

Supplementation with crajiru [*Fridericia chica* (Bonpl.) L. G. Lohmann] alters physical performance parameters in rats

ANTÔNIO MARIO GALVÃO E SILVA¹, OZANILDO VILAÇA DO NASCIMENTO², SPARTACO ASTOLFI FILHO³, LEONARD DOMINO ROSALES ACHO⁴, PATRÍCIA PRODORUTTI ALVES⁵, TATIANE PEREIRA DE SOUZA⁶, EMERSOM SILVA LIMA^{7*}

^{1,2} Faculty of Physical Education and Physiotherapy, Federal University of Amazonas, Manaus, Amazonas, BRAZIL.

³ Institute of Biological Sciences, Federal University of Amazonas, Manaus, Amazonas, BRAZIL.

^{4,5,6,7} Faculty of Pharmaceutical Sciences, Federal University of Amazonas, Manaus, Amazonas, BRAZIL.

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Abstract

Introduction: Athletes typically try to enhance performance and reduce fatigue, and certain plants may offer performance-enhancing and recovery-accelerating benefits, as well as other health benefits. *Fridericia chica* (Bonpl.) L. G. Lohmann is a species that is commonly used as a herbal medicine, mainly because of its healing and anti-inflammatory properties. The effect of *F. chica* on physical performance parameters has not yet been investigated, though there is evidence that this species can act in this sense. **Purpose:** This study investigated the effect of an aqueous extract of *F. chica* leaves on physical performance parameters in Wistar rats. **Materials & Methods:** The animals were treated during 28-days with and without continuous swimming exercise, with an aqueous dried extract from *F. chica* leaves administered via gavage. Doses of 15 or 30 mg/kg of *F. chica* extract were compared with the negative control (no treatment) or 5 mg/kg of caffeine (positive control). Biochemical and hematological blood markers were evaluated at the end of the experiment. **Results:** In the forced swim test, there was an increase in swimming time in the groups that consumed the *F. chica* extract at doses of 15 or 30 mg/kg of weight. Lactate and creatine kinase values were reduced in the group that consumed *F. chica* at 30 mg/kg ($p \leq 0.05$) and 15 mg/kg for the exercise group ($p \leq 0.05$), when compared to the other groups investigated. **Conclusions:** The aqueous extract of *F. chica* leaves influences the aerobic performance parameters and markers of fatigue in rats. This is the first study to report the ergogenic effect of *F. chica* extract, which can be explored in the development of products to increase the physical performance of athletes.

Keywords: *Fridericia chica*, Crajiru, Physical Performance, Forced swim test, Ergogenic Effect

Introduction

Increased muscle fatigue is a common event in athletes after intense physical exercise. The use of natural or synthetic substances to improve physical performance is a relatively common practice in professional and non-professional athletes (Lippi et al., 2014). Training sessions with a focus on aerobic capacity and endurance require extreme performance of the muscles, which can induce fatigue often due to lack of energy stored in the form of ATP. In this sense, it is essential to use nutritional strategies that help conserve energy before, during and after training sessions, thereby increasing the capacity for physical performance (Juszkiewicz et al., 2019).

In this context, the active ingredients and antioxidants present in different plants and fruits such as cocoa, pomegranate, grape, blackcurrant, cherry, guarana and green tea have been used to supply ATP reserves. These compounds can modulate muscle recovery levels and damage to cellular structures caused by oxidative stress, which lead to the onset of fatigue, thereby reducing aerobic endurance capacity and decreased performance among athletes (Batalha et al., 2022; Bloedon et al., 2022; Craddock et al., 2020).

Furthermore, studies have indicated that flavonoids present in certain foods have an effect on aerobic metabolism, both in animals and in athletes, by increasing the oxidation of substrates in tissues. Their mechanisms of action are not yet fully understood, but it is believed that these flavonoids stimulate mitochondrial biogenesis in muscle tissue. With more mitochondria, the athlete increases lipid oxidation and, consequently, there is an increase in energy generation (Lee et al., 2022).

