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Original Article

Courbaril extract (*hymenaea courbaril l.*) improve the explosive strength more than monohydrate creatine and decreases the muscle damage

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Abstract

Problem statement: The Courbaril extract (*Hymenaea courbaril L*) has been used for several objectives including to improvement of the sexual and sportive performance in the Amazon region. However, no scientific data were displayed about the use of this natural product to sportive performance improvement. **Approach:** Fifteen participants participated In a experimental Study. They performed three experimental sessions, one day after the end of each supplementation protocol with 9 days of washout between then. First, the participants intake courbaril extract, in the second placebo, and in the third creatine monohydrate, all by five days before the experimentation. The exercise sessions were composed by warm-up included a gentle 5-minute walk in the university parking followed by dynamic stretching exercises with low intensity and less than 10 seconds of insistence in the final joint position. The main part of the exercise tests was composed by: (1) Pull-up (PULL); (2) Push-up (PUSH); (3) Sit-up (SIT); (4) 50m Sprint (50MS). Ever between 8 and 11am, blood samples were collected before and immediately after the physical test sessions to biochemical determinations. High-performance liquid chromatograph (HPLC) to characterization of the extract compound and DPPH to antioxidant effect *in vitro* were performed. ANOVA ONE WAY and Post Hoc Test of Tukey's with a significance of 5% were used. **Purpose:** to examine and compare the effects of courbaril extract (Hymenaea courbaril L.) with monohydrate creatine in muscular explosive strength of young adults. **Results:** Both supplements improved the performance if compared to placebo, however, courbaril extract was more effective than creatine in the pull-ups, push-ups, sit-ups, and the 50 meters sprints. The creatine kinase and lactate dehydrogenase were improved with courbaril supplementation immediately post the exercise challenge without reach pathological levels. In regard the explain the possible mechanism under our observations, the HPLC display a rich antioxidant compound in courbaril extract and the DPPH display a potent dose dependent antioxidant effect. Additionally, aromatases were found which can probably decrease the conversion of the testosterone in a female hormone. Both mechanisms have a potential relation with the observed improved performance. **Conclusion:** Both supplementations provoke enhancement on the performance of young adults, but the courbaril was more effective than creatine to muscle strength which allowed higher exposition to the high-level effort, explaining the increase of muscle damage-related enzymes observed like a pertinent overview of the work.

Key Words: Natural Products; Amazon Biome; Supplementation and Sportive Performance, Antioxidant Effect; Creatine and Performance.

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Introduction

Since the antiquity the humans are searching for way of improve the performance, and different possibilities have been described to positively influence, such as nutrients as that caffeine and taurine stand out for a central and peripheral activity (Chester, 2018; Rodrigues et al., 2022; Stavsky & Maitra, 2019). However, lack about the acute effect of short-exhaustive exercise need be investigated because, acute exercise in high intensity are present in several professions as Police, Fireman, or, to several sports as soccer, basketball players, or martial arts that before on he may be forced to make a quick decision to motor action, which is fundamental in many day-by-day, sportive, or professional tasks.

More in-depth studies on natural compounds have been performed by associating commonsensical information with the corroboration or not of the biological effect. In this context, plants should be investigated to provide further insights on their properties, safety, and biological efficiency. For example the caffeine has a large amount of studies displaying positive effects on physical and mental performance (Rodrigues et al., 2022), immunological and hematological health (Valentim-Silva et al., 2014; Wilk et al., 2019). Another natural products as, for example, BCAA, whey protein, and maltodextrin has been studied about their proprieties over the human performance, however, this field have many controverse results (D'Angelo et al., 2020; M. A. R. Da Silva et al., 2020), which indicates need of more investigations.

Hymenaea courbaril L.(courbaril tree), popularly known in Brazil as "Jatobá," is a tree whose roots, leaves, fruits and bark used in folk medicine to treat various types of diseases (R. Silva & Da, 2021). The plant of the courbaril tree is used as an anti-inflammatory agent, appetite stimulant and energy tonic, while the bark is used for treating coughs and anemia and the resin is used as a pectoral, a tonic and, in a higher dose, as a vermifuge (A. F. Silva et al., 2015).

Phytochemical studies about courbaril showed the presence of phenolic compounds, such as flavonoids, tannins, and terpenes, in the resin produced by the trunk, as well as in the extracts of the bark (Brunelli et al., 2014). These compounds have antioxidant activity which is mainly related to their reducing properties and chemical structure. They act by donating hydrogen atoms or electrons, thus transforming radicals into stable substances. Phenolic compounds also play an important role in neutralizing free radicals, moreover, they may act at the beginning or in the propagation of the oxidative process (Santos et al., 2017; R. Silva & Da, 2021). Therefore, in this context, the hypothesis of the present study is that the phenolic compounds of courbaril can improve exercise performance. In addition, the courbaril have an antioxidant effect due their flavonoid content, what, in according to recent evidence, are being associated at enhancements of the athletic performance (Iannone et al., 2019).

