



CELLULAR AND MOLECULAR BIOLOGY

Juicing up performance: The impact of pitaya on biochemical and oxidative responses in acute swimming exercise

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Abstract: Aerobic exercise boosts antioxidant defenses, but intense acute exercise can increase oxidative stress, reducing performance and accelerating muscle fatigue, especially in untrained individuals. Acute swimming is often used to model such stress, making it ideal for evaluating antioxidant interventions. With rising interest in natural antioxidants, pitaya (*Hylocereus polyrhizus*), rich in polyphenols and betacyanins, presents a promising alternative. This study assessed the effects of pitaya pretreatment on mice subjected to acute swimming. Mice were divided into: Control, Exercise, Pitaya, and Pitaya + Exercise groups, with the latter two receiving 200 μ l of pitaya juice daily for 30 days. After an acute swimming protocol, animals were euthanized for biochemical analysis. Glucose, triglycerides, total cholesterol, alkaline phosphatase (ALP), TBARS, reactive species (RS), glutathione levels (GSH), and activity of catalase (CAT), superoxide dismutase (SOD), and related enzymes were measured. In our results, the Pitaya + Exercise group showed a significant reduction in RS compared to Pitaya, and the Exercise group increased CAT and GST activity, while Pitaya pretreatment attenuated these effects and elevated GSH. ALP was significantly higher only in the Exercise group. These results suggest that pitaya pretreatment has antioxidant potential in acute exercise, though further research is needed to explore additional mechanisms.

Key words: Pitaya, antioxidant, exercise, oxidative stress.

INTRODUCTION

Aerobic physical exercise plays a vital role in mitigating reactive oxygen species (ROS) and preventing diseases associated with oxidative stress (Lesmana et al. 2022). Long-term regular training has been shown to enhance antioxidant defense mechanisms, limiting mitochondrial oxidative damage (Powers et al. 2022). Chronic exercise induces adaptive physiological changes, where the elevated generation of reactive species (RS) is counterbalanced by the body's adaptive response to exercise (Torma et al. 2019). Zarrindast et al. (2020) highlighted that moderate-intensity aerobic training over

eight weeks, whether on land or water, improves antioxidant status, thereby increasing resilience against oxidative stress. In contrast, intense acute exercise in untrained individuals is linked to heightened oxidative stress compared to moderate, consistent aerobic exercise (Lu et al. 2021). This exacerbated oxidative stress results in reduced strength, accelerated muscle fatigue due to diminished calcium sensitivity, and reduced calcium-activated maximal force (Arazi et al. 2021).

The primary cause of this oxidative stress is the overproduction of RS coupled with the insufficient neutralizing capacity of the antioxidant defense system (Sahoo

et al. 2023). Oxidative stress is closely tied to various physiological conditions, including inflammation, chronic diseases, aging, and exercise (El Assar et al. 2022). As an endogenous source, exercise increases oxygen consumption and activates metabolic pathways, leading to a surge in ROS production, especially during strenuous high-intensity exercise, potentially contributing to oxidative stress by altering the cellular redox state. The complexity of the redox balance depends on multiple factors, such as training level, gender, age, exercise duration, and intensity (Drăgoi et al. 2024). While moderate, regular exercise benefits health, acute and strenuous sessions of both aerobic and anaerobic exercise can trigger an overproduction of ROS (Espinosa et al. 2023). For instance, acute swimming exercise is often used as a model to induce oxidative stress, which can be instrumental in evaluating potential antioxidant interventions (Amiri et al. 2023).

In recent years, the use of supplemental antioxidants has surged among athletes, aiming to restore the balance between oxidative and antioxidant agents (Mason et al. 2020). Dietary supplementation is an extrinsic factor that may mitigate muscle damage, enhance physical performance, and reduce oxidative stress. Furthermore, it lowers the risk of pathological outcomes associated with strenuous exercise (Cheng et al. 2020). Given the central role antioxidants play in reducing the risk of developing a wide array of pathologies by preventing oxidative damage at the cellular level (Jaganjac et al. 2022), certain fruits stand out due to their rich antioxidant content. With oxidative stress increasingly associated with chronic diseases, boosting the intake of fresh foods with antioxidant properties is a key strategy (Mason et al. 2020). The literature consistently links regular consumption of fruits and vegetables with the prevention of chronic

conditions, such as diabetes, cancer, stroke, and age-related functional decline (Stanaway et al. 2022). Moreover, fruits, vegetables, and other plant-based foods contain bioactive phytochemicals, compounds recognized for their health benefits and for reducing the risk of chronic disease (Sorrenti et al. 2023).

One such fruit, Pitaya (*Hylocereus spp.*), belonging to the genus *Hylocereus* or *Selenieryus* (*Cactaceae*), is notable for its rich composition of polyphenols, flavonoids, betacyanins, and fibers. Native to Central and South America, Pitaya boasts nutritional, medicinal, and industrial potential (Shah et al. 2023). There are at least three cultivars: *H. undatus* (white pulp, red skin), *H. polyrhizus*/*H. monacanthus*/*H. costaricensis* (red pulp, red skin), and *H. megalanthus* (white pulp, yellow skin). These cultivars are native to specific regions, such as Colombia, Mexico, and South America (*H. undatus*), Bolivia, Peru, Ecuador, Colombia, and Venezuela (*H. megalanthus*), and Mexico (*H. polyrhizus*). According to Joshi & Prabhakar (2020), Pitaya's phenolic compounds, vitamin C, vitamin E, carotenes, and betanin contribute significantly to its antioxidant properties, and Luo et al. (2014) attributed this activity primarily to its polyphenolic content. The antioxidant potential of Pitaya has been demonstrated in the DPPH radical scavenging assay, further solidifying its role as an effective antioxidant (Luo et al. 2014). While its physicochemical properties and bioactive constituents are well characterized, the impact of Pitaya on antioxidant effects during physical exercise remains largely unexplored. Thus, this study aims to bridge that gap by investigating the antioxidant properties of Pitaya juice in animals subjected to acute swimming exercise, offering a novel perspective on its potential as a functional food for exercise-induced oxidative stress mitigation.