Among the 55,000 species cataloged in the Amazon region is *Fridericia chica*, which belongs to the Bignoniaceae family. The species has been widely reported to be used as a medicinal plant by local populations in the Amazon. The extract from its leaves produces an intense red dye and, because of its high iron content, it has also been used to treat anemia. Moreover, *F. chica* is reported to have antioxidant, antiproliferative,

antimicrobial, antiseptic, antifungal, enzyme modulation and anti-inflammatory actions (do Nascimento et al., 2023; Servant-Medina et al., 2015).

F. chica stands out for its antioxidant potential, which is related to the action of C-glucosyl xanthenes, phenylpropanoids, anthocyanins, allantoin, triterpenes, alkaloids, anthraquinone, steroids, phenols, flavonoids, flavanones, saponins, tannins and catechins. In this context, the anthocyanins present in the leaves seem to be mainly responsible for most of the activities reported for the species and also for its characteristic color. This leads to this species being widely researched in human health applications (Servant-Medina et al., 2015).

To date, in the literature, there are no reports of any effect of *F. chica* on physical performance. Therefore, the aim of the present study was to evaluate whether the aqueous extract of *F. chica* leaves modifies physical performance parameters in rats after forced swimming.

Materials & Methods

Botanic material and the extract production

F. chica leaves were collected in the medicinal plants and vegetables sector of Embrapa Western Amazonia, located on Highway AM 010, Km 29, Manaus, AM, Brazil. All ethical aspects of animal care were based on Directive DBCA/2013 (CONCEA, 2013) and endorsed by CEUA/UFAM under process number 004/2022. The preparation of the dry extract was performed following the protocol described by (Siraichi et al., 2013). In summary, an aqueous infusion of 10% of the crushed dried leaves was performed in water and, after filtration, the solution was dried using a spray dryer (MDS 1.0, Ribeirão Preto, SP, Brazil).

Determination of total phenolic content

The concentration of total phenols was quantified using the method described by Granato et al., (2016), with modifications. Initially, 10 μ L of the sample solution was used at a concentration of 1 mg/mL with 50 μ L of Folin-Ciocalteu reagent (Sigma-Aldrich, St Louis, USA) (1:3 diluted) and 200 μ L of Milli-Q water were added. This mixture was incubated in the dark for 8 min at room temperature. Shortly after, a saturated 0.4% sodium carbonate solution was added, and the mixture was incubated again for 3 min. The reaction was performed in triplicate. The calibration curve was obtained using gallic acid (8-1,000 mg mL⁻¹). The reading was performed in a spectrophotometer (DTX 800, Beckman Coulter, Indianapolis, USA) at 630 nm.

Determination of flavonoid content

To determine the concentration of total flavonoids, a previously described colorimetric method was applied Chang et al., (2002). A 5% methanol solution of aluminum chloride (AlCl₃) was incubated together with the extract and the reading of the reactions was conducted in a UV-Vis spectrophotometer (Lambda 35, Perkin Elmer) at 425 nm. Quercetin concentrations (Merck) were used as standards, and the results expressed as a percentage of quercetin.

DPPH assay

This assay was conducted according to the methodology described by Molyneux et al., (2004) with some adaptations for execution in a 96-well microplate. The DPPH solution was prepared at a concentration of 1 mg/mL in 96% alcohol. In a 96-well plate, 30 μ L of 96% alcohol and 270 μ L of DPPH solution were added, and the plate was then analyzed in a spectrophotometer at an absorbance of between 0.9 and 1.0 at 492 nm. The samples were prepared at a concentration of 5 mg/mL in 96% alcohol. Then, 60 μ L of the samples were added to the wells, with a subsequent serial dilution of 30 μ L. Following this, 30 μ L of 96% alcohol and 270 μ L of DPPH solution were added. The plate was incubated for 30 min and analyzed in a spectrophotometer (DTX 800, Beckman Coulter, Indianapolis, USA), at an absorbance of 492 nm. From the results, the percentage of DPPH remaining in the reaction medium of Rufino et al., (2009) was calculated. The IC₅₀ values (amount of antioxidant needed to reduce the initial concentration of DPPH by 50%), determined by linear regression analysis, were compared to the gallic acid standard (1 mg/mL), as per Mensor et al., (2001).

