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# High-Intensity Resistance Exercise and *Schinus terenbinthifolius* Supplementation Attenuate Oxidative Stress and Muscle Damage Biomarkers

## El Ejercicio de Resistencia de Alta Intensidad y la Suplementación con *Schinus terenbinthifolius* Atenúan los Biomarcadores del Estrés Oxidativo y Daño Muscular

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**SUMMARY:** High-intensity physical exercises can cause oxidative stress and muscle damage. Several medicinal plants have been used as antioxidant and anti-inflammatory agents. The present study evaluated high-intensity resistance exercise (HIRE) associated with Schinus Terebentifholius ethanolic extract (EE) on oxidative parameters and muscle damage in Wistar rats. Animals were divided into 04 groups (n=10/group): 1. Control (CG) - animals that did not undergo HIRE and were treated with vehicle (distilled water, orally); 2. Acute exercise (AE) – animals submitted to acute exercise session; 3. Exercise + vehicle (EV) - animals that underwent HIRE and were treated with vehicle and 4. Exercise + extract (EX) animals administered with *Schinus terebenthifolius* EE (100mg/Kg, orally) and submitted to the exercise session. *Schinus terebenthifolius* EE showed high *in vitro* antioxidant activity (13.88 ± 0.36 mg/mL). Before the experimental period, lactate was measured at pre and post moments of AE ($p<0.0001$) and EX ($p<0.0001$) groups. After the acute session, the following were evaluated: oxidative stress {malondialdehyde (MDA), sulfhydryl groups (SH) and ferric reducing antioxidant power (FRAP)}, muscle damage (creatine kinase (CK) and lactate dehydrogenase (LDH)), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In the *in vivo* analyses of the EX group compared to AE and EV groups, respectively: hepatic (MDA: $p<0.0001$ and SH: $p=0.0033$, in both; FRAP: $p=0.0011$ and $p=0.0047$), muscle (MDA, SH and FRAP: $p<0.0001$, in both; CK: $p=0.0001$ and $p<0.0001$; LDH: $p<0.0001$, in both), serum levels (MDA: $p=0.0003$, $p=0.0012$, SH: $p=0.0056$, $p=0.0200$, FRAP: $p=0.0017$ and $p=0.0165$) were significant. There was no significant difference in ALT and AST markers. It could be concluded that *Schinus terebenthifolius* EE associated with HIRE attenuated oxidative stress and muscle damage in rats.

**KEY WORDS: Physical Training; Supplements based on Medicinal Plants; Natural Product; Oxidative Lesion; Damage Reduction.**

## INTRODUCTION

High-intensity resistance physical exercises can trigger ischemia and reperfusion events due to increased muscle activity and energy demand, and, as a consequence, increase in the production of reactive oxygen species (ROS) due to a substantial increase in oxygen consumption (VO2) (De Araújo *et al*., 2019). Physiological ROS production can promote health benefits on the other hand, when production is exacerbated (physiological imbalance), that is, when the

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intracellular regulatory capacity is not able to normalize ROS concentration close to acceptable physiological values, the phenomenon called oxidative stress (OE) occurs (Gutteridge & Halliwell, 2018).

The effects arising from the practice of physical exercises on OE indicators, in their various mechanisms, play an important role in the biological, cellular and tissue adaptation processes, whether in pathological or physiological conditions (Hoshino *et al*., 2013), as well as in OE markers, which have been extensively investigated in order to verify alterations between ROS production and the antioxidant capacity of neutralizing them (Parker *et al*., 2018). However, several types of physical exercises and models (human and animal models) have been evaluated and applied in order to clarify the impacts generated by both acute and chronic physical exercise with regard to the different OS biochemical markers (Slusher *et al*., 2018). Furthermore, ROS are also associated with post-exercise inflammatory responses that can promote muscle damage (Dos Santos *et al*., 2014). However, such muscle damage and inflammation are proportional to the type of exercise as well as its intensity (Souza *et al*., 2020).

In this sense, the association of medicinal plants with physical exercise has been used for several purposes, such as attenuation of muscular and oxidative damages and as hyperglycemic agents (Dos Santos *et al*., 2014; De Araújo *et al*., 2019; Santos *et al*., 2020). Among the various mechanisms reported to elucidate, at least in part, the physiological effects arising from medicinal plants, the antioxidant capacity of phenolic compounds stands out (Dos Santos *et al*., 2022). In view of the above, there is a popular plant from northeastern Brazil belonging to the Anacardiase family, which has antioxidant compounds such as flavonoids, with the species *Schinus terebenthifolius* Raddi known as "Aroeira", as its main exponent (Oliveira *et al*., 2020). In addition, it has anti-inflammatory, antitumor, healing, antipyretic and analgesic properties (De Lima Glória *et al*., 2017). The hypothesis is that, due to its antioxidant characteristics, this plant plays a redox attenuating role in damage markers. In this sense, the present study verified the effects of high intensity resistance exercise associated with the *Schinus Terebentifholius* ethanolic extract on oxidative parameters and muscle damage in Wistar rats.