In another hand creatine has been widely researched and it has been the object of several reviews of the literature which have reported its efficacy and safety in sports practice. It has been well-established that creatine supplementation can increase strength performance and improve body composition measurements, through a reduction in body fat percentage and an increase in lean body mass (Antonio et al., 2021)Creatine is produced endogenously from three amino acids (i.e. glycine, arginine and methionine) and the synthesis begins mainly in the kidneys and is completed in the liver. Daily production of creatine is approximately 1-2 g with approximately 95% being distributed to the muscles. In addition, creatine is a non-essential dietary compound which is found mainly in red meat and seafood (Poortmans et al., 2010). Although the supplementation can enhance the performance, the effect of several compounds to the health is unclear, and, data under exercise situations are much scarce, that in fact, coats this work of importance (de Guingand et al., 2020; Kaviani et al., 2020).

Different studies have shown an increase in sarcolemma permeability with consequent releases of muscle enzymes when high-intensity physical exercises are performed (Fouré & Bendahan, 2017), which can be attenuated by natural compounds (Narciso et al., 2018). Thus, it can be assumed that the enzymes Creatine Kinase (CKMM) and Lactic Dehydrogenase type 5 (LDH5) can be considered indirect biochemical markers of muscle injury (Neubauer et al., 2008) is important to monitor this exercise-induced muscle injury, physical performance is impaired and thus, research is required on ergogenic and nutritional strategies that can affect both aspects (Bemben & Lamont, 2005). Studies have been shown that the natural compounds associated with the exercise can cause impairments in different tissues, such as in the white blood cells (Bassini-Cameron et al., 2007) which can be critical and turn the supplementation with Hymenaea courbaril L dangerous, and due to this, the investigation of the muscle and liver toxicity is crucial to determine the safety of this extract.

Within this context, the flavonoids have been shown to be endowed with many biological activities, leading to cytostatic, apoptotic, anti-inflammatory, anti-angiogenic, and hepatoprotective effects (Iannone et al., 2019; Middleton et al., 2000; R. Silva & Da, 2021) and attracting attention also as possible chemoprotective or chemotherapeutic agents (Rosenberg Zand et al., 2000). Nevertheless, their low concentration in food and non-optimal oral bioavailability, the effects of dietary flavonoids are moderately low and difficult of evaluation. Among them, isoflavones, often classified as "phytoestrogens" due to their weak estrogenic activity, may be of interest in sports for their estrogenic and/or antioxidant properties (Iannone et al., 2019). Considering the aspects here related, the objective of the present study is to examine and compare the effects of courbaril extract (*Hymenaea courbaril L.*) with monohydrate creatine in muscular explosive strength of young adults.

Material & methods

Participants

After collection of contact information and a preliminary conversation with those students, 392 potential respondents were short-listed for participation in the research. The previously selected individuals were asked to perform the physical exercises and undergo the anthropometric assessments proposed in the present study.

The objective was to select only volunteers with similar results in the exercises for strength, speed, body fat percentage and lean body mass to yield a lower standard deviation in the sample. Exclusion criterion included use of ergogenic resources, lower limb injuries which totally or partially prevented the execution of the exercises, being a smoker, use of anabolic steroids, being a vegetarian and/or following any dietary restrictions. Thus, 15 volunteers, male, self-reported as physically active were selected. All the volunteers were enrolled in strength training exercise for at least one year, and they had a medical history interview. The figure 1 summarize the selection procedures.