ABTS assay

The antioxidant capacity of the samples was determined using the ABTS radical capture method, as described by Re et al., (1999) and Ferreira et al., (2022) with modifications. Antioxidant activity was evaluated in 96-well microplates. The ABTS solution was 1.8 mM diluted in Milli-Q water with 5 mM sodium persulfate. This mixture was incubated at room temperature and in the dark for 16 h to allow a complete reaction. Then, 270 μ L of ABTS diluted in water (1:7) and 30 μ L of the diluent were added to the wells and the absorbance was adjusted to $\pm$ 1,000, at a wavelength of 630 nm, using a spectrophotometer (DTX 800, Beckman Coulter, Indianapolis, USA). Samples were prepared in test tubes at the concentration of 1 mg mL⁻¹ in Milli-Q water. A 30 μ L aliquot of each sample was added to a microplate well, followed by 270 μ L of ABTS^{•+} solution, which was then incubated for 15 min. The experiment was performed in triplicate and, based on the results obtained, the percentage of ABTS^{•+} remaining in the reaction medium was determined. The results were compared with the Trolox standard (Sigma-Aldrich, St. Louis, USA).

LC-MS/MS analysis

All the chemical analyses were performed in a LC-MS/MS system consisting of a surveyor liquid chromatography system (Thermo Fisher, USA) coupled to a LCQ Fleet ion trap mass spectrometer equipped

with an electrospray ionization (ESI) source. To achieve the chromatographic separation, a Phenomenex (Torrance, CA, USA) Luna-C18 column was used (5.0l m, 4.6 mm i.e. 9.150 mm) using a binary mobile phase. Solvent A was water, and solvent B was acetonitrile (Tedia, Mexico City, Mexico). The gradient elution at 35 °C was as follows: 0–2 min, 10% B; 2–19 min, 10–70% (v/v) B; 19–21 min, 70–100% (v/v) B; and 21–24 min, 100 % B at a flow rate of 0.8 mL/min. The autosampler temperature was maintained at 15 °C and the injection volume was 10 µL. The ESI source parameters were previously optimized. In summary: spray voltage, 5 kV; sheath gas, 10 arb; auxiliary gas, 5 arb; sweep gas, 0 arb; capillary temp, 200 °C; capillary voltage, 40 V; tube lens, 120 V; microscans, 4; max, injection time (ms) 100.000. Helium (Praxair, Danbury, CT, USA) was used as the collision gas and the collision energies ranged from 15 to 35%. Tentative identifications were performed by manual interpretation of MS/MS spectral data and comparison with previously published data (Bataglion et al., 2014; Paceli et al., 2012).

Forced swim test

The study was conducted with the use of 62 male rats (*Rattus norvegicus*) of the adult Wistar lineage, weighing between 200 and 350 grams, which were provided by the bioterium at the National Institute for Amazonian Research.

The 64 adult Wistar rats were divided into 8 groups (Figure 1). After adaptation, the rats started the continuous swimming training protocol (CST). For the adaptation to the water, the following procedure was performed: on the first day the rats swam for 20 min, on the second and third day for 40 min, and from the fourth to the seventh day 60 min, following the protocol of Paceli et al. (2012).

After adaptation to the water, on the ninth day, the forced swim test (FST) was performed to divide the groups and then the rats began the continuous swimming training protocol. For this purpose, the rats were weighed and received a metal ring on the tail with a weight corresponding to 5% of body weight, indicating the intensity of the training. The continuous swimming protocol was performed in a water tank (φ 45 cm, 90 cm deep filled with water heated to 37 °C to 38 °C) five times a week, in the morning, and lasted for 28 days (Figure 1).