MATERIALS AND METHODS

Chemicals

The reagents listed below were purchased from Sigma-Aldrich (St. Louis, MO, USA): 2',7"-dichlorofluorescein diacetate (DCFH-DA), thiobarbituric acid (TBA), (-)-Epinephrine (E4250), glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), O-phthalaldehyde (OPA) and 4-(2-hydroxyethyl) piperazine-ethanesulfonic acid (HEPES). The other reagents used come from other suppliers and are available at the Laftambio Pampa Laboratory, UNIPAMPA Campus, Itaqui.

Preparation of Red Pitaya Juice

The elaboration of Pitaya juice occurred with the use of red Pitaya (*Hylocereus polyrhizus*) from producers of a farm located in the interior of the municipality of Itaqui-Rio Grande do Sul ("Pitayas Tapera do Coqueiro" located in Fundo Grande-RS 529; Latitude: -29.1525, Longitude: -56.5507 29°9'9" South, 56°33'3" West). The fruits were harvested in the commercial maturation phase. The first stage consisted of cleaning, peeling, grinding, and, finally, were homogenized. The pulps were obtained and prepared in a concentration (1:1) using distilled water for this dilution. After the preparation, the finished and diluted juice was stored at -20°C for later use in the experimental design (Madalosso et al. 2023).

Animals

Male mice of the Swiss lineage, seven weeks old, and from the Central Vivarium of the Federal University of Santa Maria (UFSM) were used. Before the beginning of the experimental protocol, the acclimatization of the animals took place, following the ideal conditions for animal experimentation. Thus, the mice were randomly separated into polypropylene boxes under controlled conditions such as a 12-hour light/dark cycle, controlled temperature (25 ±

2°C), and ad libitum feeding and water. It is noteworthy that the procedures conducted in this research follow the guidelines of the Committee for the Care and Use of Experimental Animal Resources. This research was previously approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Pampa (code 024/2022).

Exercise protocol

For the acute swimming exercise protocol in mice, the animals underwent an adaptive phase, where they were exposed to the water environment for 15 minutes daily over the course of seven days (Voltarelli et al. 2002). Each swimming session was conducted individually in a dedicated basin, with continuous monitoring of the water temperature (34°C ± 1°C). The acute exercise protocol involved swimming with a small weight attached to the dorsal region of the animals, equivalent to 5% of their body weight, until they reached the point of physical exhaustion. Exhaustion was defined as the inability of the mice to stay afloat, characterized by a cessation of swimming and failure to remain at the water's surface. Upon reaching this state, the animals were immediately removed from the water, dried, and returned to their respective experimental groups. At the conclusion of the protocol, on the 30th day, both the Exercise and Pitaya + Exercise groups were subjected to a final single session of swimming until exhaustion. The latency period for each swim session was recorded to assess performance differences between the groups following Pitaya consumption. This protocol was adapted from the methodology established by Wang et al. (2008), with minor modifications.

Experimental design

The total sample of 24 mice was determined to be the minimum number required to assess the relationship between Pitaya juice consumption

and acute swimming exercise (Fig. 1). After an acclimatization period, the animals were randomly assigned to one of four groups ($n = 6$ per group): Control Group (200 μ L of NaCl 0.9% [vehicle]), Exercise Group (200 μ L of vehicle + exercise), Pitaya Group (200 μ L of Pitaya juice), and Pitaya + Exercise Group (200 μ L of Pitaya juice + exercise). The vehicle (water) or Pitaya juice (1:1 concentration) was administered orally via gavage for 30 consecutive days. Initially, the animals received Pitaya juice as a pre-treatment for 30 days, after which the exercise protocol was implemented. The start and end times of the acute exercise session for each animal were recorded, and euthanasia was performed with a pentobarbital dose (180 mg/kg, intraperitoneally) one hour after the exercise session concluded. This period was carefully monitored for each mouse to evaluate the physiological effects of acute swimming exercise. Following euthanasia, blood samples were collected via cardiac puncture, transferred to tubes containing heparin (as an anticoagulant), and centrifuged at 3,000 rpm for 10 minutes to isolate the serum. Both blood serum and dissected liver tissues were stored at -80°C for subsequent analyses, including biochemical and oxidative stress assessments.

Ex vivo assays

For liver preparation and homogenization, the samples were processed in 50 mM Tris-HCl buffer (pH 7.4) at a ratio of 1:10 (w:v), followed by centrifugation at 2,500 rpm for 10 minutes. The resulting supernatants (referred to as S1) were used for all *ex vivo* analyses, except for glutathione (GSH) measurements. For GSH determination, homogenization was performed in 0.1 M perchloric acid (HClO_4) at the same 1:10 (w:v) ratio, followed by centrifugation at 3,000 rpm for 10 minutes to obtain the supernatant.

Glucose, triglycerides, and total cholesterol

The evaluation of glucose, triglycerides, and total cholesterol levels in the mice's blood serum was obtained through the use of biochemical kits, following the instructions provided by the manufacturer (Labtest, Diagnostica S.A., Minas Gerais, Brazil). These levels were expressed in mg/dL.

