ORIGINAL ARTICLE

Brazilian Amazonian Jatobá sap (*Hymenaea Courbaril L.*) as a hormonal modulator in soccer athletes

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ABSTRACT

To investigate the effects of Jatobá sap for eight weeks as an immunometabolic modulator in male soccer athletes. Phase 1: Phytochemical characterization (HPLC); Phase 2: (Jatobá) x (placebo). C-reactive protein (CRP), creatine phosphokinase (CPK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), urea, creatinine, cortisol, and testosterone were evaluated. The present study identified propelargonidin dimer and the flavonoids proanthocyanidin dimer, proanthocyanidin trimer, catechin, taxifolin, and caffeoylquinic acid glycoside. The placebo group showed a significant decrease in testosterone levels after the game about the pre-game time, a fact that did not occur in the supplemented group. Also, the supplemented group showed a significant increase in the levels of this variable in the 24-hour time about the post-game time, which was not observed in the placebo group. Regarding cortisol, both groups showed an increase in the post-game time with a return to basal levels in 24 hours. However, the stress of the match caused the placebo group to increase blood concentrations of this hormone by 150.5%, while the supplemented group increased blood concentrations by 59.3%. Supplementation modulated the testosterone/cortisol hormonal balance, with significant anabolic control between the groups after the match, and increase testosterone, reduced cortisol levels, and positively modulated the testosterone/cortisol ratio.

Keywords: Sport medicine, Amazonian plant, Immunometabolism, Sportomics, Health.

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INTRODUCTION

There are cyclical sports, such as running, cycling, and swimming, and acyclic sports, such as soccer. In this high-intensity acyclic sport, immunometabolism is required to the maximum, impacting the athlete's performance and health (Oliveira *et al.*, 2017). A promising approach in this context is using ergogenic resources, such as natural compounds with anti-fatigue and anti-inflammatory properties (Mucha *et al.*, 2021).

Previous studies have shown that oxidative stress generated by physical exertion can lead to complications such as fatigue, inflammation, and muscle injuries in athletes (Taherkhani *et al.*, 2021). In this scenario, plant-based antioxidant compounds have demonstrated the ability to offer protection against oxidative damage and inflammation (Maury *et al.*, 2020). Therefore, the intersection between different forms of sport and exercise methods and the action of natural compounds has aroused increasing interest in research into health and human performance (Nieman; Wentz, 2019).

In this context, the sap of the Jatobá tree, which belongs to the Fabaceae family, is a natural compound that has attracted attention for its antioxidant and anti-inflammatory properties, such as Propelargonidin dimer, proanthocyanidin dimer, proanthocyanidin trimer, catechin, taxifolin and caffeoylquinic acid glycoside (Boniface; Ferreira; Kaiser, 2017; Costa et al., 2021A; Costa et al., 2021B). In addition to other functions, these antioxidant compounds slow or inhibit the formation of reactive oxygen species (ROS) (Costa *et al.*, 2021A; Costa *et al.*, 2021B). ROS accumulates during intense exercise, and the antioxidant defence system may be unable to minimize its deleterious effects. As a result, a redox imbalance occurs that can cause impairment of the musculoskeletal system, thus leading to peripheral muscle fatigue (Fransson *et al.*, 2018).

The present study aimed to investigate the effects of eight weeks of ingestion of the sap of the Brazilian Amazon Jatobá tree as a modulator of biochemical and hormonal markers in male soccer players.

MATERIAL AND METHODS

Type of scientific research

This is a randomized, placebo-controlled clinical trial, with a quantitative approach, previously approved by the Research Ethics Committee (REC) of the União Educacional do Norte (UNINORTE), under opinion no. 5,159,860 and CAAE: 53503321.0.0000.8028 and following the standards present in resolution 674/2022.

Participants

Thirty male professional soccer players from a team in northern Brazil participated in the study. The selected participants were randomly divided into two groups of 15 people each, with one group using 50ml of Jatobá sap per day for 8 weeks (age 27.5±3.65) and the other group using a placebo (maltodextrin) 50ml per day for 8 weeks (age 24.8±3.43). Maltodextrin has been used as a placebo to study the effect of a wide variety of interventions, especially dietary supplements (Moreno-Pérez *et al.*, 2018).

