zulaini 1

1Department of Sports Sciences, Faculty of Sports Sciences, Universitas Negeri Medan, Medan, North Sumatra, 20221, Indonesia
2Department of Pharmacology, Faculty of Medicine, University of Sumatra Utara, Medan, North Sumatra, 20155, Indonesia
3Department of Physiology, Faculty of Medicine, Padjajaran University, Bandung, West Java, 45363, Indonesia
4Surgery Division, Dr. T. Mansyur Tanjung Balai Hospital, Tanjungbalai, North Sumatra, 21312, Indonesia
5Department of Public Health, Faculty of Medicine, University of Sumatra Utara, Medan, North Sumatra, 20155, Indonesia

Abstract

**Background:** Oxidative stress from exercise can contribute to damaging cells, increasing heat shock protein 70 (HSP70) and suppressing the immune system in the body. This research aimed to determine the antioxidant potential of red-fleshed pitaya extract on HSP70 and cortisol expression in rats which were subjected to strenuous exercise.

**Methods:** The subjects of this research were 32 Sprague Dawley male rats, aged 3 months, with an average weight of 200 g. Red-fleshed pitaya extract was obtained from methanol extraction process; a maceration technique was performed and the extract was concentrated using an air-drying method. Rats were randomly divided into four groups. Group 1 were subjected to strenuous exercise and treated with distilled water only; while Groups 2, 3 and 4 were subjected to strenuous exercise and treated with 100 mg/kg body weight, 200 mg/kg body weight and 300 mg/kg body weight of red-fleshed pitaya extract, respectively. Strenuous exercises in rats was performed by intense swimming of 20 min/day, 3 days a week for 3 weeks. HSP70 expression and cortisol were measured with Enzyme-Linked Immune Sorbent Assay (ELISA) method.

**Results:** There was a significant reduction of HSP70 (p=0.000) and cortisol expression (p=0.000) between the groups. Also, there was a significant difference in the average decreasing of HSP70 expression between group 4 and either groups 1 or 2 (p=0.000). However, a significant difference between groups 4 and 3 was not observed (p=0.813). Lastly, a significant difference was found in the average decrease of cortisol expression between groups 4 and 1 (p=0.000), 2 (p=0.000), and 3 (p=0.000) respectively.

**Conclusion:** Red-fleshed pitaya is potential to be utilized as antioxidant to decrease the HSP70 and cortisol expression.
Keywords
Red-fleshed pitaya, HSP70, cortisol, strenuous exercise

Corresponding author: Novita Sari Harahap (novitahrp74@gmail.com)

Author roles: Harahap NS: Conceptualization, Investigation, Methodology, Resources, Writing – Original Draft Preparation; Lelo A: Investigation, Methodology; Purba A: Resources, Supervision; Sibuea A: Resources; Amelia R: Resources; Zulaini Z: Formal Analysis

Competing interests: No competing interests were disclosed.

Grant information: The authors would like to acknowledge Ministry of Research, Technology and Higher Education of Republic of Indonesia for funding this research via Research Grant of Universitas Negeri Medan with contract number of 027/UN33.8/LL/2018. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2019 Harahap NS et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: 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]
F1000Research 2019, 8:130 (https://doi.org/10.12688/f1000research.17533.1)

First published: 30 Jan 2019, 8:130 (https://doi.org/10.12688/f1000research.17533.1)
Introduction

Physical activity is an activity which has various influences and significant effects on the body. The effect of regular physical activity is a positive influence on biological functions, and will improve health and the antioxidant defense system in order to protect the body from the negative effects of oxidative damage\(^1\). Strenuous exercise tends to trigger free radical compound production. Moreover, this impairs the balance of free radicals and antioxidants as a result of oxidative stress\(^2\). Research has discovered that oxidative stress from strenuous exercise reduces performance as it damages cells\(^7\), causing pain and muscles fatigues\(^8\), lowering antioxidant levels\(^9\), increasing the expression of heat shock protein 70 (HSP70)\(^10\) and suppressing the immune system\(^11\).