Alkaline Phosphatase (ALP) Levels

The levels of alkaline phosphatase (ALP) in the mice's blood serum were obtained using kits, with a procedure carried out according to the manufacturer's instructions (Labtest, Diagnostica

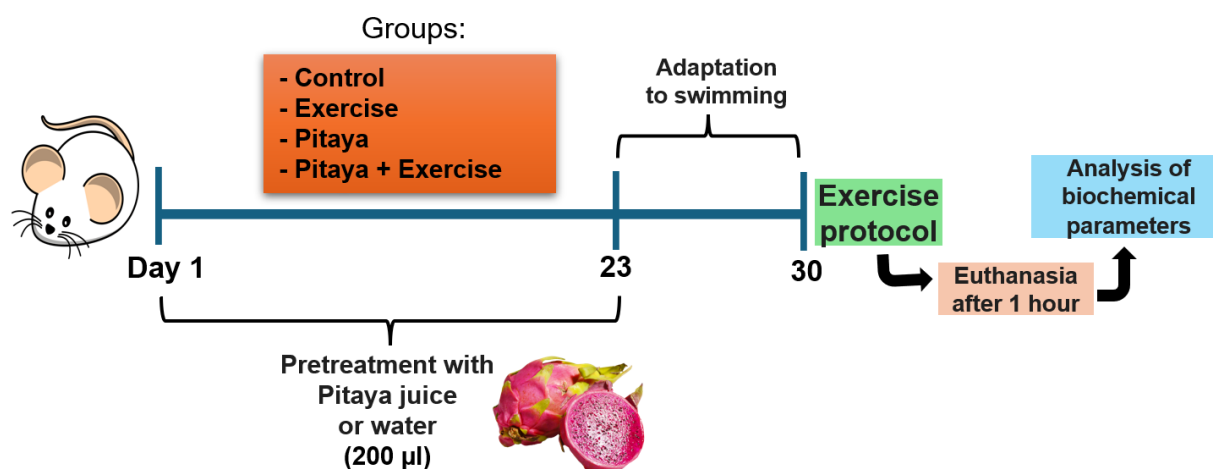


Figure 1. Experimental design of the study.

S.A., Minas Gerais, Brazil). The expression of ALP enzymatic activity is demonstrated in U/L.

Thiobarbituric acid reactive species (TBARS)

Lipid peroxidation levels were measured following the method described by Ohkawa et al. (1979). Thiobarbituric acid reactive substances (TBARS) were quantified in liver tissue by assessing malondialdehyde (MDA) levels, a final product of fatty acid peroxidation that reacts with thiobarbituric acid (TBA) to produce a colored complex. For the assay, the following reagents were added to test tubes: liver supernatant, acetic acid buffer (2.5 M, pH 3.4), 0.8% TBA (pH 3.2), 8.1% SDS, and distilled water. The mixture was then incubated at 95°C for 2 hours in a water bath, with glass beads used to prevent evaporation. After incubation and subsequent cooling, absorbance was measured at 532 nm. The TBARS results were expressed as MDA (nmol/g tissue).

Reactive Species (RS)

Following the protocol established by Pérez-Severiano et al. (2004), reactive species (RS) levels in the S1 were analyzed. This analysis measures the conversion of DCFH-DA (the chemically reduced form of fluorescein) into dichlorofluorescein (DCF), resulting in fluorescence following the oxidation process. To quantify RS levels, S1 was mixed with 10 mM Tris-HCl buffer (pH 7.4) and DCFH-DA (1 mM), and the mixture was incubated for 1 hour. Both the blank and samples were then analyzed using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies) with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. The production of RS is directly related to the intensity of the emitted fluorescence, and the results are expressed as a percentage relative to the control group (% control).

Catalase (CAT) Activity

The protocol established by Aebi (1984) was employed to evaluate the activity of the catalase (CAT) enzyme, which is responsible for neutralizing hydrogen peroxide (H_2O_2). In this enzymatic reaction, liver supernatant (S1, diluted 1:10) and H_2O_2 served as substrates in a potassium phosphate buffer (50 mM, pH 7.0). The CAT's ability to remove H_2O_2 was quantified by measuring absorbance at 240 nm for 120 seconds using a microplate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer) at a controlled temperature of 37°C. The enzymatic activity of CAT was expressed in the graphs as Units (U) per mg of protein.

Superoxide Dismutase (SOD) Activity

To assess the enzymatic activity of superoxide dismutase (SOD), we employed the methodology outlined by Misra & Fridovich (1972). This approach evaluates the ability of SOD to inhibit the auto-oxidation of epinephrine to adrenochrome. Sodium carbonate buffer (Na_2CO_3 ; 57.7 mM) and the liver supernatant were added to the reaction mixture, and the SOD kinetics were initiated by the addition of epinephrine (6 mM). Following the addition of epinephrine, the color reaction was immediately measured using a microplate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer) at 480 nm. Each unit of SOD activity is defined as the amount of enzyme required to inhibit the auto-oxidation of the substrate (epinephrine) by 50% at a temperature of 30°C. The results for SOD activity were expressed as U/mg of protein.

Glutathione-S-Transferase (GST) Activity

From the liver supernatant, an aliquot of the homogenized tissue was prepared by adding 0.1 M potassium phosphate buffer (pH 7.5), along with 100 mM glutathione (GSH) (1 mM), and 1 mM CDNB, which served as the substrate for

the glutathione S-transferase (GST) enzymatic reaction. The enzymatic activity of GST was quantified following the methodology described by Habig et al. (1974) and measured using a spectrophotometer at 340 nm for 2 minutes (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer). This method involves the conjugation of glutathione with the substrate CDNB, and the results were expressed as CDNB (nmol/min/mg of protein).

Glutathione (GSH) levels

The measurement of glutathione (GSH) levels was conducted following the methodology established by Hissin & Hilf (1976), with adaptations from Sies (1999) and Forman et al. (2009). In this fluorimetric assay, a portion of the liver was excised, weighed, and homogenized in 0.1 M perchloric acid (HClO_4) at a ratio of 1:10 (w:v). After that, 100 μL of the tissue supernatant was combined with 800 μL of 0.1 M potassium phosphate buffer (pH 8). Subsequently, 100 μL of o-phthalaldehyde (OPA) solution (1 mg/mL) was added as the fluorophore. This mixture was incubated at room temperature for 15 minutes, after which fluorescence readings were taken at wavelengths of 420 nm (emission) and 350 nm (excitation) using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies). A calibration curve was also established for quantification, with the results expressed as GSH concentrations (nmol/g tissue).