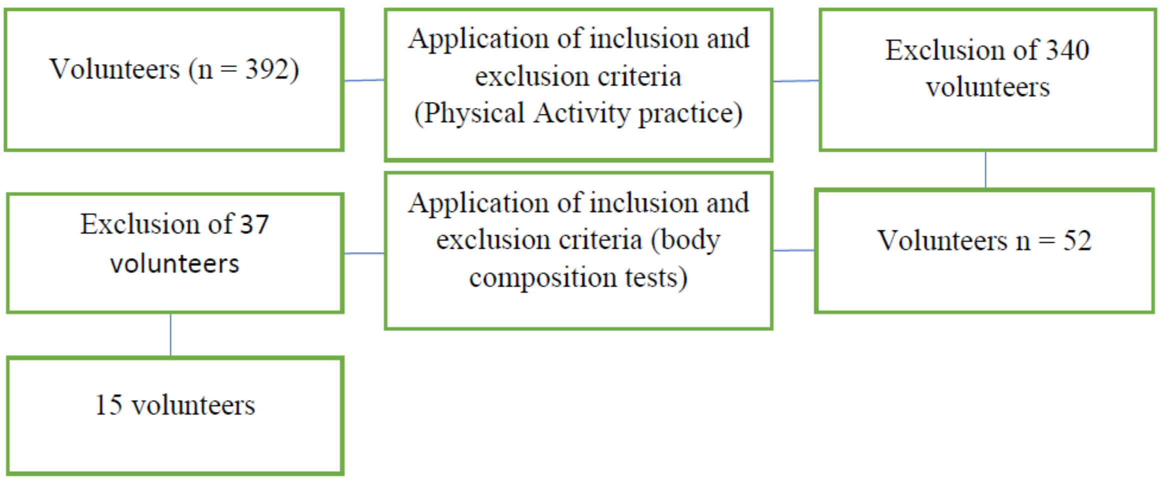


Figure 1: Volunteers selection scheme. From 392 volunteers was submitted at inclusion and exclusion physical performance criteria and 340 were excluded and the least 52 volunteers were submitted at the body composition inclusion and exclusion criteria, and after excluding 37. The final volunteer's number after the application of all criteria of selection were 15 volunteers.

The anthropometric characteristics of the participants were acquired and exposed in the table 1. Body mass and stature were obtained by means of a Filizolla mechanical platform scale with an accuracy of 0.1 kg. Based on these measurements it was possible to calculate the body mass index using the equation 1. The methodology of these conjunct of experiments was previously described (de Oliveira et al., 2017; Lameira-de Oliveira et al., 2018; Marques et al., 2018; Nogueira et al., 2014).

$$BMI = \frac{Body\ mass}{Height\ x\ Height} \quad (1)$$

Equation 1: Calculi of the body mass index. The body mass in kilograms divided by the the height squared.

Variable	M±SD
Age (years)	22.83±2.33
Height (cm)	173±0.03
Weight (kg)	72.79±6.49
BMI (kg/m^2)	24.16±1.95

Table 1: Anthropometric characteristics of the participants of the present study. Values in mean and standard deviation.

Experimental design

This investigation was quasi-experimental with use of placebo because have control group, experimental group, but, although the group composition followed a very criterious procedure, the acquisition of the participants follows a convenient way.

In regard the experimental proceedings, first, the participants became familiar with the warm-up protocol used before all experiments. Warm-up included a gentle 5-minute walk in the university parking followed by dynamic stretching exercises with low intensity and less than 10 seconds of insistence in the final joint position. The volunteers performed the proposed exercises in a gym and in flat area demarcated in the parking lot at the Federal University of Acre (UFAC), on separate days, in the Gym of this same university ever at the same order, as follows: (1) Pull-up (PULL); (2) Push-up (PUSH); (3) Sit-up (SIT); (4) 50m Sprint (50MS) ever between 8 and 11am. Blood of the volunteers was sampled before and immediately after the exercises, in all the meetings. They were instructed to ingest either the supplement or the placebo, according to the previously established dosage, times and days. The figure 2 show and explain the experimental design of this study.

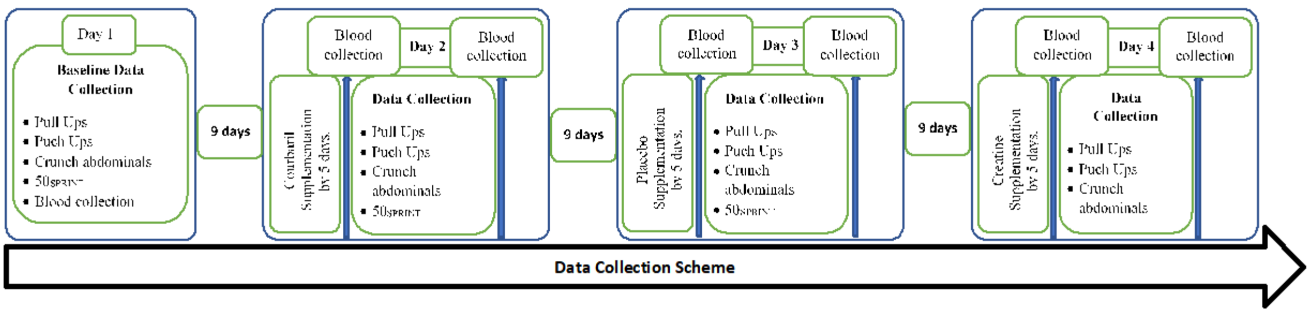


Figure 2: Scheme of data collection. Volunteers (n=15) were submited at four days of data collection where (FPU; A), push ups (GPU; B), crunch abdominals (CA; C) number of repetitions and (D) 50 meters sprint performance (50SPRINT). The first day (Day1) was used as the baseline data, nine days after, the Jatobá supplementation was biggened and after five days new data collection was performed (Day2). In follow of nine days, the supplementation with placebo (Maltodextrin) was done by five days, and in the day immediately after new data collection were performed (Day3). Finally, after nine days, the supplementations with creatine by five days was done and, in the day, immediately after, the least data were acquired (Day 4). Blood sampling were acquired before and after the exercise challenge.

