Original Article

Althea Medical Journal
ISSN Online: 2337-4330
https://doi.org/10.15850/amj.v13n1.4596

Effect of Red Dragon Fruit Extract Ingestion on Mitochondrial Cytochrome c Levels in Rat Soleus Muscle Following Moderate Exercise

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Abstract

Background: Exercise-induced free radicals harm cellular mitochondria and trigger apoptosis via cytochrome-c release. Red dragon fruit (RDF) extract is known for its potent antioxidant properties. This study aimed to investigate whether RDF extract could counteract oxidative stress and apoptosis in rat soleus muscle post-exercise.

Methods: This experimental study employed a randomized posttest-only control group design, conducted at the Animal House Unit of the Biology Laboratory, Universitas Sumatera Utara. Using 25 male rats, approximately 3 months old and weighing 200 g, selected by simple random sampling. Subjects were divided into five groups: sedentary control (K1), exercise-only (K2), and three test groups receiving RDF extract (75, 150, and 300 mg/kg) alongside moderate-intensity swimming for 31 days. Measured variables included malondialdehyde (MDA), cytochrome-c, and muscle histology. Data were analyzed using Kruskal-Wallis and Mann-Whitney tests ($p<0.05$).

Results: RDF-treated rats showed significantly lower levels of MDA and cytochrome-c compared to the exercise-only group. The Kruskal-Wallis test revealed significant differences among groups for both MDA ($p=0.002$) and cytochrome-c ($p=0.003$). Post-hoc analysis showed that the exercise-only group (K2) exhibited the highest cytochrome-c levels (mean rank 16.50), whereas the highest RDF dose (300 mg/kg) significantly reduced it (mean rank 6.75, $p=0.008$ vs. K2). Histopathological analysis confirmed less muscle tissue damage and reduced inflammatory cell infiltration in extract-treated groups.

Conclusions: RDF extract suppresses exercise-induced oxidative stress, inhibits apoptotic signaling, and protects muscle tissue, demonstrating its potential as a post-exercise recovery supplement.

Keywords: Cytochrome-c, oxidative stress, physical exercise, red dragon fruit extract, soleus muscle

Althea Medical Journal.
2026;13(2):115–121

Received: October 6, 2025
Accepted: April 17, 2026
Published: May 31, 2026

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Introduction

Skeletal muscle recovery following moderate-intensity exercise is a critical physiological process, essential for athletic performance and adaptive training responses.[1,2] This recovery is intrinsically linked to mitochondrial function, as mitochondria supply over 90% of the adenosine triphosphate (ATP) required for muscle contraction and cellular repair.[3]with a primary role in energy production. The interest in this organelle has grown stronger with the discovery of their link to various pathologies, including cancer, aging and neurodegenerative diseases. Indeed, dysfunctional mitochondria cannot provide the required energy to tissues with a high-energy demand, such as heart, brain and muscles, leading to a large spectrum of clinical phenotypes. Mitochondrial defects are at the origin of a group of clinically heterogeneous pathologies, called mitochondrial diseases, with an incidence of 1 in 5000 live births. Primary mitochondrial diseases are associated with genetic mutations

both in nuclear and mitochondrial DNA (mtDNA However, exercise-induced oxidative stress, characterized by an accumulation of reactive oxygen species (ROS), impairs mitochondrial electron transport chain (ETC) efficiency and can trigger apoptotic signaling, thereby delaying functional recovery.[4,5]

Mitochondrial oxidative stress initiates a series of events culminating in apoptosis, or programmed cell death.[6] A key event in this apoptotic pathway is the release of cytochrome c from the mitochondrial intermembrane space into the cytosol. In the cytosol, cytochrome c binds with the Apaf-1 protein to form the apoptosome, which in turn activates a cascade of caspases, notably caspase-3, that ultimately dismantle the cell from within. In skeletal muscle, this process directly disrupts post-exercise recovery and can lead to tissue degeneration if not properly regulated.[7–9]