Glutathione Peroxidase (GPx) Activity

The spectrophotometric measurement of glutathione peroxidase (GPx) activity was performed according to the methodology described by Wendel (1981). The liver homogenate was evaluated by introducing the S1 fraction into a reaction system composed of GSH, NADPH, and glutathione reductase, and sodium azide (CAT inhibitor). Subsequently,

100 μL of 4 mM hydrogen peroxide (H_2O_2) was added to catalyze the enzymatic reaction. The dismutation of H_2O_2 was monitored at 340 nm, with the GPx activity expressed in nanomoles per minute per milligram of protein (nmol min/mg of protein).

Glutathione reductase (GR) activity

Glutathione reductase (GR) activity was assessed following the protocol outlined by Carlberg & Mannervik (1975). An aliquot of the S1 fraction was added to a reaction system containing 0.15 M potassium phosphate buffer (pH 7.0), 1.5 mM EDTA, and 0.15 mM NADPH. After obtaining the baseline absorbance reading, a second measurement was taken following the addition of the substrate, 20 mM glutathione disulfide (GSSG). The GR activity was quantified and expressed as nanomoles per minute per milligram of protein (nmol min/mg protein).

Statistical analysis

The data were processed using the normality test using the Shapiro-Wilk test. After checking normality, a two-way analysis of variance (ANOVA) was performed to compare all groups, followed by Tukey's post hoc test. Data were expressed as mean and standard error of the mean (S.E.M). Statistical results were defined using GraphPad Prisma 9.0 Software, considering p values <0.05 statistically significant.

RESULTS

Effect of pitaya on glucose, triglycerides and total cholesterol levels

In the analysis of biochemical parameters in mice plasma, glucose levels (Fig. 2a) did not differ significantly among experimental groups (Control [mean = 233.3], Pitaya [mean = 242.0], Exercise [mean = 264.5], Pitaya + Exercise [mean = 268.5]). Similarly, triglyceride levels (Fig. 2b)

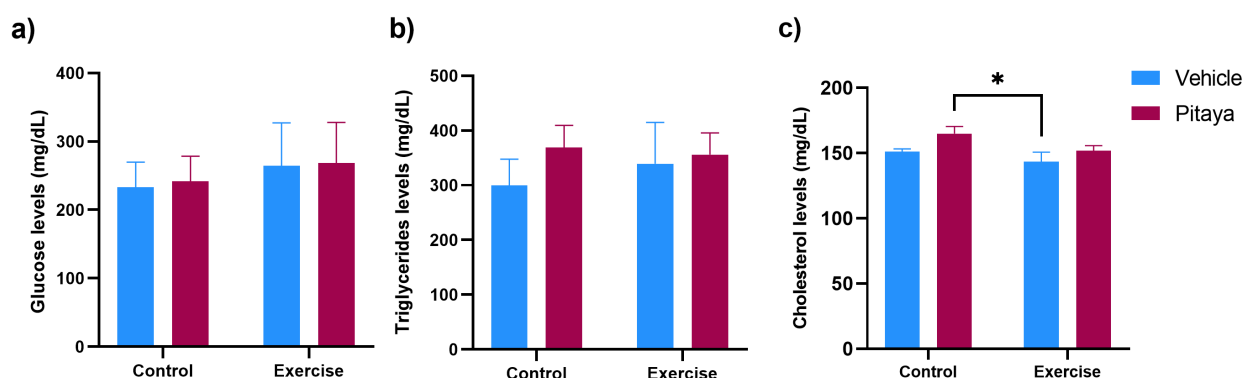


Figure 2. Effects of pre-treatment with Pitaya juice on plasma levels of parameters a) Glucose, b) Triglycerides, and c) Total cholesterol in mice subjected to acute swimming exercise. The significance level was obtained by analysis of variance (ANOVA, two-way) with Tukey post hoc. Data expressed statistically as mean $\pm$ SEM. Significant values when $p < 0.05$. *indicates significance level for $p < 0.05$.

remained stable across all groups (Control [mean = 299.9], Pitaya [mean = 369.0], Exercise [mean = 339.3], Pitaya + Exercise [mean = 356.2]). Notably, total cholesterol levels (Fig. 2c) exhibited a significant reduction in the Exercise group [mean = 143.4] compared to the Pitaya group [mean = 164.9] ($p = 0.0402$), suggesting a positive effect of exercise on lipid profile indicators. However, no significant differences in total cholesterol were observed between the Control [mean = 151.3] and Pitaya + Exercise [mean = 151.8] groups.

Effect of pretreatment with pitaya on TBARS and RS

The assessment of lipid peroxidation, indicated by MDA levels (Fig. 3a), revealed no statistically significant differences among the study groups (Control [mean = 632.8], Pitaya [mean = 642.6], Exercise [mean = 732.7], Pitaya + Exercise [mean = 809.0]). Animals pre-treated with Pitaya juice for 30 days exhibited unchanged levels of TBARS, as evidenced by consistent MDA formation. In contrast, the levels of RS (Fig. 3b) demonstrated a significant reduction in the Pitaya + Exercise [mean = 109.9] compared to the group receiving only Pitaya [mean = 163.2] ($p = 0.0292$), indicating that the combination of both treatments effectively lowered the production of RS. Thus,

the 30-day pre-treatment with Pitaya juice, in conjunction with the acute swimming exercise protocol, resulted in a notable decrease in RS levels, highlighting the positive impact of these interventions on this parameter.