During an intense workout, self-defense and self-adaptation depend on the body condition which can be observed from HSP70 protein expression. Increases of HSP70 in muscles indicates a response to protect muscles cells from oxidative stress. HSP70 expression is an adaptation mechanism and a sign of damaging cells caused by oxidative stress\(^12\). Previous research reported that workouts increase HSP70 expression\(^10\). Strenuous exercise is a physical stressor in the body and as a result, adrenocorticotropic hormone (ACTH) is secreted by hypothalamus hypo-physis anterior and triggers the adrenal cortex to produce cortisol\(^13\). The escalation of cortisol is influenced by the intensity and duration of training that leads to a suppression of the immune system, resulting in a decline of antibody. Cortisol can be a sign that the body encounters a decline in the immune system due to heavy training\(^14\). Antioxidants can detoxify the lipid peroxide produced during exercise, which can eliminate radicals and reduce the inflammatory response to exercise. Therefore, it can prevent a muscle damage from exercise\(^15\).

The body needs exogenous antioxidants to neutralize and prevent chain reactions from free radicals formed from heavy physical trainings\(^16\). Sources of exogenous antioxidants are Vitamin E, C and also beta-carotene. External antioxidants from food or supplements can help the body to fight an excess of free radicals. In a previous study, proanthocyanidin from grape seed was given to rats for 2 weeks. As a result, it lowered malondialdehyde level and increased the superoxide dismutase and glutathione peroxidase was activated significantly. Furthermore, it reduced fatigue after physical activity\(^17\).

Red-fleshed pitaya (\textit{Hylocereus polyrhizus}) is a unique fruit with a lot of benefits. The fruit is recently popular among Indonesians and appears to be a natural antioxidant. Several \textit{in vitro} studies have revealed that red-fleshed pitaya extract has the potential to be an antioxidant\(^18\). This research aimed to investigate the antioxidant potential in red-fleshed pitaya extract on HSP70 and cortisol expression in rats that were subjected to strenuous exercise.

Methods

Experimental animals

The subjects of this research were 32 Sprague Dawley male rats, aged 3 months, with an average weight of 200 g, were obtained from the Animal Holding Unit of the Pharmacy laboratory, University of Sumatera Utara, Indonesia. All rats were sustained and maintained in groups (four mice per cage) in experimental animal cages of the Pharmacy laboratory, University of Sumatera Utara, Indonesia. The cage was made of plastic (30 x 20 x 10 cm) and covered with fine wire mesh. The base of the cage is covered with rice husk as thick as 0.5 - 1 cm and replaced every day during the research. The room light was controlled to be exactly at 12 hours light and 12 hours dark cycle, while the temperature 25–27°C and humidity of the room were adjusted to a normal range and fed with standard rat pellets 551, and drink (tap water) was given \textit{ad libitum}.

Study design

The research applied a laboratory experiment method with random group posttest-only design. The male rats were obtained from the Pharmacy Laboratory, University of North Sumatra. The experimental animals were simple random sampling divided into four groups: Group 1 was subjected to strenuous exercise and treated with distilled water only; Group 2 was subjected to strenuous exercise and treated with dosage 100 mg/kg body weight of red-fleshed pitaya extract; Group 3 was subjected to strenuous exercise and treated with dosage 200 mg/kg body weight of red-fleshed pitaya extract; Group 4 was subjected to strenuous exercise and treated with dosage 300 mg/kg body weight of red-fleshed pitaya extract.

Red-fleshed pitaya extract

Red-fleshed pitaya fruit, obtained from farmers, in Indonesia, was peeled, washed, cut into small pieces and then dried in a drying cabinet. After that, the fruit was blended using a blender. The fruit extract was isolated through maceration method by using ethanol 96% which has been distilled as much as 10 times the weight of red-fleshed pitaya powder. Red-fleshed pitaya fruit powder in a container had 96% ethanol added to it (ratio 1:7, fruit powder: ethanol), and then was soaked for 3 days then filtered and sealed. The macerates were collected in a container and then processed with rotary evaporator at a temperature of 45°C until the extract was thickened. After that, the same process of were repeated the remaining ethanol 96% for 3 days. The less thickened extract was then evaporated in a water bath until a thick extract was obtained.