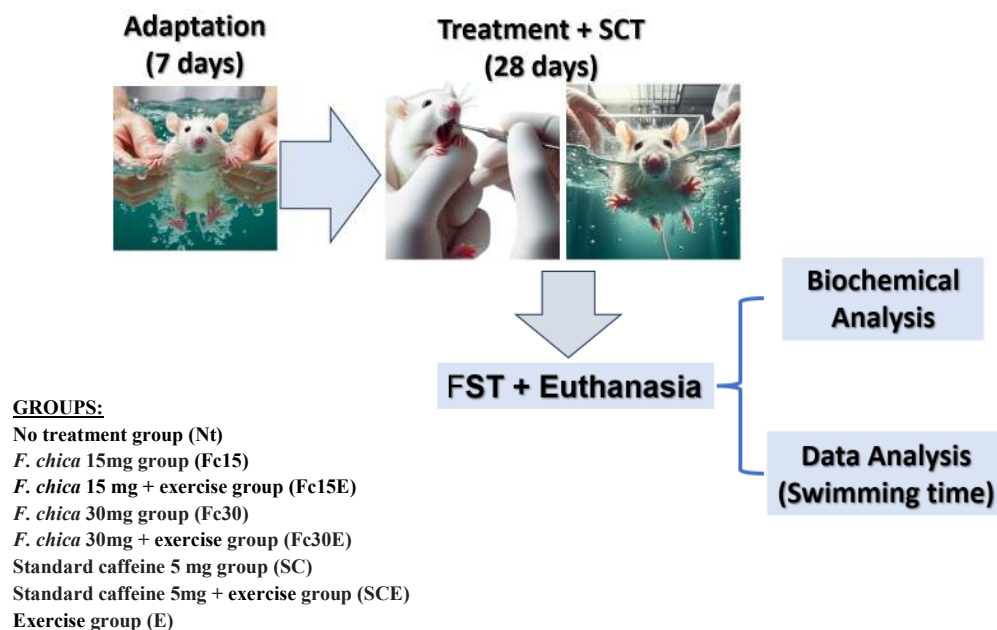


Figure 1: Experimental design of the study. CST= swimming training protocol; FST=forced swim test.

The Fc15, Fc15E, Fc30, Fc30E, SC and SCE groups were supplemented via gavage for 28 days according to the protocol of Ribeiro et al. [19]. On the 30th day, the second forced swim test was applied according to Toda et al., (2016).

On the 30th day, the rats underwent the forced swim test in a water tank (φ 45 cm, 90 cm deep filled with water heated to 37 °C to 38 °C). The test was performed with continuous swimming with extra weight attached to the tails of approximately 3% of their body weights until the animal reached exhaustion Wu et al., (2014).

The timing of the test was started after the animal's body was submerged in the water. The animal was considered to have reached exhaustion when it presented the loss of coordination of movements and failed to return to the surface within 10 sec three consecutive times (Veskoukis et al., 2018).

Twenty-four hours after the test, the animals were euthanized using a mixture of ketamine hydrochloride (130 mg/kg; Frank Tar, Virbac, Fort Worth, TX, USA) and xylazine hydrochloride (6.8 mg/kg, 2% Virbaxyl; Virbac). Blood samples were taken via cardiac puncture for the biochemical analysis.

Biochemical analysis

The biochemical blood tests (lactate and creatine kinase) were performed using an automatized biochemical analyzer (ChemWell 2910, Awareness Technology, EUA) and commercially available kits (Labtest Diagnóstica, Minas Gerais, Brazil), according to the manufacturer's recommendations.

Statistical analysis

The results were described using the mean, standard deviation, frequency and percentage and were analyzed using the statistical software Statistical Package for the Social Sciences (SPSS) Verano 26.0. The variables were tested for normality using the Kolmogorov-Smirnov test. A one-way analysis of variance (ANOVA) was followed by the Tukey test for comparisons between groups. Values with $p < 0.05$ were considered statistically significant.

Results

An aqueous extract of *F. chica* was produced by infusion, with an approximate yield of 3%, after drying using a spray drier. The extract was analyzed for its content of total phenolics and flavonoids, and antioxidant activity was also evaluated using DPPH and ABTS radical inhibition assays, as shown in Table 1.

Table 1: Content of flavonoids, phenols and antioxidant activity (DPPH and ABTS) in the crude extract of *F. chica*.

	Phenols (%)	Flavonoids (%)	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)
<i>F. chica</i> extract	12.7±0.4	15.5±0.9	34.6±1.6	19.45±0.5

The dried extract was also submitted to phytochemical analysis using LC-MS/MS to elucidate the main compounds present and those possibly responsible for the biological activities. Figure 2 presents the chromatogram with the retention times of the main peaks identified and Figure 3 presents a suggestion of the compounds based on their m/z fragmentation patterns.