Pretreatment with pitaya reduced CAT activity altered by acute exercise

The antioxidant enzyme system is crucial for understanding the effects of natural substances. Regarding superoxide dismutase (SOD) (Fig. 4a), no statistical differences were observed among the experimental groups (Control [mean = 6.534], Pitaya [mean = 6.951], Exercise [mean = 6.613], Pitaya + Exercise [mean = 7.149]). In contrast, the catalase (CAT) activity (Fig. 3b) analysis revealed that the Control [mean = 26.89] and Pitaya [mean = 17.22] did not significantly affect enzyme activity. However, the Exercise [mean = 44.23] exhibited a notable increase in CAT activity compared to the Pitaya [mean = 17.22] ($p = 0.0116$), Pitaya + Exercise group [mean = 19.93] ($p = 0.0165$). This finding indicates that the acute swimming exercise protocol effectively enhanced CAT enzymatic activity, while the pre-treatment with Pitaya juice mitigated this increase. Consequently, the results suggest that the 30-day pretreatment with Pitaya juice led to

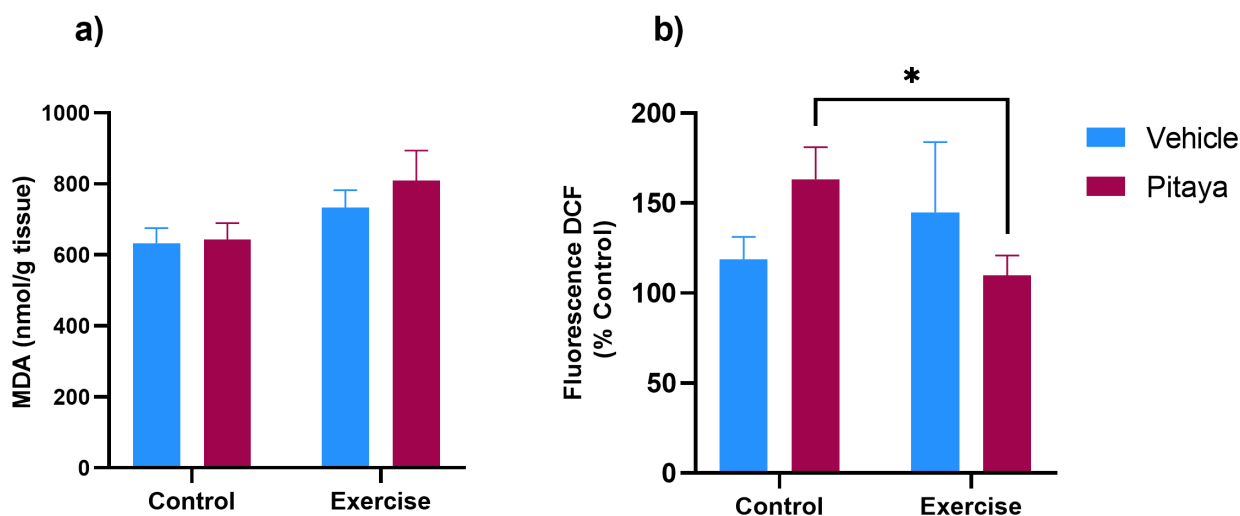


Figure 3. Effect of pre-treatment with Pitaya juice on the levels of a) TBARS and b) Reactive species in the liver of mice subjected to acute swimming exercise. The significance level was obtained by analysis of variance (ANOVA, two-way) with Tukey post hoc. Data expressed statistically as mean $\pm$ SEM. Significant values when $p < 0.05$. *indicates significance level for $p < 0.05$.

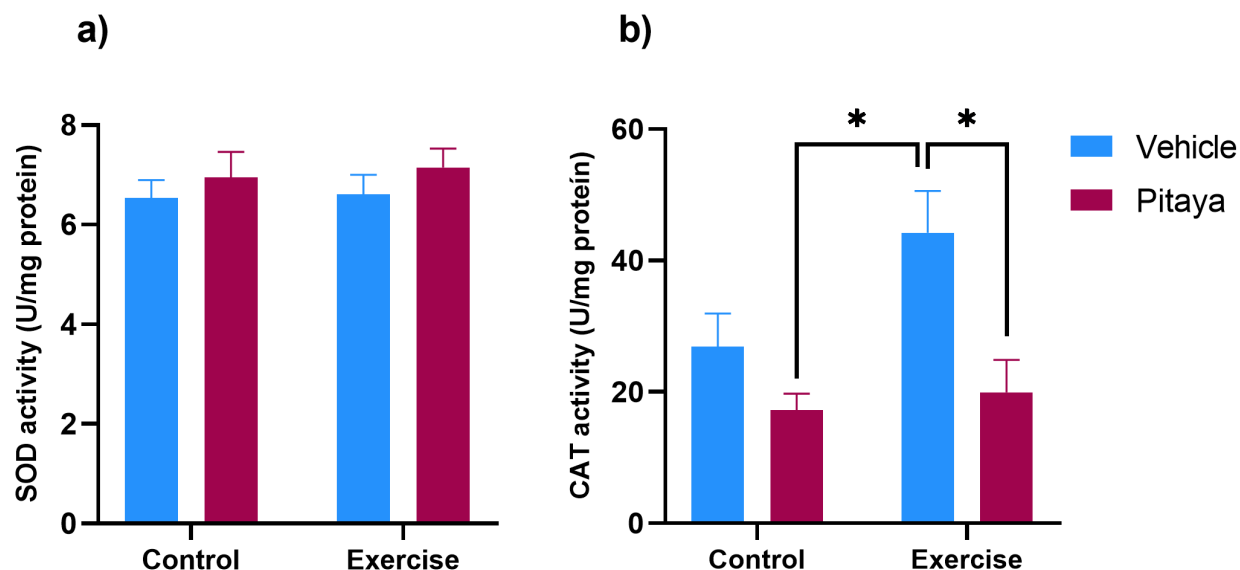


Figure 4. Pre-treatment for 30 days with Pitaya juice and its effect on the activity of antioxidant enzymes a) SOD and b) CAT in the liver of mice subjected to acute swimming exercise. The significance level was obtained by analysis of variance (ANOVA, two-way) with Tukey post hoc. Data expressed statistically as mean $\pm$ SEM. Significant values when $p < 0.05$. *indicates significance level for $p < 0.05$.

a reduction in CAT activity, aligning the values closer to those observed in the Control group.