100 mg red-fleshed pitaya extract was weighed. Then, it was gently ground using a pestle and mortar. After that, carboxy methyl cellulose (CMC) Na 0.5% solution was slowly added and ground until a homogeneous phase was achieved. Finally, the suspension was added to a 10 mL measuring flask until it reached the mark line. The allocation of red-fleshed pitaya extract, dosage of 100 mg/kg body weight, for instance: weight of 200 g, volume taken: 2 ml extract suspension. Dosage of 200 mg/kg body weight, for instance: weight of 200 g, volume taken: 4 ml extract suspension. Dosage of 300 mg/kg body weight, for instance: weight of 200 g, volume taken: 6 ml extract suspension.

Experimental procedures

Strenuous exercise given to all rats was a morning swim between 8–9 am for 20 minutes/day, 3 days a week over 21 days. The equipment used in this research was a 10-cm length and 25-cm diameter bath as a pool. Group 1, the rats received distilled water only; Group 2, the rats received 2 ml red-fleshed pitaya extract suspension. Dosage of 100 mg/kg body weight, for instance: weight of 200 g, volume taken: 2 ml extract suspension. Dosage of 200 mg/kg body weight, for instance: weight of 200 g, volume taken: 4 ml extract suspension. Dosage of 300 mg/kg body weight, for instance: weight of 200 g, volume taken: 6 ml extract suspension.
extract suspension; Group 3, the rats received 4 ml red-fleshed pitaya extract suspension; Group 4, the rats received 6 ml red-fleshed pitaya extract suspension. Administration of red-fleshed pitaya extract suspension and water was performed orally once daily for 21 days.

Testing for HSP70 and cortisol was conducted two days after the rats had completed a strenuous exercise, 3 days a week over 21 days. During the test, the rats were given a maximum training session by swimming as hard as they could until the rats drowned or showed fatigue symptoms such as the entire body almost dipped into water and limb movements slowed down. After that the rats were sacrificed by placing them in a jar containing cotton which was moistened with 10 ml of chloroform. After that, 2–3 ml blood was taken from the heart.

Blood samples were collected in micro tubes and centrifuged at 3000 rpm for 15 minutes. The serum was separated and stored at a temperature of 20°C until the analysis process would be carried out. Cortisol was measured with enzyme-linked immune sorbent assay (ELISA); Mouse Cortisol Elisa kit (catalog: E1483Mo, Brand Bioassay TL) and UV spectrophotometry at a wavelength of 450 nm. The HSP70 expression was recorded with ELISA; Mouse HSP70 Elisa Kit (catalog: E0302Mo, Brand Bioassay TL); the absorbance was indicated at 405 nm. All efforts were made to reduce any suffering of the rats during the experiments by following careful procedures and also by anaesthetizing the animal prior to sacrifice to prevent experiencing any pain.

Results
A normality test indicated that the data are normally distributed (Table 1). HSP70 expression was decreased across all groups (69.57 vs 46.04 vs 31.47 vs 27.65 pg/mL). Group 4 had the lowest expression compared with the other groups. This research reveals a significant decrease in HSP70 expression (p=0.000) between the groups (Table 2).

Cortisol expression was also decreased across all groups (119.02 vs 86.11 vs 62.94 vs 40.86 pg/mL). Group 4 had the lowest expression compared with the other groups. An ANOVA test revealed a significant decrease in cortisol expression (p=0.000) between the groups (Table 2).