In regards to the supplementation protocol and controls, although discussion about the creatine effect on muscle power has been performed, a recent work concludes that mono-hydrated creatine is the best choice because while some forms of creatine may be more soluble than creatine monohydrate when mixed in fluid, evidence-based research clearly shows creatine monohydrate to be the optimal choice, and a variety of athletic events, including resistance/power activities (Antonio et al., 2021), which may benefit from creatine supplementation which justify our choice by creatine to comparison with Jatobá. However, the choice by maltodextrin followed the rationale that this supplement could alter the glucose availability, and in consequence, their metabolism, which, in part could increase the muscle fuel to power activities.

The Participants were instructed to follow a feeding routine guided by a nutritionist and distributed according to Table 2. Is important comment that this feeding routine do not break the routine of the volunteers.

Breakfast	Lunch	Afternoon snack	Dinner
Coffee, fresh milk or Fruit juice, bread, cheese, ham	Salad (ad libitum), rice, beans, pasta and steak	Fruits for free choice	Vegetable's soup

Table 2: Feeding Routine.

The participants were also instructed to perform a standard physical exercises routine, organized per joint methodology called agonist/antagonist because have the exercise to agonist muscle and, in follow, one exercise to their antagonist muscle [21]. The objective was based in a hypertrophy objective, with intensity between 65%-85% of the maximal power output, composed by 4 Sets, and with 90 seconds of recovery between sets. To assess more details of the training, see the supplementary material (Supplement1).

Supplementation

Supplementation was provided at three moments. In first moment, with five days' length, the participants followed a diet of 100mL of courbaril extract per day, administered in two doses of 50 milliliters (ml), according to popular usage, coinciding with the main meals (lunch and dinner). In second moment, with five days' length, the participants followed a diet of 20 grams (g) of maltodextrin (placebo) (Altimari et al., 2010; Carvalho, Molina, et al., 2011; Carvalho, Rassi, et al., 2011; Outlaw et al., 2014; Pereira et al., 2012), administered in doses of 5 grams (g), coinciding with the main meals (breakfast, lunch, afternoon snack and dinner). Maltodextrin was ingested after dilution of the container in 250 ml of water. In third moment, creatine monohydrate supplementation was used, following the same protocol as placebo.

Exercise challenge

The protocol for execution of the pull-up exercise was performed as described. The horizontal bar was installed at enough horizontal height so that the volunteers could be suspended with extended elbows and avoid touching their feet on the ground. They used a pronated grip, with hands separated at a distance like the biacromial distance. The volunteers were instructed to raise their body by means of elbow flexion, until they brought their chin over the level of the bar. Then, they returned to the initial position. In that occasion, they were considered to have completed one repetition. The test was dynamic; hence, volunteers were not allowed to interrupt between repetitions in order to rest (MILITAR & GERAL, 2014).

According to the protocol for execution of the pull-up exercise, the volunteers had to lie flat on the floor in prone position, hands outstretched and resting on the ground, arms outstretched with hand separation being a little greater than biacromial width, legs extended and pulled together and toes touching the ground. Upon hearing the voice command "Start", volunteers were supposed to flex their elbows and raise their chest to approximately 5 cm above the floor. No contact between the body and the floor was permitted, except for their toes and the palms of their hands. Next, they had to extend their elbows completely, an occasion in which they will have completed an exercise (MILITAR & GERAL, 2014).

According to the protocol for the execution of the sit-up exercise, the volunteers had to lie on the floor in supine position, with knees flexed and arms crossed at chest height, so that their right hand could hold their left shoulder and their left hand could hold their right shoulder, with external support on the dorsum of their feet. Upon hearing the voice command "Start", the volunteers were supposed to perform sit-ups with their forearms touching their thighs and their scapula touching the floor, thus completing one exercise. Then, they could initiate the implementation of a new repetition (MILITAR & GERAL, 2014).

The protocol for execution of the 50 meters running (C50Ms) exercise began after the volunteers were facing the runway and got into the starting stance, without contact between their upper limbs and the floor. At the start signal, volunteers were instructed to run as fast as possible for 50 meters in a straight line. Disqualification factors included doing a false start, exceeding the time limit, and having occasional accidents (falls) which could prevent the completion of the test (Noll et al., 2013).

Antioxidant Potential of *Hymenaea courbaril L.*

The extract was submitted to subjected to the sweep test of the radical 1,1-difenil-2-picrilhidrazil (DPPH), performed in accordance to Brand and Williams (Brand-Williams et al., 1995) with modifications to use in places in 96 well plates. This method is based in the electrons transfer phenomenon by a free radical antioxidant, o DPPH, with purple color, is reduced and form difenil-picril-hidrazina, with yellow color and consequent decreasing of the absorption (Noll et al., 2013).