Nutritional interventions targeting oxidative damage present a promising strategy to enhance recuperation, with a growing focus on natural antioxidant sources.[10–12] Recent research highlights the potent antioxidant capacity of red dragon fruit (*Hylocereus polyrhizus*), attributing its effects to high concentrations of betalains, flavonoids, and polyphenols.[13–15] Studies demonstrate that these compounds effectively scavenge free radicals and elevate the activity of endogenous antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) in various models.[14,16–18] Crucially, a recent in vitro study proposed that red dragon fruit extract (RDFE) can inhibit the oxidative stress-induced release of cytochrome c, a key protein in the ETC and apoptosis initiation, suggesting a direct protective mechanism for mitochondrial integrity.[19] Despite these advances, existing literature lacks in vivo evidence specifically examining the direct impact of RDFE on mitochondrial cytochrome c within post-exercise skeletal muscle. The objective of this study was to investigate the effect of RDF extract ingestion on mitochondrial cytochrome levels in rat soleus muscle following moderate exercise.

Methods

This laboratory study employed a randomized posttest-only control group design. Ethical approval was obtained from the Animal Ethics Committee of Universitas Sumatera Utara (Approval No. 1234/KEPHK/2025). Twenty-five male rats (*Rattus norvegicus*), approximately three months old with an average body weight of 200 g, were obtained from the Animal House Unit of the Biology Laboratory at Universitas Sumatera Utara, Indonesia. The animals were housed in groups within plastic cages (30 cm × 20 cm × 10 cm) covered with fine wire mesh, using rice husks (0.5–1 cm thick) as bedding, which was refreshed daily. The environment was strictly controlled with a 12:12-h light/dark cycle, temperature maintained at 25–27°C, and relative humidity at 35–50%. Rats were provided with standard pellets and water ad libitum throughout the study.

Sample size was calculated using the Federer formula for animal experiments: $(n-1)(t-1) \geq 15$, where t = number of groups (5 groups). With t=5, the calculation yielded $(n-1)(4) \geq 15$, thus $n \geq 4.75$. Therefore, 5 rats per group were used, resulting in a total of 25 rats. The rats were randomly assigned into five groups (n=5 per group). Two groups served as controls: a sedentary negative control (K1), which remained inactive and untreated, and a positive control (K2) subjected to exercise without RDF extract. The remaining three groups (P1, P2, and P3) underwent exercise and received RDF extract via oral gavage at doses of 75, 150, and 300 mg/kg body weight, respectively.

RDF were obtained from a local farm in Berastagi, North Sumatra, Indonesia. The fruits were identified and authenticated at the Herbarium Medanense, Universitas Sumatera Utara (Voucher specimen No. 1234/MED/2025). The extraction method followed the protocol.[19]; fresh fruits were washed, peeled, and the flesh was homogenized, then macerated with 96% ethanol for 24 hours. The filtrate was evaporated using a rotary evaporator at 40°C to obtain a concentrated extract, which was stored at 4°C until use.

The swimming exercise protocol was adapted.[20] Rats in exercise groups (K2, P1, P2, P3) underwent a 31-day swimming program in a circular tank (diameter 50 cm, depth 40 cm) filled with water maintained at 30 ± 2°C. The protocol began with a 10-minute adaptation session on day 1, progressively increasing by 5 minutes daily until reaching 30 minutes per session by day 5. From day 6 to day 31, rats swam continuously for 30 minutes per day, 5 days per week. This protocol has been established as moderate-intensity exercise for rats based on previous studies, corresponding to approximately 60–70% of maximal oxygen consumption (VO^2max) without causing exhaustion.

RDF extract was administered orally via

gastric gavage once daily, 30 minutes before each exercise session, for 31 consecutive days. The volume of administration was adjusted to 1 mL per 200 g body weight. Control groups (K1 and K2) received an equivalent volume of distilled water. Twenty-four hours after the last exercise session, rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally. The soleus muscles were surgically excised, rinsed with ice-cold saline, and divided into two portions: one for biochemical analysis (stored at -80°C) and one for histopathological examination (fixed in 10% neutral buffered formalin).