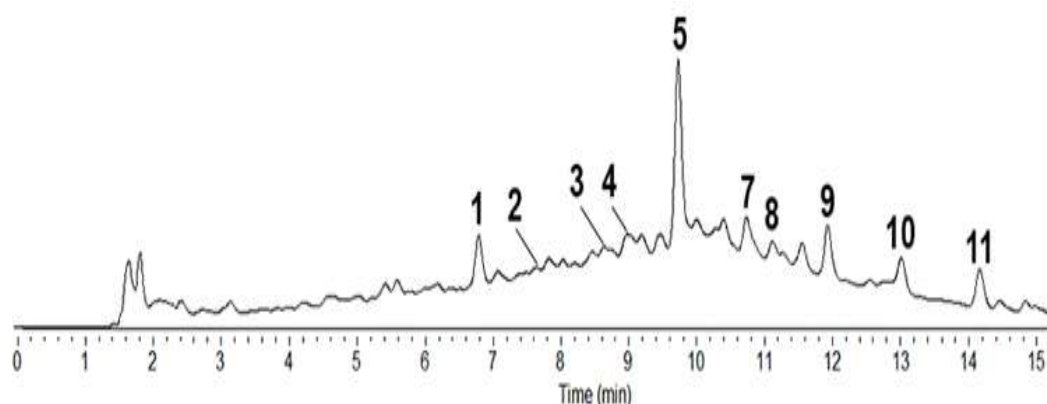


Figure 2: Chromatogram of the *F. Chica* extract obtained via HPLC

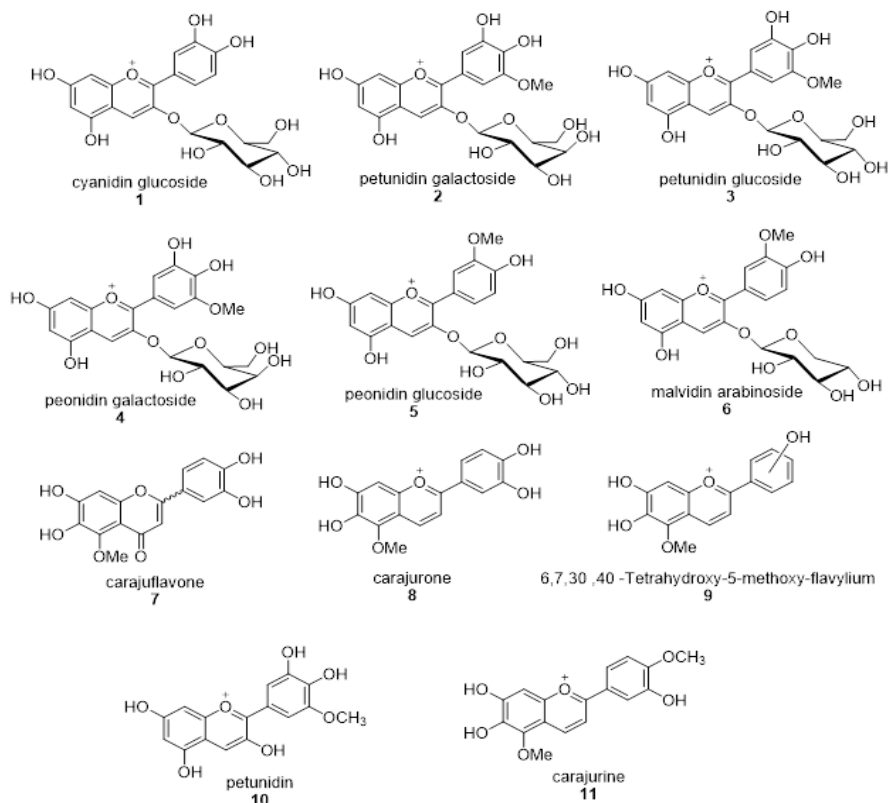


Figure 3: Proposition of the main phytochemical compounds present in the aqueous extract of *F. chica*, analyzed using LC-MS/MS electrospray.