Pretreatment with pitaya increases GSH levels

The enzymatic activity of glutathione S-transferase (GST) and the levels of glutathione

(GSH) are crucial indicators of the antioxidant response to exercise and dietary interventions. The enzymatic activity of GST (Fig. 5a) was significantly elevated in mice subjected to acute exercise [mean = 57.62] compared to the Control [mean = 35.61] ($p = 0.049$). The Pitaya +

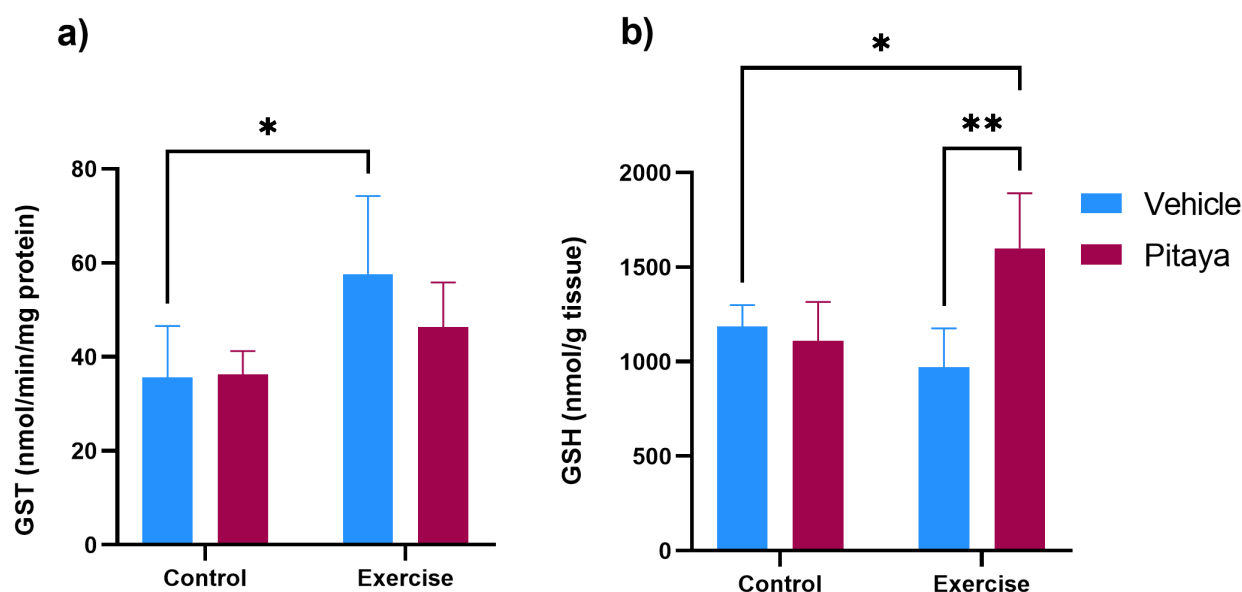


Figure 5. Effect of pre-treatment with Pitaya juice on a) GST enzyme activity and b) GSH levels in the liver of mice subjected to acute swimming exercise. The significance level was obtained by analysis of variance (ANOVA, two-way) with Tukey post hoc. Data expressed statistically as mean $\pm$ SEM. *indicates significance level at $p < 0.05$ and **indicates significance level at $p < 0.01$.

Exercise [mean = 57.62] did not show a statistical difference in GST activity when compared to the Exercise [mean = 57.62] or pitaya group [mean = 36.29]. In GSH levels (Fig. 5b), the Pitaya + Exercise [mean = 970.1] demonstrated a significant increase compared to the Control [mean = 1599.0] ($p = 0.0482$). Notably, the GSH levels in the Pitaya + Exercise [mean = 1599.0] was also significantly higher than those in the Exercise [mean = 970.1] ($p = 0.0044$), but not for pitaya group [mean = 1110.0]. These results underscore the role of pre-treatment with Pitaya juice in sustaining GSH levels, highlighting its protective effects in comparison to both the Control and Exercise groups within this study.

GPx and GR levels were not affected by exercise or Pitaya

The assessment of the antioxidant system, focusing on the enzymatic activities of glutathione peroxidase (GPx) (Fig. 6a) and glutathione reductase (GR) (Fig. 6b), was conducted to evaluate the effects of a 30-day

pretreatment with Pitaya prior to acute swimming exercise. No significant differences in GPx activity were observed among the experimental groups (Control [mean = 6.754], Pitaya [mean = 6.885], Exercise [mean = 6.374], Pitaya + Exercise [mean = 6.125]). Similarly, GR activity also showed no significant differences across groups (Control [mean = 6.037], Pitaya [mean = 6.263], Exercise [mean = 6.024], Pitaya + Exercise [mean = 5.373]).

Exercise increased ALP, but only compared to Pitaya

To assess hepatic metabolism, we analyzed ALP levels (Fig. 7). The Exercise group [mean = 556.2] exhibited significantly higher ALP levels compared to the Pitaya group [mean = 381.0] ($p = 0.0482$). However, no significant differences were observed between the Exercise group and the Control [mean = 413.5] or Pitaya + Exercise [mean = 413.7]. Although the intention is not to compare the Pitaya and the exercise group, an isolated effect is demonstrated in these groups. Therefore, the increase in ALP was not