Figure 1 indicates a significant difference in the average decrease of HSP70 expression between group 4 and either group 1 or 2 (p=0.000). However, a significant difference between group 4 and group 3 (p=0.813) was not found. This means that group 4, with strenuous exercise and given 300 mg/kg body weight red-fleshed pitaya extract was indicated to be more effective for

### Statistical methods
Data was analyzed using SPSS 22 for Windows and displayed in tables and diagrams. Normality test was conducted through Shapiro-Wilk test (P > 0.05) in order to determine the average of normal distribution of sample data which is presented as mean ± SD. The result of the normality test was used for next analysis; parametric analysis was used for normal distribution, otherwise non-parametric analysis was used. The ANOVA statistical analysis was performed to indicate the effects of treatments for each group. If the significant result is obtained, then the procedure is followed by Least Significance Difference or Bonferroni tests.

### Ethical approval
The research was performed on the animal subjects were in accordance with the ethical standards by the Animal Research Ethics Committees/AREC, Faculty of Mathematics and Natural Sciences University of Sumatera Utara, Indonesia (approval number 0011/KEPH-FMIPA/2018).
reducing HSP70 expression compared to group 1 and 2, with no big variance from group 3.

Figure 2 confirms that a significant difference is revealed in the average decrease of cortisol expression between groups 4 and 1 (p=0.000), 2 (p=0.000), and 3 (p=0.000). It can be concluded that group 4 with strenuous exercise and 300 mg/kg body weight red-fleshed pitaya extract was more effective in reducing cortisol expression compared to the other groups.

**Discussion**

Based on the results of this research, it is found that strenuous exercise combined with daily red-fleshed pitaya extract consumption contributes to a declining expression of HSP70 and cortisol. The dosage of 300 mg/kg body weight red-fleshed pitaya extract was found to be the optimum amount in decreasing cortisol compared to 100 and 200 mg/kg body weight dosage. However, for both 300 mg/kg body weight and 200 mg/kg body weight dosage of red-fleshed pitaya extract could not provide any difference to HSP70 expression. Therefore, the red-fleshed pitaya extract can be categorized as a potential exogenous antioxidant to eliminate free radicals formed during strenuous exercise.

Free radicals are an element that possesses one or more unpaired electrons in its outermost orbital. Consequently, it is very reactive to cells or cell components in its surroundings. Commonly, a reactive element finds its pair by attacking and binding with adjacent electrons. Then, if this element reacts with another radical element, a new radical element will be formed. This will consistently continue to occur leading to an unavoidable chain reaction. During strenuous exercise, oxygen consumption rises by 20 times. The excess oxygen triggers the formation of free radicals with electrons released from the respiratory chain. Free radicals production from activity, especially superoxides, increases in the mitochondria. When an imbalance happens due to the excess of free radicals, oxidative stress occurs and damages the DNA. Moreover, proteins will lose their structure and function like enzymes and membrane receptor. Also, there will be damage in the lipid bilayer structure.

Strenuous exercise triggers oxidative stress to occur, therefore, HSP70 expression will elevate, resulting in a decrease of endogenous antioxidant activity. HSP70 is an important protein molecule for cell healing and preventing homeostasis, and also increased expression as a cyto-protective effect. HSP70 is induced strongly due to oxidative stress as cyto-protective to prevent oxidative damage and heal broken proteins. The increase of HSP70 expression is aimed to balance between ischemic condition, high temperature and increased production of free radical. In this research, it is found that HSP70 expression tends to decrease in a group with strenuous exercise combined with red-fleshed pitaya extract.

The red-fleshed pitaya extract combined with strenuous exercise is proven to be able to inhibit the increasing HSP70 expression. This occurred due to the potential of red-fleshed pitaya as an antioxidant that is able to balance the increasing amount of free radicals formed and impairs HSP70 synthesis induced by strenuous exercise. Therefore, HSP70 expression is lower compared to the groups that are not given by red-fleshed pitaya extract. The results of this research is in accordance to a research conducted by Petiz et al. (2017) which discovered that Vitamin A combined with high intensity activities could prevent tissue damage and reduce endogenous antioxidant defense regulation in rats, which also suppressed HSP70 expression.