## MATERIAL AND METHOD

Collection, identification and processing of the plant material. *Schinus terebinthifolius* leaves were collected in the municipality of São Cristóvão, Sergipe, Brazil at coordinates (10º 55' 14.8" S, 37º 06' 11.9" W) registered in the National Genetic Heritage Management System (SIGEN) No. A6AC079. A specimen was collected, deposited, identified and registered in the Herbarium of the Federal University of Sergipe (UFS) located in the Department of Biology under voucher ASE 39748. Soon after, they were transported to the Laboratory of Chemistry of Natural Products and Biochemistry (LQPNB), Department of Physiology (UFS), where they were placed in an oven (model MA-037) at 37°C, with air circulation and renewal for 48 hours until complete dehydration.

After the drying process, leaves were reduced to powder using a knife mill with fine mesh sieve (Willey MA430). Subsequently, leaves were submitted to extraction in 95 % ethanol for 5 days at room temperature and in a closed container. Subsequently, the extract was filtered and concentrated in rotary evaporator (Logen Scientific®, Lagos, Nigeria), under reduced pressure at 50 ºC to eliminate the solvent and to obtain the ethanolic extract (EE).

***In vitro* antioxidant activity.** The protocol used in this assay was adapted of Cheng *et al*. (2006). Initially, a stock 0.208 mM DPPH (2,2-diphenyl-1-picrylhydrazyl solution) in methanol was prepared. Then, a stock EE solution at concentration of 1 mg/mL was prepared, and from this, different sample concentrations (25 - 60 mg/mL) were prepared.

Subsequently, a gallic acid curve was calculated with concentrations of 1, 2, 3, 4 and 5 mg/mL, and for the purpose of comparison with the sample, gallic acid solution at concentration of 5 mg/mL was used as positive control. About 100 mL of negative control (methanol); 100 mL of positive control (gallic acid); and 100 µL of sample were distributed in the wells of the Elisa plate (in triplicate); and then, under light protection, 100 mL of DPPH solution were added. For the blank, samples were made using 100 mL of each concentration plus 100 mL of methanol. Finally, the material was incubated for 60 minutes. After this time, absorbance was read in microplate spectrophotometer (UV/vis at 515 nm).

The effective antioxidant concentration needed to decrease the initial DPPH radical concentration by 50 % (EC50) was calculated using the %DPPHREM at a time of 60 minutes, as opposed to the sample concentrations. Results were expressed as standard error of the mean (S.E.M.). Antioxidant activity was also expressed by the antioxidant activity index (AAI), calculated accordingly to Scherer & Godoy (2009) based on the equation: AAI = DPPH stock (mg/mL) / EC50 (mg/mL). The antioxidant activity is considered weak when the AAI value is less than 0.5, moderate when AAI is between 0.5 and 1.0, strong when

AAI is between 1.0 and 2.0 and very strong when the AAI value is greater than 2.0 (Scherer & Godoy, 2009).

***In vitro* cytotoxicity analysis.** Fibroblasts (L929) were distributed in 96-well plates (2 x 104 cells/well) and incubated for 24 h in 5 % CO2 atmosphere at 37 °C. After this period, the medium was removed and adhered cells were treated with S. terebinthefolius extract at concentrations of 100 and 200 mg/mL-1 for 24h under the same incubation conditions. Untreated cells were used as control and considered to have 100 % cell viability. After the treatment period, cell viability was determined by the MTT assay {[3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide]} as described in ISO 10993-5 (International Standard ISO 10993–5, 2009), with modifications. For this, the cell monolayer was washed twice with PBS (pH 7.4) and then 200 ml of MTT (0.5 mg mL-1 in PBS, Sigma-Aldrich, St. Louis, MO, USA) were added to each well. Plates were again incubated under the same conditions as above for a period of three hours. After the incubation time, the MTT was aspirated and the formazan crystals were solubilized in 200.0 mL dimethylsulfoxide - DMSO (Vetec by Sigma-Aldrich, USA). After 10 minutes, the optical density was measured in a microplate reader at wavelength of 570 nm. Results were expressed as viability percentage, according to the following equation:

$$\text{Viability} = \frac{\text{absorbance (treated cell) x 100}}{\text{absorbance (control cell)}}$$

Each experiment was carried out in quadruplicate and repeated at least twice. Data were expressed as mean ± standard deviation (± SD).