The extract was used in the forced swim test in conditioned or unconditioned animals, as detailed in the methodology. The results of the forced swim test revealed the performance levels through the amount of time the rats of the investigated groups could remain swimming until exhaustion. This demonstrated the superiority of the *F. chica* group at the dose of 15 mg (Figure 4) and *F. chica* group at the dose 30 mg with exercise (Figure 5).

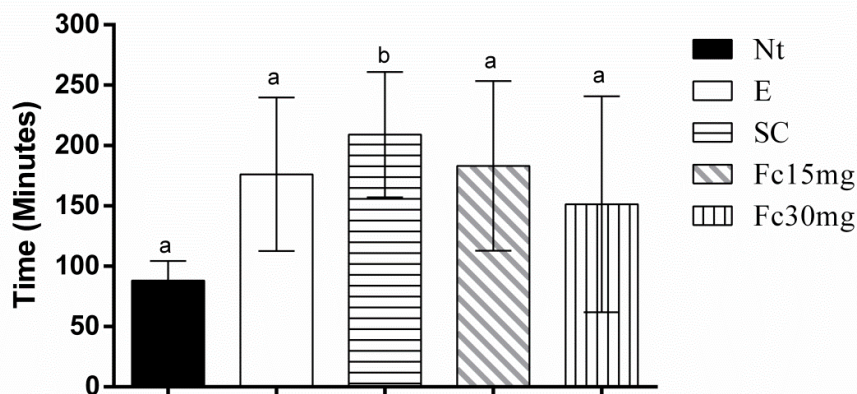


Figure 4: Forced swim test among Wistar rats with consumption or not of *F. chica* extract. Results presented after the Tukey test $*p < 0.05$ when comparing the groups, and different letters indicate significant difference between groups: Nt: no treatment group; E Exercise group without treatment; Fc15mg: *F. chica* extract 15 mg group; Fc30mg: *F. chica* extract 30 mg group; SC: Standard group caffeine 5 mg.

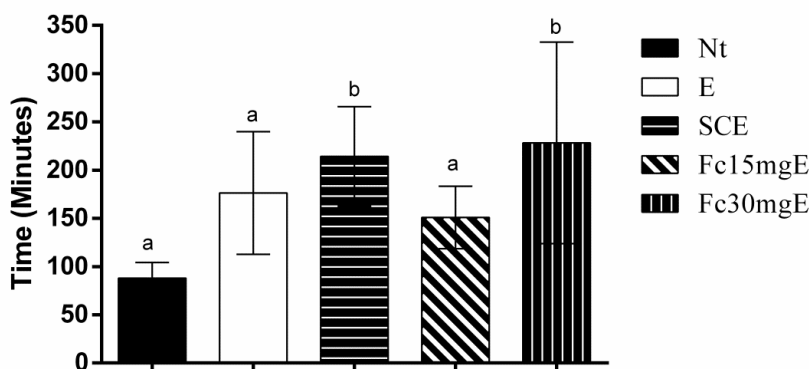


Figure 5: Forced swim test among Wistar rats with consumption or not of *F. chica* extract or caffeine after physical conditioning. Results presented after the Tukey test $*p<0.05$ when comparing the groups, and different letters indicate significant difference between groups: Nt: no treatment group; E: Exercise group; Fc15mgE: *F. chica* extract 15 mg + exercise; Fc30mgE: *F. chica* extract 30 mg + exercise; SCE: Standard caffeine + exercise.

At the end of the experiment, the animals were euthanized and the blood collected for analysis of biochemical parameters related to physical condition. Lactate and creatine phosphokinase (CK) were dosed as shown in Table 2. The values of lactate in the *F. Chica* extract 30 mg group ($p<0.05$) and creatine kinase in the *F. chica* extract 15 mg with exercise group ($p\leq 0.05$) were reduced when compared to the other groups evaluated.

Table 2 - Biochemical parameters of animals treated with *F. chica* extract or caffeine and stimulated with continuous swimming. Values are expressed as the mean and standard deviation.