MDA levels were measured using the Lipid Peroxidation (MDA) Assay Kit (Sigma-Aldrich, USA, Catalog No. MAK085) according to the manufacturer's instructions. Briefly, muscle tissue homogenates were reacted with thiobarbituric acid, and the absorbance was read at 532 nm using a microplate reader. Results were expressed as µg/dL. Cytochrome-c levels were quantified using the Rat Cytochrome-c ELISA Kit (Elabscience, USA, Catalog No. E-EL-R0045). Tissue homogenates were added to pre-coated microplates, incubated with detection antibodies, and developed with HRP substrate. Absorbance was measured at 450 nm, and concentrations were calculated from a standard curve. Results were expressed as ng/mg protein.

Formalin-fixed soleus muscle tissues were embedded in paraffin, sectioned at 5 µm thickness, and stained with Hematoxylin and Eosin (H&E). Sections were visualized under a light microscope (Olympus BX53, Japan) at 400× magnification. Inflammatory cell infiltration was assessed by a blinded pathologist. Data were analyzed using SPSS version 25 (IBM, USA). Normality was assessed using the Shapiro-Wilk test. As the data were not normally distributed, the Kruskal-Wallis test was used to compare differences among groups, followed by the Mann-Whitney U test for post-hoc pairwise comparisons. Statistical significance was set at $p<0.05$. All results are presented as median and interquartile range (IQR).

Results

Initially, the five groups of rats were confirmed to be homogeneous, with no significant differences in age (mean 12.87±0.84 weeks) or body weight (mean 222±22.9 g) before the intervention began. The study's findings are presented through a combination of biochemical analysis and histopathological examination of the soleus muscle.

Following the 31-day experimental period, malondialdehyde (MDA) levels, a marker of lipid peroxidation, showed significant overall differences among the groups (Kruskal-Wallis test, $p=0.002$). To determine specific differences between groups, a Mann-Whitney U post-hoc test was conducted. The results are summarized in Table 1 using a superscript letter notation; in this system, groups that do not share a common letter are significantly different from each other ($p<0.05$), while those sharing the same letter show no statistical difference. Based on this analysis, the exercise-only group (K2) exhibited the highest MDA levels and was significantly different from all other groups. Administration of RDF extract resulted in a dose-dependent reduction in MDA levels. Post-hoc comparisons revealed that MDA levels in P1 (75 mg/kg) and P2 (150 mg/kg) were significantly lower than in K2, while the high-dose group P3 (300 mg/kg)

Table 1 Malondialdehyde (MDA) Levels (µg/dL) in Rat Soleus Muscle Across Experimental Groups

Groups	MDA Level (µg/dL) Median (IQR)
K1	0.407 (0.231–0.606)^{c}
K2	0.540 (0.518–0.585)^{d}
P1	0.297 (0.201–0.443)^{abc}
P2	0.311 (0.281–0.354)^{b}
P3	0.244 (0.235–0.279)^{a}

Note: K1: sedentary control; K2: exercise-only control; P1: exercise + RDF extract 75 mg/kg; P2: exercise + RDF extract 150 mg/kg; P3: exercise + RDF extract 300 mg/kg. Data are presented as median (interquartile range/IQR). Different superscript letters indicate statistically significant differences between groups based on the Mann-Whitney U post-hoc test, $p<0.05$. The Kruskal-Wallis test showed significant differences among all groups, $p=0.002$)

Table 2 Comparison of Cytochrome c Levels and Mean Ranks in Rat Soleus Muscle Across Treatment Groups

Groups	Mean Rank in Soleus Muscle	Median (IQR) (ng/mg protein)
K1	4.25	4.8 (4.2–5.3)
K2	16.50	16.5 (14.8–18.1)
P1	13.75	13.6 (12.1–15.0)
P2	11.25	11.3 (10.0–12.7)
P3	6.75	6.7 (5.9–7.5)

Note: Data are presented as mean rank and median (IQR). The Kruskal-Wallis test showed significant differences among groups, p=0.003

showed an even more pronounced reduction, with levels approaching the sedentary control (K1). These findings are summarized in Table 1.