**Animals.** Forty male Wistar rats (Rattus norvegicus) with initial age of 60 days and weighing 220-250g were used, which were obtained from the Animal Facility of the Center for Research in Intracellular Signaling (NUPESIN) of UFS. Animals were kept in collective cages (4/cage) under environmental conditions of temperature from 21° to 24°C and a 12-hour light-dark cycle with free access to filtered water and specific food for rodents (Nuvilab®). The procedures used in this study were previously approved by the Ethics Committee on the use of Animals at UFS (CEUA/UFS) under protocol No. 2969150819 (2019), and were in accordance with the Guidelines of the Brazilian College of Animal Experiments (COBEA).

Animals were divided into 04 groups (n=10):

1. Control (CG): animals that did not undergo HIRE and were treated with vehicle (distilled water, orally) and included in the experiment only for evaluation of baseline levels regarding biochemical parameters;
2. Acute Exercise (AE): animals submitted to acute exercise session;
3. Exercise + Vehicle (EV): animals submitted to acute exercise session and treated with vehicle;
4. Exercise + Extract (EX): animals administered with *Schinus terebenthifolius* EE (100mg/Kg, orally) and submitted to the exercise session.

**High-intensity resistance exercise protocol.** The protocol used was adapted from Hornberger Jr. & Farrar (2004), which consists of animals climbing a vertical ladder measuring 1.1m x 0.18m with a 2cm step and inclination of 80º with a load implement attached to the base of the tail. Interventions took place in the afternoon (1p.m – 5p.m).

Previously, an adaptation protocol for climbing the stairs was applied to all groups (2 weeks, for 5 uninterrupted days), and in the first week, animals performed 04 series of 06 to 10 consecutive climbs without load and with manual stimulation, with one-minute break between series. In the second week, animals performed the same protocol, but with overload (Falcon tubes) attached to the proximal portion of the tail.

Forty-eight hours after the end of the adaptation period, animals from AE, EV and EX groups were submitted to maximum load test (MLT), which consisted of climbing with initial load of 75 % of body weight, and an additional 30g load on each climb with a one-minute rest between each climb, which was repeated until the animal completed the eighth climb or not, managed to perform a climb completely and without manual stimulation, according to protocol adapted from Raizel *et al.* (2016).

Forty-eight hours after MLT, animals, from 50 % of the body weight of the last load performed in the test, were submitted to acute exercise with progressive increase in the load by 25 % and rest of 01 minute between series composed of 04 to 08 climbs until they could not perform a complete climb in the minimum amount (Raizel *et al*., 2016).

**Blood lactate test.** Before and after MLT, approximately 25µl of blood samples were collected by puncturing the caudal end of each animal and placed on test strips for the lactate concentration quantification (BM-Lactate®). Then, these test strips were immediately introduced into the portable Accutrend® Lactate analyzer to determine lactate concentrations. Only the AE and EX groups were analyzed for the purpose of demonstrating the intensity of the exercise session.

**Euthanasia.** Forty-eight hours after the acute high-intensity resistance physical exercise session, all groups were anesthetized with ketamine/xylazine (75mg/kg + 10mg/kg i.p); then, laparotomy was performed to visualize the thoracic region and blood collection (±5 mL) was performed by means of cardiac puncture, with animals being euthanized by exsanguination. After collection, blood was immediately centrifuged at 4000 x g for 15 min at ± 4°C and the supernatant stored at ± -80°C. Simultaneously, tissues (muscle, liver) were removed and washed 3 times with 1.15 % potassium chloride (KCl) solution, dried and weighed. Soon after, they were homogenized and each gram of tissue was mixed with 5 mL of KCl + 10 µL of phenylmethylsulfonyl fluoride (PMSF –100 mmol. L-1) + 15 µL of 10 % Triton solution and centrifuged at 3000 xg for 10 min at ± -80°C for further analysis of oxidative stress markers.

***In vivo* MDA/TBARS determination.** According to method described by Lapenna *et al*. (2001), lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBARS). Aliquots of 200µL of samples (blood and tissues) were added to a 400µL mixture composed of by equal parts of 15 % trichloroacetic acid (TCA), 0.25 N HCl and 0.375 % TBA, plus 2.5 mM butylated hydroxytoluene (BHT) and 40µL of 8.1 % sodium dodecyl sulfate (SDS), being heated for 30 min at 95°C in an oven. The mixture pH was adjusted to 0.9 with concentrated HCl. BHT was used to prevent lipid peroxidation during heating. After cooling to room temperature and adding 4 mL of butanol, the material was centrifuged at 800 xg for 15 min at ± 4 °C and the supernatant absorbance was measured at 532 nm. The molar extinction coefficient used was 1.54 x 105 M -1 cm -1 and the TBARS result was expressed in nmol Eq MDA/mL for plasma and tissue samples.