This dosage of Jatobá is chosen following popular use by riverside dwellers and Indigenous peoples in the northern region of Brazil, more specifically in the State of Acre, in the city of Rio Branco.

In the first meeting, the groups received instructions on ingesting 50 ml of Jatobá (JB) and/or placebo (PLA), which was administered orally immediately before the start of each training session for eight weeks. Creatinine (CCR), Urea (URE), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma

Glutamyl Transferase (GGT), Creatine Phosphokinase (CPK), Lactate Dehydrogenase (LDH), Total Testosterone (TT), Cortisol and C-reactive Protein (CRP) were analysed, always before the start of the game, immediately after the game and 24 hours later.

Athletes who were physically fit to participate in training and games without symptoms or diagnosis of asthma, interstitial lung disease, cardiovascular, neuromuscular, or orthopaedic impairment were included. On the other hand, individuals who did not meet the inclusion criteria or were using other ergogenic resources, medications, or xanthines were excluded. After being informed of the risks and benefits of this research, all individuals signed the informed consent form (ICF).

Experimental design

1st Meeting	Presentation of the project and signing of the Free and Informed Consent Form (TCLE).
2nd Meeting	Collection of anthropometric data before the start of the season.
3rd Meeting	Ingestion of the first dose of Jatobá/Placebo and start of season training.
4th Meeting	After eight weeks of training and ingestion of Jatobá/Placebo, blood was collected in fasting conditions and immediately before the match (T1), immediately after the match (T2) and 24 hours after the game (T3).

Figure 1. Experimental design.

Extraction of Jatobá sap

The extraction of the sap of the species *Hymenaea Courbaril L.* followed the protocols of the good practices guide for extracting Jatobá sap (IPAM/USAID, 2011). The Chico Mendes extractive reserve has already adopted these protocols after authorization from Ibama under no. 57286-1. The sap was collected for exsiccation under the following GPS coordinates – 9º59,50,67º59,2,2ws and identified at the Escola da Floresta with the help of the Botanist A.D.P. SOUZA and deposited in the herbarium of the Federal University of Acre (UFAC) with registration number UFACPZ 20025, Sisgen BF45A2.

Delimitation of the initial state

All subjects came to the laboratory before the start of the training season to collect anthropometric data. Data were obtained using an Inbody® 120 bioimpedance scale and a Sanny® ES2030 Standard stadiometer and will be presented in the results section.

Intake protocol

The intake consisted of 50ml per day for eight weeks of JB and/or PLA, considering the average weight of the sample in Table 2 in the results section, which is 78.5 kg for the control group and 79.7 for the Jatobá group.

The dosage used by riverside dwellers and indigenous people in the northern region of Brazil, more specifically in the state of Acre, in the city of Rio Branco, is between 50ml and 100ml per day. However, in a study published in 2022, the researcher used two doses of 50ml per day to evaluate the explosive strength of his volunteers (Cavalcante *et al.*, 2022), which justifies the choice of dose. The intake was supervised by a nutritionist participating in the project team and administered orally always immediately before the start of training. The athletes participating in the research declared that they were not using any other type of supplementation and that they followed a traditional regional eating routine as instructed by the team nutritionist, with the same diet for the entire team. For greater control of the data, the exact times of day were respected for collection and intake.

Side effects

A side effects questionnaire was applied every day before and after training during the research. The objective was to determine the volunteers' feelings regarding tolerance to the intake of JB or PLA, as well as to know if the volunteers followed the intake protocol and if they presented any adverse symptoms during the intake period, also classifying the frequency and severity of symptoms such as dizziness, headache, tachycardia, insomnia, gastrointestinal discomfort, and unusual or adverse effects, using the following parameters: no symptoms with 0, 1 (minimum: 1-2 times), 2 (regular: 3-4 times), 3 (medium: 5-6 times), 4 (severe: 7-8 times), or 5 (very severe: 9 or more times). Those who could not follow the protocol would be removed from the research. All individuals were monitored by the project nutritionist via phone calls and messaging app, thus ensuring that there were no adverse symptoms during the intake period.