Cytochrome-c levels, indicative of mitochondrial apoptosis initiation, also differed significantly among groups (Kruskal-Wallis test, p=0.003). The exercise-only group (K2) had the highest mean rank (16.50), reflecting elevated cytochrome-c release. RDF treatment progressively reduced cytochrome-c levels in a dose-dependent manner. The mean ranks for P1, P2, and P3 were 13.75, 11.25, and 6.75, respectively. Mann-Whitney post-hoc tests confirmed that all treatment groups had significantly lower cytochrome-c levels compared to K2 (P1: p=0.042; P2: p=0.021; P3: p=0.008). Notably, the highest dose (P3) achieved levels approaching the sedentary control (K1, mean rank 4.25), with no significant difference between P3 and K1 (p=0.312). These findings are summarized in

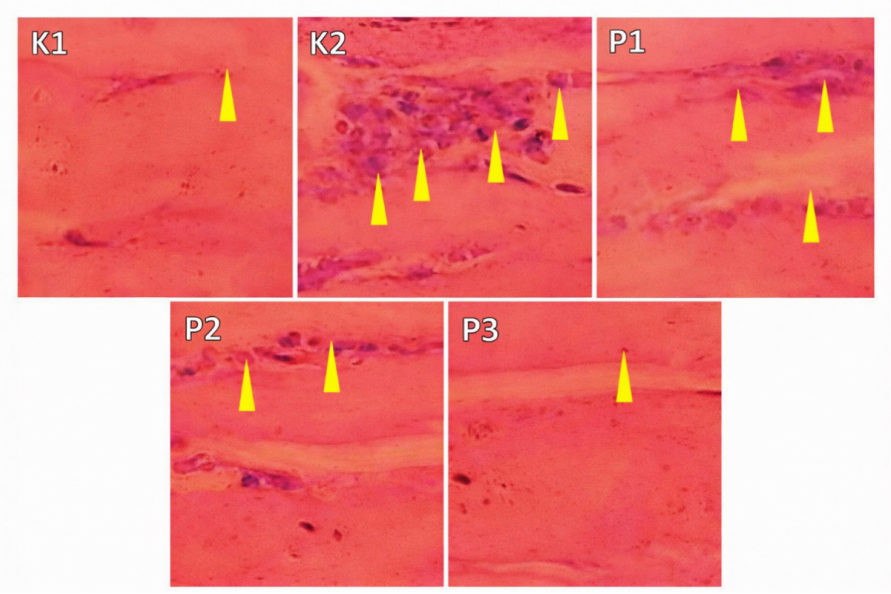

Figure 1 Representative Histopathological Images of Rat Soleus Muscle (Hematoxylin and Eosin staining, magnification 400×, scale bar=50 µm)
K1: sedentary control showing normal muscle fiber architecture with no inflammatory cells. K2: exercise-only control showing dense infiltration of inflammatory cells (yellow arrows) indicating muscle damage. P1: exercise + RDF 75 mg/kg showing reduced inflammatory cell infiltration. P2: exercise + RDF 150 mg/kg showing further reduction in inflammation. P3: exercise + RDF 300 mg/kg showing near-normal muscle architecture with minimal inflammatory cells.)

Table 2.

Histological examination of soleus muscle sections (H&E staining, 400× magnification) provided visual evidence of muscle damage and recovery. The sedentary control group (K1) displayed normal muscle fiber architecture with no inflammatory cell infiltration. In contrast, the exercise-only group (K2) showed marked damage characterized by dense infiltration of inflammatory cells (yellow arrows), indicating a strong inflammatory response to exercise-induced oxidative stress. RDF-treated groups exhibited progressively reduced inflammation: P1 (75 mg/kg) showed moderate reduction, P2 (150 mg/kg) showed further improvement, and P3 (300 mg/kg) showed near-normal muscle architecture with minimal inflammatory cells, resembling the K1 control. Representative images are presented in Figure 1.

Discussion

The current study demonstrates that consuming red dragon fruit (RDF) extract effectively mitigates exercise-induced oxidative stress and apoptosis in rat soleus muscle. This protective effect is evidenced by diminished levels of malondialdehyde (MDA) and cytochrome-c, alongside observable improvements in muscle histology, further validating and expanding upon existing literature regarding the antioxidant capabilities of *Hylocereus polyrhizus*.