The anti-radical activity determination was performed by the Radical 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) sweep test, which was based on the methodology previously described by Re et al. (Zakaria & Nasr-El-Din, 2015), with small modifications so that the test was also carried out in a 96-well microplate. After the addition of potassium persulfate, the formation of the ABTS radical, which has a greenish color, has occurred. As far as the antioxidant is mixed with this radical, the reduction of ABTS occurs, causing the loss of coloration of the reaction medium. The results are expressed as a function of the Trolox standard submitted to the same analysis conditions.

The Radical Anion Superoxide ($O2^{\bullet -}$) scanning assay was performed using the method developed by Ewing and Janero (Ewing & Janero, 1995), where sweeping activity is generated during the metabolism of aerobic organisms, either as final products of enzymatic reactions or accidentally as secondary cellular products of redox reactions. In this assay, the superoxide radical anion ($O2^{-}\bullet$) is generated by the PMS-NADH system (Phenazine methosulfate and Nicotinamide Adenine Dinucleotide), reduced by the oxidation of Nitro Blue Tetrazolium.

The dosage of total phenols was quantified by the method described by Singleton and Rossi. The measurement of total flavonoids was done using the method described by Zhishen, Mengcheng, Jianming (Zhishen et al., 1999), with the objective of evaluating the possible antioxidant potential of the sample.

Side effects assessment

During the study, a questionnaire about side effects was applied after the first meeting (courbaril supplementation), the second meeting (placebo supplementation), and the third meeting (creatine monohydrate supplementation). The questionnaire was administered to determine as follows: (i) if the participants experienced any adverse symptoms during the supplementation period and (ii) whether the participants followed the supplementation protocol. In case they present any adverse symptoms, participants were asked to classify the frequency and severity of symptoms, dizziness, headache, tachycardia, insomnia, gastrointestinal distress, and unusual or adverse effects. They also were asked to classify their symptoms with 0 (none), 1 (minimum: 1-2 times), 2 (regular: 3-4 times), 3 (average: 5-6 times), 4 (severe: 7-8 times), or 5 (very severe: 9 or more times) (Galvan et al., 2016), a 24-hour food recall was performed to assess whether volunteers followed the diet suggested during the study period, and those who failed to follow the recommendations were removed from the study.

Blood sampling and analysis

Blood sampling was performed in a room particularly prepared for the present study. Volunteers were instructed to remain seated throughout the material collection procedure. At each collection, two blood samples were collected immediately before the start of the exercise challenge and immediately after in EDTA tubes (4 mL vacutainer tube, BD Vacutainer, Franklin Lakes, NJ, USA) and stored in a refrigerated container (4 °C) until measurement, which was performed in all cases within 1 h after venipuncture.

After collection, the blood was deposited in a suitable container for transport, and immediately taken to the Biochemistry Laboratory of União Educacional do Norte - UNINORTE, Rio Branco - Acre, where it was centrifuged (Basic® / Sislab Tecnologia Laboratorial Ltda - Brazil) to separate the serum. To determine the levels of γ-glutamyl transferase (γ-GT), Creatinine, Muscle Creatine Kinase (CKMM), Lactic Dehydrogenase type 5 (LDH5). All analyses used reagent kits (Labtest Diagnóstica SA - Brazil), and a semi-automatic biochemistry analyzer (Bioplus-200S) before calibration day-by-day of experiments.

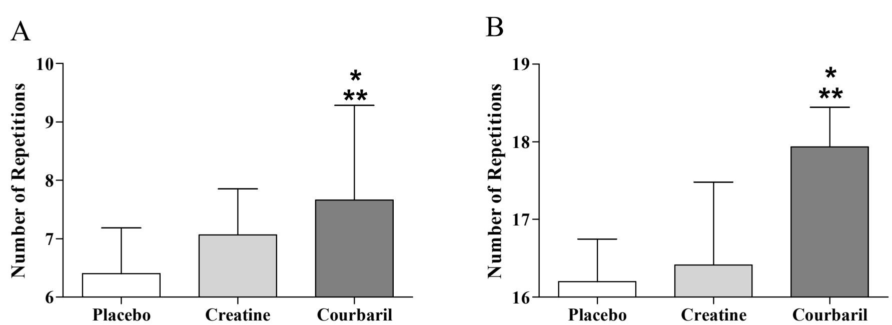
Statistical analysis

The data were analyzed using the software GraphPad Prism® version 5.0 (GraphPad, USA). Normality was conducted by performing the Shapiro-Wilk test. The data were presented as mean and standard deviation and compered by Student's t-test for parametric variables or Wilcoxon test was used to compare continuous within-group outcomes in symmetric distribution with homogeneity of variances and in symmetrical distribution with heterogeneity of variances, respectively. One-way analysis of variance (one-way ANOVA) was used to evaluate possible differences between groups over time (before and after supplementation) with Tukey's post hoc test. The Kruskal-Wallis test and Dunn's post-test were run to compare the groups together. When the test indicated differences between the groups, the Scott-Knott test was run to check these differences. According to (Borges & Ferreira, 2003), the Scott-Knott test is robust to violations of normality. All tests were set up at 5% of significance.