**Determination of total sulfhydryls (thiols).** The plasma and tissue antioxidant level was measured through the determination of sulfhydryl groups, performed according to methodology described in Faure & Lafond (1995) in which aliquots of 50 mL of samples (blood and organs) were mixed in 1 mL of tris-EDTA buffer, pH 8.2. Subsequently, the first reading (A) was performed in spectrophotometer at 412 nm. After reading, samples were transferred to test tubes and mixed with 20 mL of 10 mM DTNB diluted in methanol (4 mg/mL), left to stand in the dark. At the end of 15 min, the second absorbance reading (A2) was performed. The SH concentration was calculated according to the following equation: (A2-A1) –B x 1.57 mM x 1000, and the result was expressed in nmol.mg-1 tissue.

**Determination of tissue damage markers.** Tissue damage was determined by measuring tissue enzyme markers such as creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Labtest® kits were used (Santa Lagoa, Minas Gerais, Brazil). Serum samples (20 mL) from each animal were homogenized in specific reagents at 37 ± 0.2 °C, and readings were performed using spectrophotometer (Biospectro Modelo SP-22 UV / Visible, Minas Gerais, Brazil) at wavelength of 340 nm.

**Determination of the antioxidant capacity.** Using the Ferric Reducing Antioxidant Power (FRAP) technique, an aliquot of 9 mL of plasma and muscle and liver tissue was pipetted into a microplate, where 27 mL of distilled water and 270 mL of FRAP reagent were added. The plate was incubated at 37°C for 30 minutes and the reading was performed at 595nm. Ferrous sulfate (FeSO4) was used as standard and results were expressed in µM of ferrous sulfate equivalents (Singhal *et al*., 2014).

**Statistical analysis.** To verify the normality of variables, the Shapiro Wilk test was used. The comparison between groups was performed using the ANOVA test (One Way), with Bonferroni's post hoc. In the case of blood lactate, Student's t test was used between moments of the evaluated groups. Statistical treatment was performed using the GraphPad Prism statistical software version 7.0 (GraphPad Software, San Diego, CA, USA). The significance levels adopted were $p < 0.05$ and $p < 0.0001$.

## RESULTS

Blood lactate concentrations obtained after MLT denote that the exercise protocol used is of high intensity, since values above 4mmol/L are considered high intensity (Gobatto *et al*., 2001). The pre values (2.17 ± 0.65 mmol/L and 1.71 ± 0.65 mmol/L; for AE and EX respectively) were statistically lower ($p<0.0001$) when compared to post values (5.8 ± 0.65 mmol/L and 4.8 ± 0.65 mmol/L; for AE and EX respectively).

The S. terebinthifolius ethanolic extract showed very strong antioxidant capacity, represented by AAI, which was able to inhibit 50 % of the DPPH synthetic free radical at low EC50 concentration. Furthermore, the inhibitory potential (IP) was lower than that of gallic acid (positive control), whose values were very close to the antioxidant control, as shown in Table I.

Regarding the *in vitro* cytotoxicity assay (cell viability), the concentration of 100ug/ml (49.02 ± 2.33 mg/mL) was significantly less toxic when compared to the concentration of 200ug/ml (21.41 ± 1.97 mg/mL) (Fig. 1).

Table I. Antioxidant activity of ethanolic extract of S. Terenbinthifolius determined by the DPPH test.

| Sample | IP% | $EC_{50}$ | AAI |
|---|---|---|---|
| EE(µg/mL) | 80,06±0,65 | 2,88±0,07 | 13,88±0,36 |
| GALLIC ACID (µg/mL) | 83,00±0,10 | 2,00±0,01 | 19,98±0,10 |

Note: EE: ethanolic extract, IP%: inhibitory potential, AAI: antioxidant activity index and EC50: effective concentration. IP% and EC50 were calculated in their respective time of 60 min. One-way ANOVA was followed by the Tukey's test (r<0.05). AAI classifies the sample as weak when IAA <0.5, moderate when 0.5 <IAA <1, strong when 1> IAA <2 and very strong when IAA> 2.

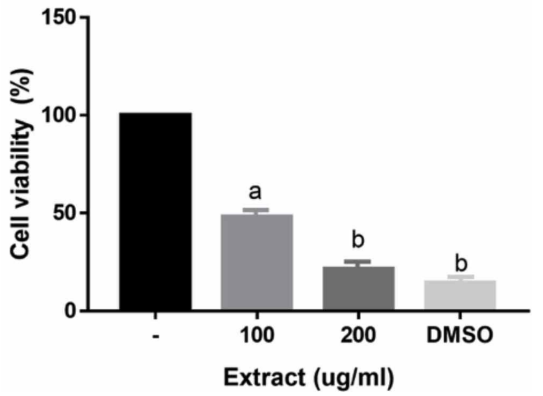


Fig. 1. Cell viability determined by the *in vitro* cytotoxicity assay. Concentrations of 100 and 200 mg/mL-1 and dimethylsulfoxide (DMSO). *Different letters in the figure represent statistically significant difference for p<0.05 between groups (n=10/sample).