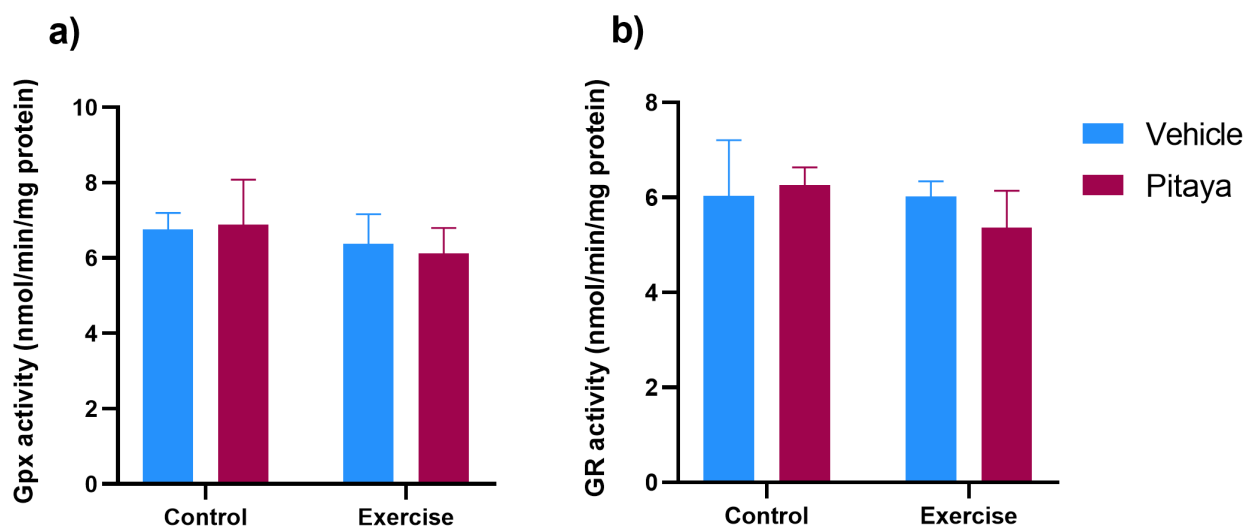


Figure 6. Effect of pre-treatment with Pitaya juice on the activity of the enzyme a) Gpx and b) GR in the liver of mice subjected to acute swimming exercise. The significance level was obtained by analysis of variance (ANOVA, two-way) with Tukey post hoc. Data expressed statistically as mean $\pm$ SEM. Significant values when $p < 0.05$.

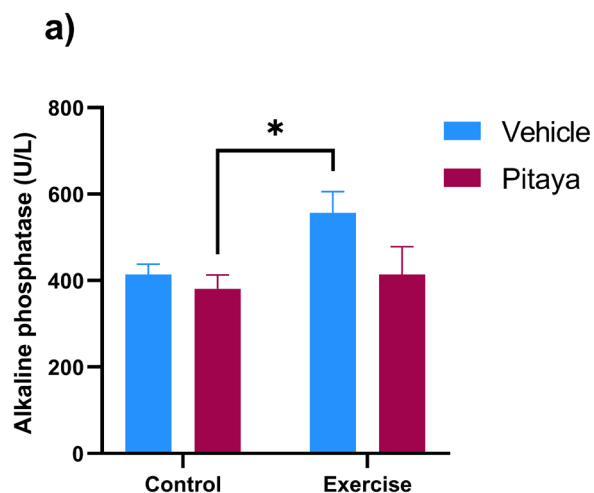


Figure 7. Effect of pre-treatment with Pitaya juice on the activity of the alkaline phosphatase enzyme (ALP) a) in the liver tissue of mice subjected to acute swimming exercise. The significance level was obtained by analysis of variance (ANOVA, two-way) with Tukey post hoc. Data expressed statistically as mean $\pm$ SEM. Significant values when $p < 0.05$. *indicates significance level for $p < 0.05$.

significantly elevated compared to the control group, being only significant compared to the Pitaya group.

DISCUSSION

In the nutritional field, research involving natural substances or bioactive compounds often includes hypotheses and experimental protocols aimed at elucidating their role in human nutrition. One such study focused on the ingestion of red Pitaya juice (*Hylocereus polyrhizus*) and aimed to investigate its effects in a mouse model subjected to an acute swimming exercise protocol after a 30-day pre-treatment with Pitaya juice. The primary objective was to determine whether the bioactive compounds in Pitaya would influence various biochemical parameters, particularly oxidative stress, with the hypothesis suggesting that prior supplementation would mitigate the effects of acute exercise or exert a favorable impact on the analyzed markers.

Although our tests did not show significant results regarding glucose and triglyceride levels, previous studies indicate that red pitaya holds therapeutic promise for metabolic syndrome. For instance, Omidizadeh et al. (2014) demonstrated that fresh Pitaya (*Hylocereus polyrhizus*)

effectively reduced insulin resistance and hypertriglyceridemia. Supporting this, Lodi et al. (2023) found that the peel and aqueous extract of *Hylocereus lemairei* exhibited antihyperglycemic and anti-obesity activities in vitro, attenuating oxidative stress and inflammation caused by high glucose concentrations. Additionally, Song et al. (2016) reported that betacyanins from *Hylocereus polyrhizus* significantly reduced serum triglycerides, cholesterol, and LDL-C in male C57BL/6J mice fed a high-fat diet, while HDL-C levels remained unaffected. In our study, total cholesterol levels significantly decreased in the exercise group compared to the Pitaya group, though not relative to the control. A longer pre-treatment period or alternative models may yield different outcomes, highlighting the potential of red pitaya as a protective agent against dyslipidemia and cardiovascular disease.

In terms of antioxidant activity, our findings revealed no significant differences in lipid peroxidation levels. However, it was noted that RS levels were significantly lower in the Pitaya + Exercise group compared to the Pitaya group, suggesting a positive interaction between physical exercise and dietary intake. Interestingly, while the Exercise group exhibited a slight increase in RS levels, this change was not statistically significant. These results point to the synergistic effect of diet and physical exercise, particularly evident in the Pitaya + Exercise group. In prior research conducted by our group, exposure to a single dose of aflatoxin B1 (AFB1) resulted in a significant increase in MDA levels compared to the control group. Pretreatment with Pitaya juice effectively reduced MDA levels in the Pitaya + AFB1 group, mitigating the adverse effects of AFB1 and offering protection against lipid peroxidation. Furthermore, AFB1 significantly elevated RS levels, which were successfully reduced by Pitaya juice in the Pitaya + AFB1 group. This protective effect underscores

the antioxidant properties of Pitaya against AFB1-induced hepatic damage, as previously detailed by Madalosso et al. (2023). In our results, only CAT activity exhibited a significant decrease in the Pitaya + Exercise group compared to the Exercise group, bringing these values closer to the control group. Pre-treatment with Pitaya juice appeared to attenuate the exercise-induced increase in CAT activity. The requirement for exogenous antioxidants to counteract reactive species chain reactions emphasizes the importance of antioxidant consumption in preventing muscle damage, particularly through detoxifying lipid peroxides produced during exercise and mitigating inflammatory responses (Silva et al. 2022). The red Pitaya (*Hylocereus polyrhizus*) stands out as a valuable antioxidant source, containing higher levels of polyphenols relative to other species (Khoo et al. 2022).