Results

Courbaril supplementation promote more improvement on physical performance than creatine

The analysis of the pull-up exercise (Fig. 3A) display that the supplementation with courbaril promote higher performance (7.66±2.92) than placebo (6.40±3.04) ($p<0.0002$) and creatine (7.06±1.67) ($p<0.03$). In regarding to push-up exercise (Fig. 3B) the courbaril supplementation improved the performance (17.93±1.98) to placebo (16.20±2.11) ($p<0.0007$), and creatine (17.27±2.31) ($p<0.01$). In relation the sit-up exercise (Fig. 3C) the supplementation with courbaril improve the performance (12.87±2.44) to placebo (11.27±2.25) ($p<0.0001$) and creatine (11.27±2.25) ($p<0.0007$). To the C50Ms exercise (Fig. 3D) the group when supplemented with courbaril reached (7.31±0.55) displaying more performance than placebo (7.74±0.59) ($p<0.0001$), and creatine (7.47±0.51) ($p<0.01$).



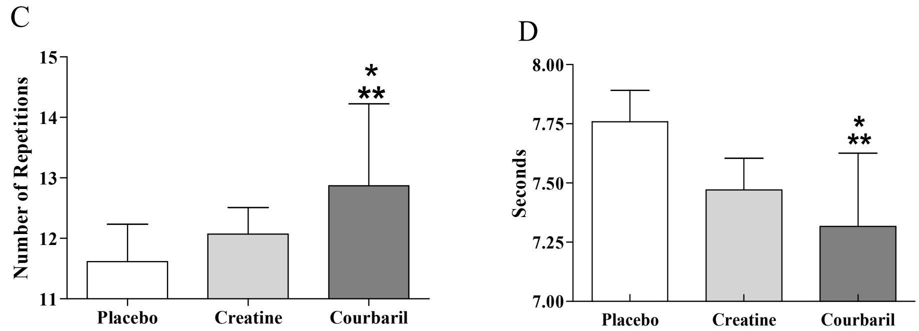


Figure 3: Exercise performance tests. Fifteen volunteers were submited at four days of data acquisition. All graphs are normalized, and the line y is the baseline performance of the group. (A) pull-up, (B) push-up, (C) Sit-up, (D) 50 meters running. The placebo supplementation (open bar), creatine (middle bar), and Jatobá (closed bar). The Wilcoxon Test followed by Dunn's and ANOVA ONE followed by Tukey's both tests set up at 5% of significance. Data are presented in mean±SD.

Liver profile (Figure 4A), GGT pre-exercise and post-exercise was different form each other creatine was higher than placebo ($p<0.0005$). Muscle profile (Figure 4B), CKMM post-exercise was different from each other creatine was bottom than courbaril and placebo ($p<0.05$). LDH5 (Figure 4C), post-exercise was different from each other creatine was bottom than courbaril ($p<0.001$). Kidney profile (Figure 4D), creatinine (Cr) pre-exercise and post-exercise was different from each other courbaril was bottom than creatine ($p<0.05$).