Figure 2 demonstrates the effects of high-intensity resistance exercise associated with S. Terenbinthifolius EE on tissue and serum TBARS levels. In liver tissue, no statistical difference between CG (498 ± 52.1 nmol EqMDA-1/mg) and EX (525.6 ± 50.05 nmol EqMDA-1/mg) groups was observed; however, the latter was significantly different when compared to AE (671.6 ± 66.05 nmol EqMDA-1/mg, p=0.0008) and EV (668.1 ± 52.05 nmol EqMDA-1/mg, p=0.0008) groups, respectively (Fig. 2A). With regard to the quadriceps muscle, the TBARS concentration in the EX group (97.61 ± 13.01 nmol EqMDA-1/mg) was lower when compared to AE (197.9 ± 39.02 nmol EqMDA-1/mg, p<0.0001) and EV (227.2 ± 20.75 nmol EqMDA-1/mg, p<0.0001) groups, respectively (Fig. 2B). Serum MDA levels (EX: 74.03 ± 9.54 nmol EqMDA-1/mg; EA: 105.70 ± 6.73 nmol EqMDA-1/mg, p=0.0003) and EV: 104 ± 17.34 nmol EqMDA-1/mg, p=0.0012) (Fig. 2C).

Figure 3 shows the concentrations of sulfhydryl groups in liver tissue, quadriceps muscle and blood. In liver tissue (Fig. 3A), no significant differences between CG (170.20 ± 20.71 nmol/mg) and EX (165.40 ± 33.76 nmol/mg) groups were observed; however, the latter showed significant attenuation when compared to AE (371.60 ± 43.65 nmol/mg) and EV (286.20 ± 20.38 nmol/mg) groups. In addition, significant difference (p=0.0033) between AE and EV groups was observed.

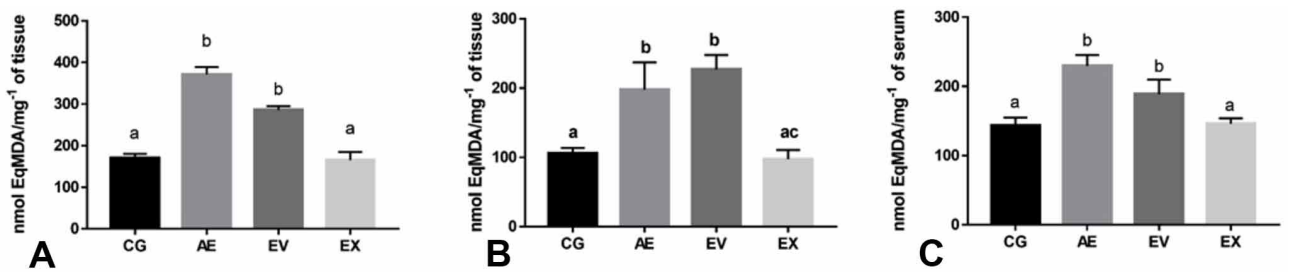


Fig. 2. Effect of high-intensity resistance exercise associated (HIRE) with S. Terenbinthifolius Ethanol Extract (EE) on oxidative stress and lipid damage biomarkers in liver tissue (A), quadriceps skeletal muscle (B) and blood (C). Values presented as mean ± standard error of the mean and expressed as nmol MDA/mg tissue for muscle and liver and nmol MDA/mg serum for blood. One-way ANOVA was followed by the Bonferroni test (p<0.05) and (p<0.0001). *Different letters in the figure represent statistically significant difference between groups.