Group	Lactate (mmol/L)	CK (U/L)
Nt	3.0±1.1	12.0±3.3
Fc15	11.0±1.4	26.6±32.1
Fc15E	10.0±30.5	10.0±30.5*
Fc30	0.11±1.0*	21.6±12.1
Fc30E	9.2±3.1	25.6±10
SC	26.6±32	26.6±32.1
SCE	4.6±1.3	13.2±7.0
E	5.2±1.7	14.4±7.1

Results presented mean ± SD. $*p<0.05$ after the Tukey test when comparing the groups: Nt: no treatment group; Fc15mg: *F. chica* extract 15 mg group; Fc15mgE: *F. chica* extract 15 mg + exercise group; Fc30mg: *F. chica* extract 30 mg group; Fc30mgE: *F. chica* extract 30 mg + exercise group; SC: Standard group caffeine 5 mg; SCE: Standard caffeine + exercise group; E: Exercise group.

Discussion

Considering that to date there are no studies in the literature that have verified the effect of *F. chica* extract on the levels of physical performance among animals and athletes, the discussion of the results in this context becomes difficult. What is proposed in the present study is the possibility of comparing the results found in this experiment with other studies that demonstrate the biological activities of the active ingredients present in *F. chica*, among them the flavonoids.

Intense exercise leads to changes in the body's oxidative system, leading to an overproduction of reactive oxygen species (ROS). This can disrupt the redox balance and antioxidant defense systems, producing oxidative stress that can reduce levels of adaptation and response to training, thereby reducing the physical performance of athletes (Cook et al., 2015).

Most research investigating the action of flavonoids on performance levels in athletes and in animals subjected to exercise models indicate in part a protective action against oxidative stress that can lead to fatigue (Decroix et al., 2018). *F. chica* extract has antioxidant potential that is related to the action of C-glucosyl xanthonones, phenylpropanoids, anthocyanins, allantoin, triterpenes, alkaloids, anthraquinone, steroids, phenols, flavonoids, flavanones, saponins, tannins and catechins (Vasconcelos et al., 2021; Batalha et al., 2022).

The results found in the DPPH and ABTS assay, 82.9 ± 1.8 and 80.5 ± 0.5 , respectively, in this experiment are identical to or superior to those found by other authors (Campos et al., 2019), who also described a significant antioxidant activity of the plant ($IC_{50}=34.6\pm 1.6$ µg/mL). The antioxidant activity may be due to the

redox characteristics present in the flavonoids of *F. chica* extract (Servant-Medina et al., 2015). In this sense, due to their antioxidant properties, flavonoids such as flavonols, among others, have been successfully shown to neutralize the oxidative stress produced by exercise, thus raising the levels of perception of effort and resistance to fatigue (Cichon et al., 2020).

In this experiment, the forced swim test evaluated the performance levels of the rats. Their performance was revealed by the amount of time the rats of the investigated groups could remain swimming until exhaustion. The results demonstrated the superiority of the *F. chica* group at a dose of 15 mg (Fig 4) and *F. chica* at a dose of 30 mg with exercise (Fig 5) when compared with the other groups. Therefore, it is believed that the results achieved by the treated groups could be partly related to the antioxidant action of the plant. The analyses of the hexane extract of the leaves of *F. chica* revealed the presence of alkaloids, anthraquinones, terpenes and anthocyanins, which are related to medicinal actions on biological systems, with emphasis on antioxidant and anti-inflammatory activities (Servant-Medina et al., 2015).

Among the groups treated and exercised, an active ingredient present in the extract could reduce the perception of fatigue. Konrad et al. (2011) evaluated 20 runners (11 men and 9 women) in a randomized, crossover trial. Each participant ingested four capsules of the flavonoid quercetin (totaling 1,000 mg) or a placebo 15 min before the running test. After ingestion, the subjects completed 2 h of running on a treadmill at 70% of maximum VO_2 . According to Devi et al. (2021), citrus flavonoids such as naringenin and hesperidin can cross the blood-brain barrier, whereas the flavonoid luteolin protects dopaminergic neurons by reducing oxidative stress. This result may confirm the findings in this experiment, as the extract of *F. chica* contains luteolin (De Cássia et al., 2016) and this may have contributed to greater swimming resistance, thereby increasing the residence time of the animals in the forced swimming test. Previous studies have confirmed that nutritional supplements containing polyphenols can effectively increase maximum oxygen consumption, exercise performance and exhaustion time (Pinto et al., 2019; Abbey et al., 2011).