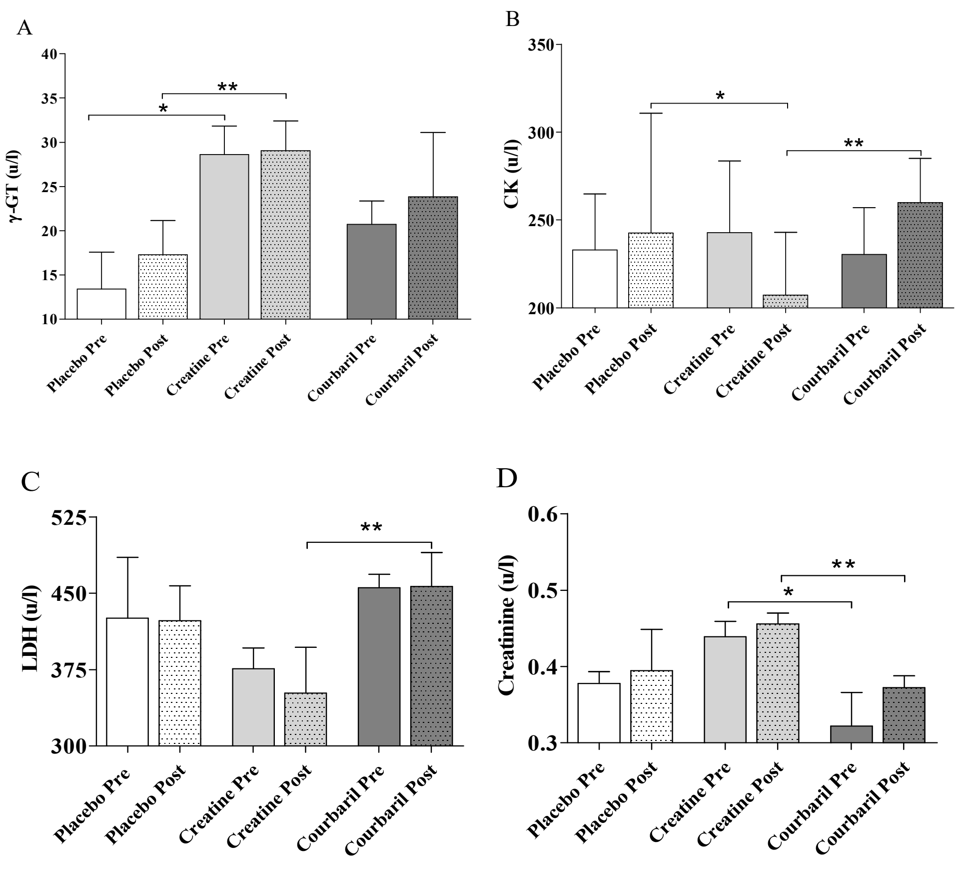


Figure 4: Liver, kidney, and muscle enzyme quantification. Fifteen volunteers were submitted at four days of data acquisition. (A) γ-GT, (B) creatine kinase, (C) Lactate dehydrogenase, and (D) Creatinine. The placebo supplementation (open bar), creatine (middle bar), and Jatobá (closed bar). The Wilcoxon Test followed by Dunn's and ANOVA ONE followed by Tukey's both tests set up at 5% of significance.

The courbaril extract have a dose dependent antioxidant effect

The courbaril extract showed that at 50µg of concentration 83.54±1.86%, at 25µg 71.01±1.55%, at 12.50µg 58.61±4.56%, and at 50.11±2% of antioxidant activity.

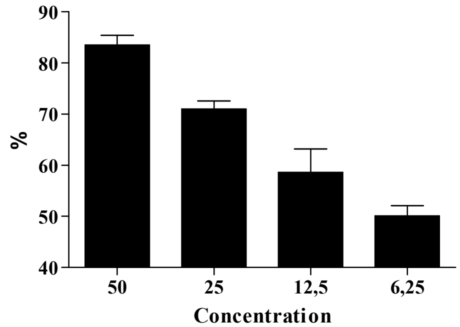


Figure 5: The antioxidant effect of the courbaril. The extract was submitted to subjected to the sweep test of the radical 1,1-difenil-2-picrilhidrazil (DPPH). The anti-radical activity determination was performed by the Radical 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) sweep test in a serial concentration from 50µg, 25µg, 12,5µg, and 6,25µg. The Wilcoxon Test followed by Dunn's set up at 5% of significance. Data are presented as mean±SD.

Discussion

This is the first study which that examine and compare the effects of courbaril extract (*Hymenaea courbaril L.*) with monohydrate creatine in muscular explosive strength of young adults. The main finding of this study display that the courbaril extract, when used as an ergogenic resource, improved the explosive performance of all the exercises proposed, in comparison to placebo. Is very important note that the placebo like control of this study was done by Maltodextrin. This fact is important because help to exclude the discussion about the glucose availability and the effect of this supplement on the first anaerobic moment of the energetic metabolism, what could have influence on the explosive muscle power output and strength, what, in fact is the interest of this investigation. If compared with creatine monohydrate, the results are very interesting due to the clear advantage of the courbaril extract supplementation over another two supplements.

There was improvement of creatine in the pull-up, sit-up and 50MS exercises, although there was a trend of improvement in mean of repetition in the sit-up exercises. The hypothesis formulated in the present study is that the improvement in performance of the group supplemented with courbaril can be related to flavonoids due the antioxidant effect here observed (Fig 4). For example, they are antioxidants which delay or inhibit the formation of reactive oxygen species (ROSs) (R. Silva & Da, 2021). During intense exercise, ROS accumulate, and the antioxidant defense system may not be able to minimize the deleterious effects induced by explosive-strength exercises. As a result, there occurs redox imbalance which has proven to cause impairment of the skeletal muscle, thus leading to peripheral muscle fatigue (Fransson et al., 2018).

Previous studies have reported that courbaril has a high content of flavonoids (Bermejo et al., 2021), that may promote redox balance, reduce muscle fatigue (Ives et al., 2017), and, perhaps, enhance the physical performance. To assess the flavonoids content of the courbaril, a high-performance liquid chromatograph (HPLC) was performed to determinate the composition of the plant extract. These compounds may have effect that can explain the enhancement of the athletic performance. These effects were reported in previous studies (Iannone et al., 2019; Lecompte et al., 2014). Perhaps, one between several effects related to the flavonoids supplementation on sports could be the androgen promotion because their proprieties of inhibition of aromatase (CYP19) (Pelissero et al., 1996; Ta & Walle, 2007) that is the catalytic enzyme that convert of androstenedione and testosterone to estrone and estradiol, respectively.