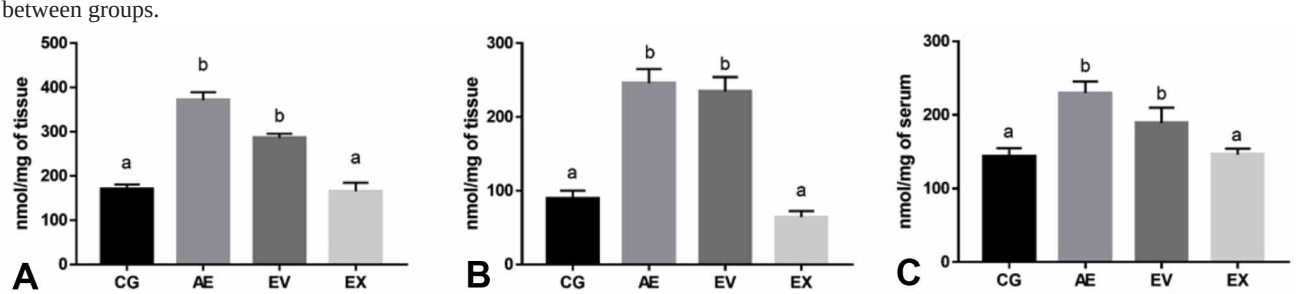


Fig. 3. Effect of high-intensity resistance physical exercise (HIRE) associated with S. Terenbinthifolius Ethanol Extract (EE) on the sulfhydryl groups in liver tissue (A), quadriceps skeletal muscle (B) and blood (C). Values presented as mean ± standard error of the mean and expressed as nmol MDA/mg tissue for muscle and liver and nmol MDA/mg serum for blood. One-way ANOVA was followed by the Bonferroni test (p<0.05) and (p<0.0001). *Different letters in the figure represent statistically significant difference between groups.

In the quadriceps muscle (Fig. 3B), the concentration of sulfhydryls was similar between CG (89.25 ± 10.78 nmol/mg) and EX (64.15 ± 8.18 nmol/mg) groups; on the other hand, both presented lower values ($p<0.0001$) when compared to AE (245.80 ± 19.39 nmol/mg) and EV (234.60 ± 19.53 nmol/mg) groups. In serum levels (Fig. 3C), no difference between CG (143.50 ± 25.33 nmol/mg) and EX (146 ± 16.27 nmol/mg) groups was observed; however, both were lower when compared to AE (229.70 ± 38.44 nmol/mg; $p=0.0056$) and EV (188.90 ± 51.10 nmol/mg; $p=0.0200$) groups.

Figure 4 shows the antioxidant capacity assessment data, denoted by the FRAP method. In liver tissue, no statistical difference between CG (116.30 ± 14.47 μM ferrous sulfate/g) and EX (118.30 ± 8.50 μM ferrous sulfate/g) groups was observed; however, the latter presented significantly different values compared to AE (201.70 ± 19.42 μM ferrous sulfate/g; $p=0.0011$) and EV (176.9 ± 12.99 μM ferrous sulfate/g; $p=0$ .0047) groups (Fig. 4A). Regarding the quadriceps muscle, the EX group (78.83 ± 9.55 μM ferrous sulfate/g) showed no significant difference compared to the CG group (62.01 ± 8.48 μM ferrous sulfate/g); however, EX showed significant values ($p<0.0001$) when compared with AE (240.40 ± 20.07 μM ferrous sulfate/g) and EV (247.30 ± 12.58 μM ferrous sulfate/g) groups, respectively (Fig. 4B). Serum ferrous sulfate concentration was evaluated and no difference between EX (100.70 ± 8.32 μM ferrous sulfate/g) and CG (85.67 ± 11.5 μM ferrous sulfate/g) groups was observed. However, statistical difference between EX when compared to AE (159.90 ± 10.53 μM ferrous sulfate/g; $p=0.0017$) and EV (138.50 ± 17.83 μM ferrous sulfate/g; $p= 0.0165$) groups was observed, respectively (Fig. 4C).

Figure 5 shows data referring to muscle and liver tissue damage markers. No statistical difference in serum CK concentration between CG (221.1 ± 22.91 iu/l) and EX (191 ± 18.21 iu/l) groups was observed; however, the latter showed statistically significant difference when compared to AE (256.10 ± 23.48 iu/l; $p=0.0001$) and EV (267.5 ± 22.37 iu/l; $p<0.0001$) groups, respectively (Fig. 5A). For serum LDH concentration, significant differences were observed when the EX group (115.90 ± 6.31 iu/l) ($p<0.0001$) was compared to AE (285.20 ± 13.31 iu/l) and EV (309.60 ± 6.39 iu/l) groups, respectively (Fig. 5B).

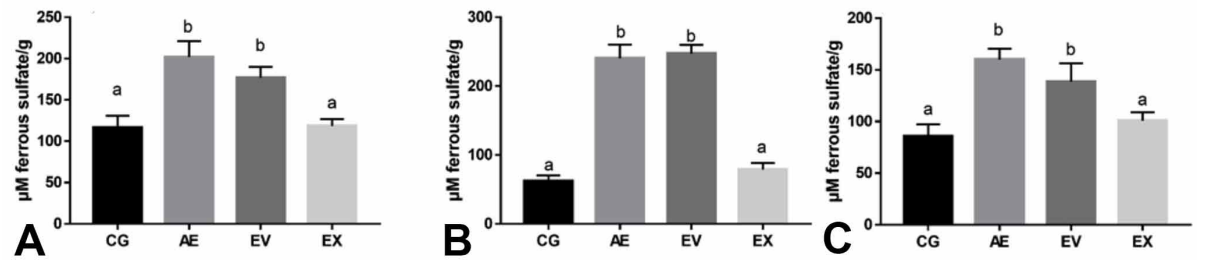


Fig. 4. Effect of high-intensity resistance exercise associated with *S. Terenbinthifolius* Ethanol Extract (EE) on the FRAP antioxidant capacity in liver tissue (A), quadriceps skeletal muscle (B) and blood (C) of Wistar rats. Values presented as mean ± standard error of the mean. One-way ANOVA was followed by the Bonferroni test ($p<0.05$) and ($p<0.0001$). *Different letters in the figure represent statistically significant difference between groups.

