Potential of consumption of red dragon fruit extract on mitochondrial cytochromes in the gastrocnemius muscle after strenuous exercise

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ABSTRACT

Background. Skeletal muscle activity with heavy intensity causes an increase in free radicals causing oxidative stress that causes damage to cell function to mitochondrial dysfunction and plays a role in the cytochrome regulation process as an induction of apoptosis which normally cannot be released from the mitochondria. This study aims to determine the antioxidant potential of red dragon fruit (RDF) to delay the occurrence of oxidative stress, which increases cell function in mitochondria in the gastrocnemius muscle. **Methods.** This study involved 25 three-month-old male rats with an average weight of 200 g. The RDF extract was obtained via 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 RDF] and K2 [exercise, no RDF] and three test groups (P1, P2, and P3; subjected to exercise and treated with 75, 150, and 300 mg kg−1 body weight of RDF, respectively). The exercise was swimming for 20 min three times per week for 31 days. Cytochrome-C and MDA were measured via an enzyme-linked immunosorbent assay, and histopathological examinations were performed via hematoxylin and eosin staining of rat muscles. **Results.** Compared the MDA levels after the ingestion of RDF extracts were 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 the occurrence of oxidative stress and cytochrome-c (Identified as an essential mediator in the apoptotic pathway, unlocking mitochondrial cytochrome-c into an apoptotic pathway), Cytochrome-C levels were compared between the K2 (16.1 ng/mg protein) group and the P1(13.5 ng/mg protein), P2 (10.6 ng/mg protein), and P3 (7.2 ng/mg protein) groups. The results showed significant differences ($p < 0.05$ for P1 and P2, and $p < 0.01$ for P3). with features of damaged muscle cells based on histopathology. Ingestion of the RDF extract improved gastrocnemius muscle cells, resulting in cell function repair. **Conclusio**n. Red dragon fruit (antioxidant) in exercise suppresses oxidative stress on cytochrome-C so that apoptotic signals occur significantly in muscles, can prevent the apoptotic process, reduce tissue damage, cause repair of cell function, and mitochondria can work usually. If there is a lot of cytochrome in the cytosol, it indicates cell dysfunction.

Keywords: Red dragon fruit (RDF) extract, strenuous exercise, mitochondrial cytochrome, gastrocnemius muscle

INTRODUCTION

Skeletal muscle activity with heavy intensity causes an increase in free radicals causing oxidative stress that causes damage to cell function to mitochondrial dysfunction and plays a role in the cytochrome regulation process as an induction of apoptosis which normally cannot be released from the mitochondria (Miwa, S. 2008; Wang CH et al 2013). It has been reported that oxidative stress induces the release of mitochondrial cytochrome c and other apoptogenic proteins (e.g. apoptosis-inducing factor) from the mitochondrial intermembrane space into the cytosol. These proteins then bind to Apaf-1 and activate pro-caspase-3, which leads to mitochondrial dependent apoptosis (D. Harman, 1956. 1972; Li P, 1997).

This study aims to determine the antioxidant potential of red dragon fruit (RDF) to delay the occurrence of oxidative stress, which increases cell function in mitochondria in the gastrocnemius muscles (Siu PM et al, 2008).

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 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 provided with enough drinking water.

Study Design

The experimental method in the laboratory was applied with a proper experimental design and randomized post-test-only control group. Simple random sampling was implemented with the experimental animals being divided into five groups (5 rats/group): group K1 with no activity and no RDF; group K2 subjected to strenuous exercise without RDF; 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.

Experimental Procedures

Strenuous exercise involved a morning swim between 8:00 and 9:00 am for 20 min, three times per week for 4 weeks. The rats were treated with RDF 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 analyse malondialdehyde (MDA) using the enzyme-linked immunosorbent assay (ELISA) method with spectrophotometry at a wavelength of 450 nm. The mouse malondialdehyde ELISA kit (Brand Bioassay TL, catalogue: EO625Mo) was used to analyse the MDA levels. The Cytochrome c polyclonal antibody kit (Bioenzy Brand, catalogue: BZ- 0856320F-AP) was used for Cytochrome c.

Histopathological Study

The gastrocnemius muscle tissues samples were collected by performing a biopsy to determine the degree of muscle damage based on haematoxylin and eosin (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 (Abe et al, 2017; Porter AG& Nicke, 1990).

Data Analysis

Experimental data were analysed using SPSS 25 for Windows. The Shapiro-Wilk test ($P > 0.05$) was used to determine the normality of the data. 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 Age 12.87±0.84 weeks and weight 222±22.9 gr were similar among the control (K1 and K2) and test (P1, P2, and P3) groups. Body weight was measured before treatment. Compared the MDA levels after the ingestion of RDF extracts were 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 the occurrence of oxidative stress and cytochrome-c (Identified as an essential mediator in the apoptotic pathway, unlocking mitochondrial cytochrome-c into an apoptotic pathway), Cytochrome-C levels were compared between the K2 (16.1 ng/mg protein) group and the P1(13.5 ng/mg protein), P2 (10.6 ng/mg protein), and P3 (7.2 ng/mg protein) groups. The results showed significant differences ($p < 0.05$ for P1 and P2, and $p < 0.01$ for P3). with features of damaged muscle cells based on histopathology. Ingestion of the RDF extract improved gastrocnemius muscle cells, resulting in cell function repair. 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).