Other researches have also revealed that antioxidant supplementation is proven to be effective to slow down the HSP70 synthesis caused by high intensity exercise. Physical training combined with low to medium dosage of red-fleshed pitaya extract were measured based on the ability and lead to immunomodulation effect that affect the body immune system and protect the body from cell damages, resulting in an effective condition to reduce the oxidative stress. Stress resulted from physical activity like oxidative stress is responded by hypothalamus to secrete corticotrophin realizing hormone (CRH) which then delivers a message to pituitary anterior. The pituitary produced adrenocorticotropic hormone (ACTH) which is useful to activate or affect adrenal cortex where...
Cortisol hormone is secreted. Cortisol contributes a massive influence to immune responses as a sign that the body is suffering from oxidative stress. In this research, cortisol hormone level decreased in the group with strenuous exercise combined with red-fleshed pitaya extract. This occurs since the red-fleshed pitaya extract is an effective antioxidant to reduce the risk of oxidative stress and is able to decrease the secretion of cortisol by reducing ACTH secretion in the hypothalamus and CRH in the pituitary gland.

Conclusion
Based on this research, it can be concluded that red-fleshed pitaya extract has the potential to be antioxidant with its anthocyanin content, and is able to eliminate oxidative stress due to strenuous exercise. It can be observed by the decreased pattern of HSP70 and cortisol expression in the strenuous exercise combined with red-fleshed pitaya extract group. Furthermore, the optimum result was shown in the dosage of 300 mg/kg body weight red-fleshed pitaya extract.

Data availability
Underlying data
Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Grant information
The authors would like to acknowledge Ministry of Research, Technology and Higher Education of Republic of Indonesia for funding this research via Research Grant of Universitas Negeri Medan with contract number of 027/UN33.8/LL/2018.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Figure 2. Effect of red-fleshed pitaya extract on cortisol in rats that were given strenuous exercise.


PubMed Abstract | Publisher Full Text


PubMed Abstract | Publisher Full Text | Free Full Text


Publisher Full Text


PubMed Abstract | Publisher Full Text


PubMed Abstract | Publisher Full Text


PubMed Abstract | Publisher Full Text


Publisher Full Text


Reference Source


PubMed Abstract | Publisher Full Text | Free Full Text


PubMed Abstract | Publisher Full Text | Free Full Text


PubMed Abstract | Publisher Full Text


PubMed Abstract | Publisher Full Text | Free Full Text


PubMed Abstract | Publisher Full Text | Free Full Text


PubMed Abstract | Publisher Full Text | Free Full Text


PubMed Abstract | Publisher Full Text | Free Full Text


PubMed Abstract | Publisher Full Text | Free Full Text


PubMed Abstract | Publisher Full Text


PubMed Abstract | Publisher Full Text

http://www.doi.org/10.17605/OSF.IO/MGX4K
Open Peer Review

Current Peer Review Status: ?

Version 1

Reviewer Report 08 November 2019
https://doi.org/10.5256/f1000research.19175.r55888

© 2019 Yusni Y. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Yusni Yusni
Department of Physiology, Syiah Kuala University, Banda Aceh, Indonesia

Introduction:
1. Please explain why red-fleshed pitaya is thought to act as antioxidants that will affect the heat shock protein 70 and the expression of cortisol: include theoretical and other studies that support.

2. The novelty of the article must be clearly explained in the introduction.

Methods:
1. Why use 3 doses, whereas the purpose of the study did not assess the effective dose? In the conclusion, also mention the effective dose.

2. Explain the results of the identification of the chemical content of red-fleshed pitaya and also the results of herbarium analysis because there is a possibility that Indonesia's geographical differences will affect its chemical content.

3. Explain the time of blood-sampling because cortisol production is influenced by circadian.

Discussion:
1. It is important to explain the role or mechanism of action of red-fleshed pitaya on heat shock protein 70 and cortisol expression.

Reference:
1. Use the latest references (from the last 5-10 years).

Note: There are some grammatical errors, you should use the proof-read service.

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

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

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

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?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Medical Physiology: exercise physiology; endocrine and metabolic 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.