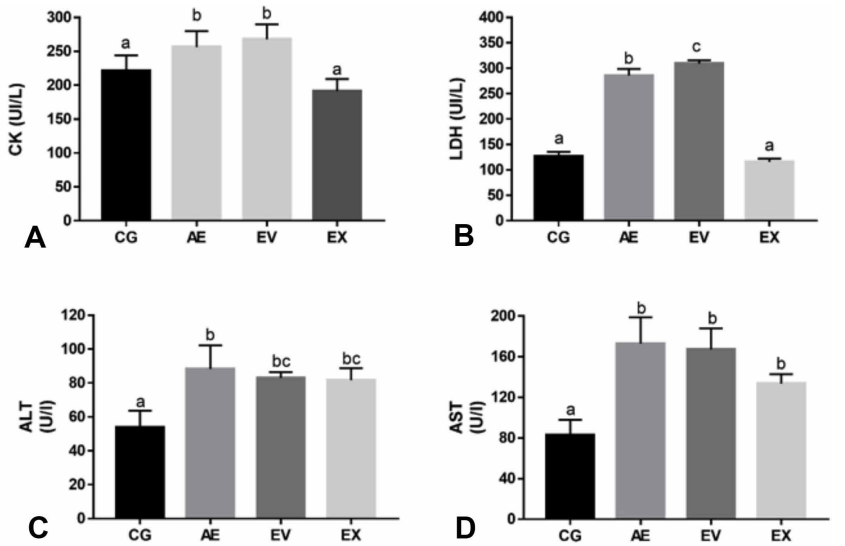


Fig. 5. Effect of high-intensity resistance exercise (HIRE associated with S. Terenbinthifolius Ethanol Extract (EE) on tissue damage markers creatine kinase - CK (A), lactate dehydrogenase - LDH (B), alanine aminotransferase - ALT (C) and aspartate aminotransferase - AST (D) in Wistar rats. Values presented as mean ± standard error of the mean. One-way ANOVA was followed by the Bonferroni test ($p<0.05$) and ($p<0.0001$). *Different letters in the figure represent statistically significant difference between groups.

With regard to ALT concentration, the EX group (81.67 ± 7.09 iu/l) showed significantly higher values compared to the CG group (53.67 ± 10.02 iu/l; p=0.0271), but no difference when compared to AE (88.20 ± 13.91 iu/l) and EV (83.03 ± 3.27 iu/l) groups. Differences between CG and AE (p=0.0087) and CG and EV (p=0.0213) groups were observed (Fig. 5C).

Regarding serum AST levels, the EX (133.05 ± 9.19 iu/l) group showed significantly higher values ??when compared to CG (82.07 ± 15.13 iu/l) (p=0.0158). On the other hand, no difference was observed when EX was compared to AE (172.90 ± 25.90 iu/l) and EV (167 ± 20.88 iu/l) groups. Significant differences between CG and AE (p=0.0117) and CG and EV (p=0.0161) groups were observed (Fig. 5D).

## DISCUSSION

The interest of the scientific community in high-intensity exercises has increased significantly in recent years, and thus, possible ways to reduce the deleterious effects that may be generated during the post-exercise recovery period (De Araujo *et al*., 2019). To the best of our knowledge, this is the first study to investigate the effects of high-intensity acute physical exercise associated with the Schinus Therebintefolius ethanol extract on oxidative stress and muscle damage markers. The main results indicated that the association between the administration of S. terebinthifolius EE and high intensity physical exercise promoted attenuation of lipid peroxidation (liver and muscle tissue) and in the sulfhydryl groups (liver, muscle and blood tissues); such findings corroborate the results obtained by other authors who submitted rats to high-intensity resistance training using plant EE with high antioxidant capacity (Dos Santos *et al*., 2014).

The cytotoxicity assay is used to verify the safest dose for application in animals (Skenderi *et al*., 2008); however, no significant differences were observed between EX and AE and EX and EV groups with regard to serum ALT and AST levels. FRAP is a parameter of plasma antioxidant activity and its increase after intense exercise may suggest increase in the antioxidant defense capacity (Chatzinikolaou *et al*., 2010) and in this study, EX was able to exert a protective function in relation to AE and EV groups.