The antioxidant potential of pitaya (*Hylocereus spp.*) has been demonstrated across diverse experimental models, revealing both conserved mechanisms and context-dependent variations. In zebrafish (*Danio rerio*) exposed to copper-induced oxidative stress, pitaya administration mitigated oxidative damage in the brain, digestive system, and whole body, accompanied by enhanced GST and CAT activities (Tamagno et al. 2022a). Similarly, in *Caenorhabditis elegans*, pitaya supplementation modulated GST, CAT, and superoxide dismutase (SOD) activities, suggesting a broad regulatory capacity over key antioxidant enzymes (Tamagno et al. 2022b). These findings align with the observed antioxidant effects in our study, particularly regarding enzymatic modulation. Notably, pitaya's bioactivity extends beyond enzymatic regulation, as evidenced by its dose-dependent protection against H₂O₂-induced oxidative stress in 3T3-L1 adipocytes, which persisted for up to 48 hours irrespective of the fruit's compositional fraction (Khoo et al. 2022).

Such multifaceted antioxidant properties may underpin its reported anticancer potential in 3T3-L1, RIN-5F, and HepG2 cell lines, though further mechanistic studies are warranted (Khoo et al. 2022).

In our experimental framework, exercise induced a significant elevation in GST activity compared to the control group; however, 30-day pitaya supplementation did not attenuate this exercise-induced response. This contrasts with prior findings where pitaya prevented GST upregulation in the context of aflatoxin B1 (AFB1) exposure (Pitaya + AFB1 group), underscoring the condition-specific modulation of enzymatic activity (Madalosso et al. 2023). The divergence may stem from differences in oxidative stressors (AFB1 vs. exercise) or tissue-specific responses, highlighting the need to contextualize pitaya's effects within experimental paradigms. Conversely, we observed a synergistic interaction between pitaya and exercise, with the Pitaya + Exercise group exhibiting significantly elevated GSH levels compared to both Control and Exercise groups. This elevation suggests a compensatory mechanism wherein dietary antioxidants potentiate intracellular defenses against ROS generated during physical activity. GSH, a critical cofactor for antioxidant enzymes, facilitates detoxification and regeneration of vitamins C and E, with plasma levels serving as a biomarker of systemic redox status (Deponte 2017). Intriguingly, while pitaya normalized GSH depletion caused by AFB1 in prior work (Madalosso et al. 2023), our results demonstrate its capacity to amplify GSH synthesis when combined with exercise, emphasizing its role in enhancing adaptive antioxidant responses. These findings collectively illustrate that pitaya's antioxidant efficacy is contingent upon the nature of the oxidative challenge and the biological system under investigation. The pitaya ability to modulate GST, CAT, and SOD activities

across species, coupled with its GSH-enhancing potential in exercise models, positions it as a versatile dietary agent for redox regulation.

On exercise protocols, increased GST enzymatic activity and elevated GSH levels have been reported in studies involving aerobic training in mice (de Sousa Fernandes et al. 2022). This research assessed oxidative balance in the prefrontal cortex of obese mice and noted no differences in MDA and CAT activity, aligning with our findings that exercise did not significantly affect lipid peroxidation or antioxidant enzyme activities. The elevation of GST and GSH has been linked to exercise-induced ROS stimulation, enhancing antioxidant capacity and maintaining homeostasis. Genes regulated by nuclear factor erythroid 2-related factor 2 (Nrf2) play a critical role in this adaptation, encompassing GST and GSH synthesis (Ross & Siegel 2021). This information helps elucidate the changes observed in GST and GSH, considering their established relationship with physical exercise. In contrast, the activities of GPx and GR were not significantly altered by the acute exercise protocol, and the impact of pitaya on these antioxidant enzymes remains inconclusive. However, a study evaluating p-anisaldehyde (PAA) revealed its effectiveness in preserving pitaya fruit quality, maintaining elevated levels of antioxidant compounds (Xu et al. 2021). Furthermore, PAA treatment enhanced the activities of key antioxidant enzymes, including SOD and CAT activity, while also increasing levels of reduced GSH and GR activity. Currently, limited information exists regarding the enzymatic activity of GPx and GR following pitaya administration.

Acute swimming exercise resulted in elevated ALP levels compared to the Pitaya group. We anticipated a significant decrease in the Pitaya + Exercise group; however, this was not observed. Conversely, Pitaya juice effectively

mitigated the increase in ALP in the Pitaya + AFB1 group post-mycotoxin exposure, highlighting its protective role against AFB1-induced liver injury (Madalosso et al. 2023). Physical activity exerts a positive influence on biological functions, bolstering antioxidant defense systems and improving metabolic profiles. Pitaya juice, renowned for its antioxidant properties, demonstrates potential for enhancing recovery after exercise, serving as a dietary intervention for optimizing performance in physically active individuals. Notably, studies suggest that Pitaya juice consumption may reduce muscle soreness and improve recovery post-exercise.

In summary, this investigation highlights the interplay between Pitaya juice and acute exercise, underscoring the importance of dietary interventions in modulating oxidative stress and antioxidant responses. Further studies are warranted to explore the intricate mechanisms underlying these interactions and their implications for human nutrition and health.

CONCLUSIONS

This study provides evidence supporting the antioxidant properties of pitaya juice in animals subjected to acute swimming exercise, and its potential as a functional food for mitigating exercise-induced oxidative stress. Our findings demonstrate that 30 days of pretreatment with pitaya juice led to favorable outcomes in certain parameters, including reduced RS levels, lower CAT activity, and increased GSH levels in the Pitaya + Exercise group. However, to fully elucidate the underlying mechanisms and support the hypotheses, further research involving chronic supplementation or higher doses is warranted, as this initial investigation clarifies the effects of pitaya juice consumption on acute exercise and lays the groundwork for

future studies exploring its potential to reduce oxidative damage associated with exercise.

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