The estrogenic/antiestrogenic activity of isoflavones may also be due to an alteration of biosynthesis and subsequent secretion of luteinizing hormone (LH), that acts stimulating the production of testosterone in male testicles, and that it is therefore included in the Prohibited List of the World Anti-Doping Agency (WADA), in section S2 (Peptide hormones, growth factors, related substances and mimetics) (Kinahan et al., 2017).

Previous studies have suggested that Creatine supplementation improves performance in short-duration high-intensity exercises (Candow et al., 2019). Therefore, "trained" individuals that make use of creatine, when submitted to short-duration high-intensity efforts in a single set, may use their phosphocreatine (PCr) reserves for ATP resynthesis (Kaviani et al., 2020). Other studies reported improvements in explosive performance with the use of creatine (Tyka et al., 2015), thus corroborating the results of the present study in all exercises, in which the treatment with creatine supplementation was different from the placebo ($p < 0.05$).

As regards to the metabolization of the supplementation here proposed, the liver functioning was investigated. So, the γ-GT (Figure 2 A) showed no difference between groups (pre-treatment vs. post-treatment). In contrast, within groups, the treatment with courbaril presented lower explosive performance than the treatment with creatine, suggesting possible liver protection, but no statistical difference was detected. There were no studies in the literature that support this statement, and there was no change in the clinical patterns of normality. The treatment with creatine proved to be different from the Placebo treatment, without significant clinical changes corroboration with another studies (Baracho et al., 2015).

Total CKMM (Figure 2 B) showed no difference within groups in the pre-exercise treatment, while in the post-exercise one, the group that was supplemented with creatine was different from the other treatments. This finding suggests muscle protection, as the use of supplementation of 5g of creatine for five days with 20g/day can maximize the concentration of creatine and muscle phosphocreatine(Van Loon et al., 2003). The parameters for CKMM are within the clinical patterns of normality. In another hand, the total volume of exercise reached by the group supplemented by courbaril was higher what can explain the improvement of the release of the muscle damage markers if compared with the Placebo.

Here was identified that lactate dehydrogenase (LDH5) was different, showing reduction of LDH5 after the use of creatine supplementation and the practice of high-intensity exercises. A similar result was found in the literature (Pereira et al., 2012). In another study, there was no change in LDH5 levels before and after creatine supplementation when high-intensity exercises were performed (Altimari et al., 2010). The supplementation protocol including a maintenance phase in addition to the loading phase (unlike the present study), which probably influenced the parameters for LDH5 (Pereira et al., 2012).

In part, these observed phenomenon's can be explained by the same probably proprieties of this extract have in the promotion of the athletic performance, the antioxidant effect due their flavonoid content. The flavonoids were previously related to anti-inflammatory, anti-angiogenic, and hepatoprotective effects (Arkhipov et al., 1997; Middleton et al., 2000) what can be relationship to the here observed on γ-GT and lactate dehydrogenase release (Figure 4A, 4B respectively).

The analysis of creatinine was made because they are indicators of renal function (Robinson, 2000). Creatinine (from the creatine treatment) increased after the supplementation period compared to Placebo treatment, this increase did not exceed the index normality, and it was not considered to be clinically significant. This result is in accordance with the literature (Carvalho, Molina, et al., 2011). In another hand, within pre- and post-exercise groups with the courbaril treatment, creatinine decreased after the supplementation period when compared with the other treatments. This was significant as regards the creatine treatment, thus indicating possible renal protection. However, no data were found the literature that can support this hypothesis.

Conclusions

Here were investigated the effect of the supplementation with *Hymenaea courbaril L* on athletic performance, and markers of muscle, and liver injure in comparison with a classical supplement, the monohydrate creatine. The data reported in this study support the hypothesis that both courbaril and creatine improve physical performance of young adults in explosive-strength exercises. Creatine supplementation, through physiological mechanisms, increase the stock of creatine and intramuscular phosphocreatine, thus optimizing the processes of ATP synthesis and resynthesis, aspects which are well-defined in the literature. Courbaril (*Hymenaea courbaril L.*) is a potential natural ergogenic resource because its antioxidant properties, well-established in the literature (*in vitro*), may have delayed or inhibited the formation of ROS, hance improving redox balance and decreasing muscle fatigue. Additionally, we displayed that this supplementation do not is hepatotoxic, which could be a problem due to the hepatic metabolization of this compound. Although more investigations are suggested, we infer that the courbaril extract can be used to improve the athletic performance without problems. However, further studies are needed for characterization of the activity of the antioxidants of *Hymenaea courbaril L.*, on body metabolism, testosterone, because it is a complex system.

Conflicts of interest - All authors declare no conflict of interest.

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