The cell membrane and other organelles are protected by several antioxidant molecules with enzymatic and non-enzymatic activity that can attenuate oxidative and muscle damage (Gutteridge & Halliwel, 2018). The increase in blood CK and LDH concentrations has been related to muscle micro-injuries, as well as the adjustments promoted by exercise in MDA, CK and LDH concentrations (Santos *et al*. 2020). Previous studies (Silvestre *et al*. 2017; De Araujo *et al*., 2019; Santos *et al*. 2020) have reported that high-intensity training can increase serum CK and LDH values and are used as parameter to assess physical effort intensity and muscle injuries. Regarding blood lactate concentrations, significant differences were found between the pre and post MCT moments in EX (p<0.0001) and AE (p<0.0001) groups, which results in relation to this metabolite are similar to those obtained by Silvestre *et al*. (2017), who also performed an acute exercise session with a ladder model and progressive load increase.

The present study showed that the administration of Schinus Therebintefolius EE attenuated the concentration of these tissue damage markers, but for future studies, it is suggested to evaluate other fractions of extracts and possible effects (beneficial or not) on the organism. In conclusion *Schinus terebinthifolius* EE reduced oxidative and muscle damage markers in the tissues of rats submitted to high-intensity resistance exercise. Therefore, it is suggested as a possible strategy in the reduction of oxidative stress when in the association between medicinal plants and physical exercise in the acute form, mainly of high intensity, which can promote an elevation in these markers.

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**VIEIRA-SOUZA, L. M.; MARÇAL, A. C.; DOS SANTOS, J. L.; MARTINS, F. J. A.; DOS SANTOS, S. B.; SILVA, A. L. S.; REIS, G. C.; CARVALHO, G. F. S.; SOUZA, R. F.; SANTOS, D. T.; SOTO, D. A. S. & BRITO, C. J.** El ejercicio de resistencia de alta intensidad y la suplementación con *Schinus terenbinthifolius* atenúan los biomarcadores del estrés oxidativo y daño muscular. *Int. J. Morphol., 40(3)*:781-788, 2022.

**RESUMEN:** Los ejercicios físicos de alta intensidad pueden causar estrés oxidativo y daño muscular. Varias plantas medicinales se han utilizado como agentes antioxidantes y antiinflamatorios. El presente estudio evaluó el ejercicio de resistencia de alta intensidad (HIRE) asociado con el extracto etanólico (EE) de *Schinus terebentifholius* sobre los parámetros oxidativos y el daño muscular en ratas Wistar. Los animales se dividieron en 4 grupos (n=10/grupo): 1. Control (GC) - animales que no se sometieron a HIRE y fueron tratados con vehículo (agua destilada, por vía oral); 2. Ejercicio agudo (AE) – animales sometidos a sesión de ejercicio agudo; 3. Ejercicio + vehículo (EV) - animales que se sometieron a HIRE y fueron tratados con vehículo y 4. Ejercicio + extracto (EX) animales administrados con *Schinus terebenthifolius* EE (100 mg/kg, por vía oral) y sometidos a la sesión de ejercicio. *Schinus terebenthifolius* EE mostró una alta actividad antioxidante *in vitro* (13,88 ± 0,36 mg/mL). Antes del período experimental, se midió el lactato en los momentos pre y post de los grupos AE (p<0,0001) y EX (p<0,0001). Tras la sesión aguda, se evaluaron: el estrés oxidativo malondialdehído (MDA), grupos sulfhidrilo (SH)

y poder antioxidante reductor férrico (FRAP), daño muscular (creatina quinasa (CK) y lactato deshidrogenasa (LDH)), alanina aminotransferasa (ALT) y aspartato aminotransferasa (AST). En los análisis *in vivo* del grupo EX frente a los grupos AE y EV, respectivamente: hepático (MDA: p<0,0001 y SH: p=0,0033, en ambos; FRAP: p=0,0011 y p=0,0047), muscular (MDA, SH y FRAP: p<0,0001, en ambos; CK: p=0,0001 y p<0,0001; LDH: p<0,0001, en ambos), niveles séricos (MDA: p=0,0003, p=0,0012, SH: p=0,0056, p=0,0200, FRAP: p=0,0017 y p=0,0165) fueron significativas. No hubo diferencia significativa en los marcadores ALT y AST. Se podría concluir que *Schinus terebenthifolius* EE asociado con HIRE atenuó el estrés oxidativo y el daño muscular en ratas.

**PALABRAS CLAVE: Entrenamiento Físico; Suplementos a base de Plantas Medicinales; Producto natural; lesión oxidativa; Reducción del daño.**

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