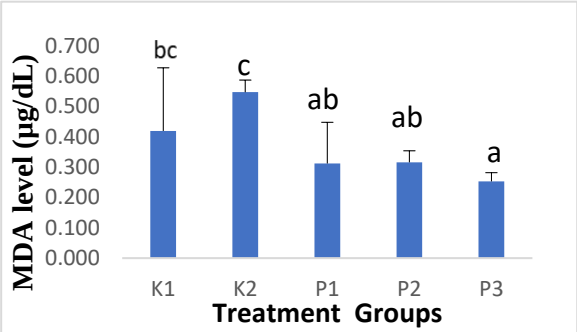
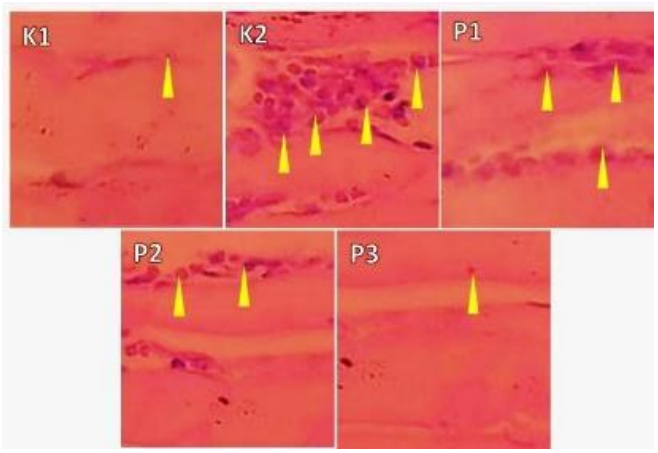


Figure 1. Correlation between RDF extract provision and MDA levels (µg/dL) between study groups; Mean ± SD

Note: The different notation letters on the bar graph are significantly different ($p < 0.05$)

No	Kelompok	Mean Rank Gastronemius muscle
1	K1	5.00
2	K2	16.13
3	P1	13.50
4	P2	10.63
5	P3	7.25

Keterangan: p<0,05 adalah perbedaan perlakuan antara ekspresi sitokrom-c otot gastrocnemius.



A picture of changes in soleus muscle cells before and after treatment of RDF extract (antioxidant exogen). Yellow arrows: inflammatory cells

DISCUSSION

Mitochondria are thought to play a central role in activating apoptosis induced by multiple stimuli. It is well known that mitochondria are both a target and source of ROS (Singh G et al., 2015). Results from our study demonstrate that RDF challenges free radicals, characterized by reduced levels of MDA. If these levels increase, it will cause the release of cytochrome-c from mitochondria to cytosol in human skeletal muscle fibroblasts (Jun Yuan et al., 2003). Cytochrome-c is synthesized as an apoprotein on cytosolic ribosomes, then translocating to mitochondrial intermembrane space. It is rarely detectable in the cytosol. However, cytochrome-c can be released from mitochondria to cytosol in apoptosis when the mitochondrial pathway is involved. In our study, the level of cytochrome-c decreased after RDF administration compared to the control, which can be shown histopathologically (Rizo-Roca D et al. l., 2015; Boatright KM & Salvesen GS., 2003). Besides that, mitochondria can also produce antioxidant in the form of superoxide in relatively constant amounts, or elicit spontaneous or environmentally-induced "superoxide flashes" and it is generally assumed that the main sites of superoxide production in the mitochondrial respiratory chain are complexes I and III. Consequently any impairment in the electron transfer process causes a reduction in upstream carriers, thus filling the Q cycle electron pool (V. Calabrese, et all, 2010 ; Gusbakti. et al., 2022). These processes cause mitochondria to become a major source of physiological or endogenous ROS production. Many studies have focused on the harmful effects of ROS, but it is now clear that mitochondrial generated ROS are also involved in regulating intracellular signal transduction pathways that result in cell activities such as proliferation. (W. Wang, et., 2008; V. Calabrese, et all, 2009; Galluzzi, et al. 2012).

CONCLUSION

The present evidence that oxidative stress by exogenous hydrogen peroxide promoted human skeletal muscle of gastrocnemius fibroblasts apoptosis. This apoptotic process is most likely through the mitochondrial cascade, in which cytochrome c is released from mitochondria to cytosol and the released cytoplasmic cytochrome c, in turn, activates caspase-3. The provision of antioxidants RDF can suppress free radicals which are characterized by decreased levels of cytochrome enzymes and the release of cytochrome-c from the mitochondria to the cytosol does not occur as the process of apoptosis is slowed down. Regulation of intracellular ROS and modification of apoptotic cascades may control apoptotic events and provide new strategies for the prevention and treatment of skeletal muscle of gastrocnemius degeneration.

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