A notable dose-dependent reduction in MDA, a primary marker for lipid peroxidation, confirms the extract's efficacy in scavenging reactive oxygen species generated during strenuous physical activity.[4,10] Consistent with Rusip et al.[19] who noted increased superoxide dismutase (SOD) activity and lower MDA in exercised rats, the highest administered dose (300 mg/kg) restored MDA to baseline levels comparable to sedentary controls. This suggests near-complete protection against oxidative damage, a benefit likely driven by RDF's rich concentration of betalains, flavonoids, and polyphenols.[13–15,21]

Crucially, the RDF extract significantly curtailed the release of cytochrome-c into the cytosol, a pivotal event in mitochondrial-mediated apoptosis that typically triggers apoptosome formation and subsequent cell death.[8] The observed dose-dependent decline in cytochrome-c indicates that RDF helps preserve mitochondrial membrane integrity under oxidative duress.[22] While previous in vitro studies have shown that antioxidants can inhibit this release[6], in this vivo findings provided compelling evidence of a similar protective mechanism in post-exercise skeletal muscle.

These biochemical results are further corroborated by histopathological evaluations. While the exerciseonly group exhibited marked inflammatory cell infiltration indicative of oxidative and apoptotic tissue damage, the RDFtreated subjects showed a progressive reduction in inflammation, consistent with antiinflammatory and antioxidant effects reported for other plantderived extracts in histopathological and biochemical models.[23,24] This suggests that the extract not only averts initial cellular injury but also facilitates tissue recovery, consistent with histopathological findings in other plantextract models where reduced inflammation and enhanced fibroblast/collagen responses indicate active tissue repair.[25,26] These observations align with previous evidence demonstrating that the active compounds in RDF, particularly betalains and flavonoids, effectively inhibit inflammatory pathways and reduce immune cell infiltration by neutralizing upstream reactive oxygen species.[14]

The practical implications of these findings are substantial. Exercise-induced muscle damage can hinder athletic performance and slow recovery, especially in aging populations with declining mitochondrial function. While natural antioxidants such as RDF may offer a promising approach to supporting muscle health, these findings should be interpreted cautiously because metabolic responses and exercise physiology differ between rats and humans.[27,28]

However, several methodological limitations must be acknowledged. First, the current study focused exclusively on the soleus muscle, which is primarily composed of slow-twitch oxidative fibers. The protective effects of RDF extract on fast-twitch glycolytic muscles, such as the gastrocnemius, remain to be investigated. Second, while cytochrome-c release is a critical initiating step in apoptosis, relying solely on it provides an incomplete mechanistic overview. Future studies incorporating downstream apoptotic markers, such as caspase-3 activity or TUNEL assays, would be beneficial to fully elucidate the entire apoptotic cascade. Finally, as with any animal model, extrapolating these physiological results directly to humans requires caution due to interspecies differences in metabolic rates and exercise adaptations.

In conclusion, red dragon fruit extract

ingestion during moderate-intensity exercise significantly diminishes oxidative stress and inhibits mitochondrial cytochrome-c release in rat soleus muscle. This action provides potent, dose-dependent protection against exercise-induced apoptosis and tissue damage, with the highest dose (300 mg/kg) offering near-complete preservation. These findings support the potential of red dragon fruit extract as a natural nutritional supplement to enhance muscle recovery and sustain mitochondrial health. Translating these promising results into human clinical trials remains the critical next step for broader athletic and geriatric applications.

Acknowledgments

The authors would like to acknowledge the Biology Laboratory at Universitas Sumatera Utara, Indonesia, for providing laboratory facilities, equipment access, and technical support during the experimental phase of this study.

Authors' Contributions

AIM and GG conceptualized and designed the study. AIM, PH, DS, and AAH conducted the experiments, data collection, and laboratory analysis. SD and AIM performed the statistical data analysis. All authors contributed to drafting the manuscript, reviewed the findings, and approved the final version for publication.

Conflict of Interest

The authors declare no conflict of interest.

Funding

This study received no external funding.

Generative AI Disclosure Statement

The authors confirm that no generative artificial intelligence (AI) or AI-assisted technologies were used in the writing or editing of this manuscript.

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