One of the forms of action of flavonoids would be as an antagonist of the adenosine receptor and, therefore, it would activate the central nervous system in a similar way to the effect of caffeine (Pinto et al., 2019). In this context, perhaps some as yet unknown active ingredient in the extract of *F. chica* may have a caffeine-like action, with this increased performance favoring the increase in the execution time of the forced swim test both in the treated animals and treated and exercised animals.

The accumulation of lactate in the blood alternates according to the intensity of the exercise, and its concentration may be a biochemical marker of muscle fatigue (Yang & Park, 2020). In the biochemical dosage, reduced values of blood lactate were present in the *F. Chica* extract 30 mg group ($p<0.05$) (Table 3). Zhang & Yang, (2021) used different doses of lemon seed flavonoids (LSF) for 4 weeks, with vitamin C as a positive control. Their aim was to investigate the effects of LSF on antioxidant and anti-fatigue capacity in mice exercised until fatigue. The results obtained in the experiment indicated that both vitamin C and LSF can significantly increase the exhaustion time during training by reducing lactate levels in a dose-dependent manner in mice. The flavonoids myricetin-3-O-glycoside, quercetin-3-O-rutinoside, quercetin-O-glycoside, camferol-3-O-rutinoside, isoramnetin-3-O-rutinoside and kaempferol from the Chinese chestnut (*Castanea mollissima* Blume) flower were used as anti-fatigue drugs in mice after the forced swim test. The flavonoids used had an excellent free radical scavenging activity, with a negative effect on the levels of lactic dehydrogenase (LDH), lactic acid, creatine kinase (CK) (Penk et al., 2021). Moreover, it has been reported that the decrease in tissue glycogen deposits elevates peripheral fatigue, thereby elevating muscle degradation levels with increased creatine kinase (Yang & Park, 2020).

In our experiment, there was a reduction in creatine kinase in the *F. Chica* extract 15mg with exercise group ($p\leq 0.05$) when compared to the other groups evaluated (Table 3). Huang et al. (2022) used *Glossogyne tenuifolia* extract in order to examine the effectiveness of the extract in improving physical fatigue and performance during exercise. Fifty male mice were randomly grouped into five groups ($n=10$) to receive *G. tenuifolia* extract orally for 4 weeks, at different concentrations (50, 100, 250 and 500 mg/kg BW/day). Swimming time, serum levels of biochemical markers, blood lipid profile and histological analysis of various organs were performed to evaluate the anti-fatigue effect and physical performance. Swimming time increased significantly in a dose-dependent manner in all groups when compared to the control, while renal markers and creatine kinase were unchanged after the experiment. Similar effects were found in rats using *Moringa oleifera* extract, which reduced the accumulation of urea ammonia and creatine kinase and caused a significant increase in swimming time (Chen et al., 2022).

In summary, supplementation with *F. chica* extract improved aerobic endurance and led to an increase in resistance time when subjected to forced swimming in conditioned or unconditioned rats. The mechanisms involved in these effects have not been fully clarified but are probably related to the presence of phenolic compounds present in the extract such as flavonoids and anthocyanins. Among the likely mechanisms that explain these effects are the effects on cellular stress and also on mitochondrial membrane potential. There is also evidence that the extract of *F. chica* may have an effect on the number and size of mitochondria (Batalha et al., 2022).

Conclusions

By carrying out this research, it was possible to demonstrate for the first time the effect of the extract of *F. Chica* leaves on the physical performance parameters in rats subjected to a forced swim test. The aqueous extract of the *F. chica* leaves used with or without physical conditioning increased the forced swim time in the Wistar rats when supplemented orally for 21 days. The extract also decreased the lactate and creatine kinase levels when compared to the other groups without using of the extract. The extract was more effective than the standard caffeine, taking into account that it was administered in a dose at least three times higher than the standard. This study opens up an opportunity to investigate plant extracts from plants such as *F. chica* as possible modulators of muscle recovery or improvers of physical performance. It is also important to highlight that other aspects that have not been fully explored, such as the possible mechanisms involved in these effects, should be investigated in future studies.

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