Blood collection and analysis

A qualified nursing professional collected blood in a room prepared for the study. The individuals remained seated throughout the entire procedure. The blood was collected (± 5 ml) in the cubital fossa under vacuum in collection tubes with a clot activator (silica) sandblasted on the walls. Disposable vacuum collection needles were used.

After collection, the blood was deposited in a container suitable for transport and immediately taken to the Bionorte Biochemistry Laboratory. For the measurement of creatinine, the Labtest colorimetric kit (Reference 35; MS 10009010034) was used, for urea, the Labtest colorimetric-enzymatic kit (Reference 27; MS 10009010011) was used, for AST, the Labtest kinetic kit (Reference 109; MS 10009010018) was used, for ALT the Labtest kinetic kit (Reference 108; MS 10009010029) was used, for CPK the Labtest kinetic kit (Reference 117; MS 10009010019) was used, for GGT the Labtest kinetic kit (Reference 105; MS 10009010004) was used, for LDH the Labtest kinetic kit (Reference 86; MS 10009010056) was used, for PCR the Labtest kit Turbiquest Plus (Reference 331; MS 10009010198) by turbidimetry, for Testosterone the electrochemiluminescence method (ECLIA) was used, Labtest kit (Reference 4027; MS 10009010399), for Cortisol the electrochemiluminescence method (ECLIA) was used, Labtest kit (Reference 4063; MS

10009010399). All analyses were performed on the "*Cobas Integra 400 Plus Analyzer and Cobas 411 Analyzer*". The determinations of laboratory procedures were provided by the responsible biochemists of the laboratory itself, and all protocols were following the manufacturers' recommendations.

Data analysis

After organizing the data using "*Data Wrangling*," descriptive statistics of the variables were performed, with measures of position (mean, median, and mode), dispersion (deviation, standard error, and variance), and, finally, measures of shape (asymmetry and kurtosis). For comparisons between times and groups, since the variance in the biomarker values of this population is unknown, it is necessary to apply comparison tests for paired groups, adopting a significance level of 5%, $p < .05$ (error α). For this, the Shapiro-Wilk test was initially applied (due to n = 30 participants) to verify the normality between the data, which could result in two possibilities: A – If the value obtained was $p < .05$, the Mann-Whitney non-parametric test would be applied for two means and the Kruskal-Wallis non-parametric test for three or more. B – If the value obtained were $p \geq .05$, the Equal Variance Test would be applied, and if this test failed ($p < .050$), the Mann-Whitney non-parametric test would be applied for two means and the Kruskal-Wallis non-parametric test for three or more. However, if this test passed ($p \geq .050$), the paired T-Test would be applied for two means or the ANOVA for three or more.

Due to a predominance of non-normal distribution for the database (variables), non-parametric association methods were selected, which, since they are quantitative data, were the Spearman correlation, the elbow method for determining the ideal number of clusters, and the distance dendrogram by the Euclidean similarity index (agglomerative hierarchical cluster).

The graphs were presented with mean and standard deviation. SigmaPlot 14.5 (Academic Perpetual License—Single User—ESD Systat® USA), R and R Studio (Free version for Windows), and Past 4.03 (Free version for Windows) were used to carry out the different statistical tests and produce the graphs.

Calculations

Cohen d

$$d = \frac{X1 - X2}{\sqrt{(S_1^2 + S_2^2)/2}}$$

d = 0.20 (Small); d = 0.50 (Medium); d = 0.80 (Large)

Percentual variation

$$\Delta\% = \frac{(final - initial)\ x\ 100}{initial}$$

Z score

$$Z = \frac{Individual\ score - Average}{Standard\ Deviation}$$

Euclidean similarity index

$$ED = \sqrt{\sum_j K = 1\ (ZXjp - ZXjq)2}$$

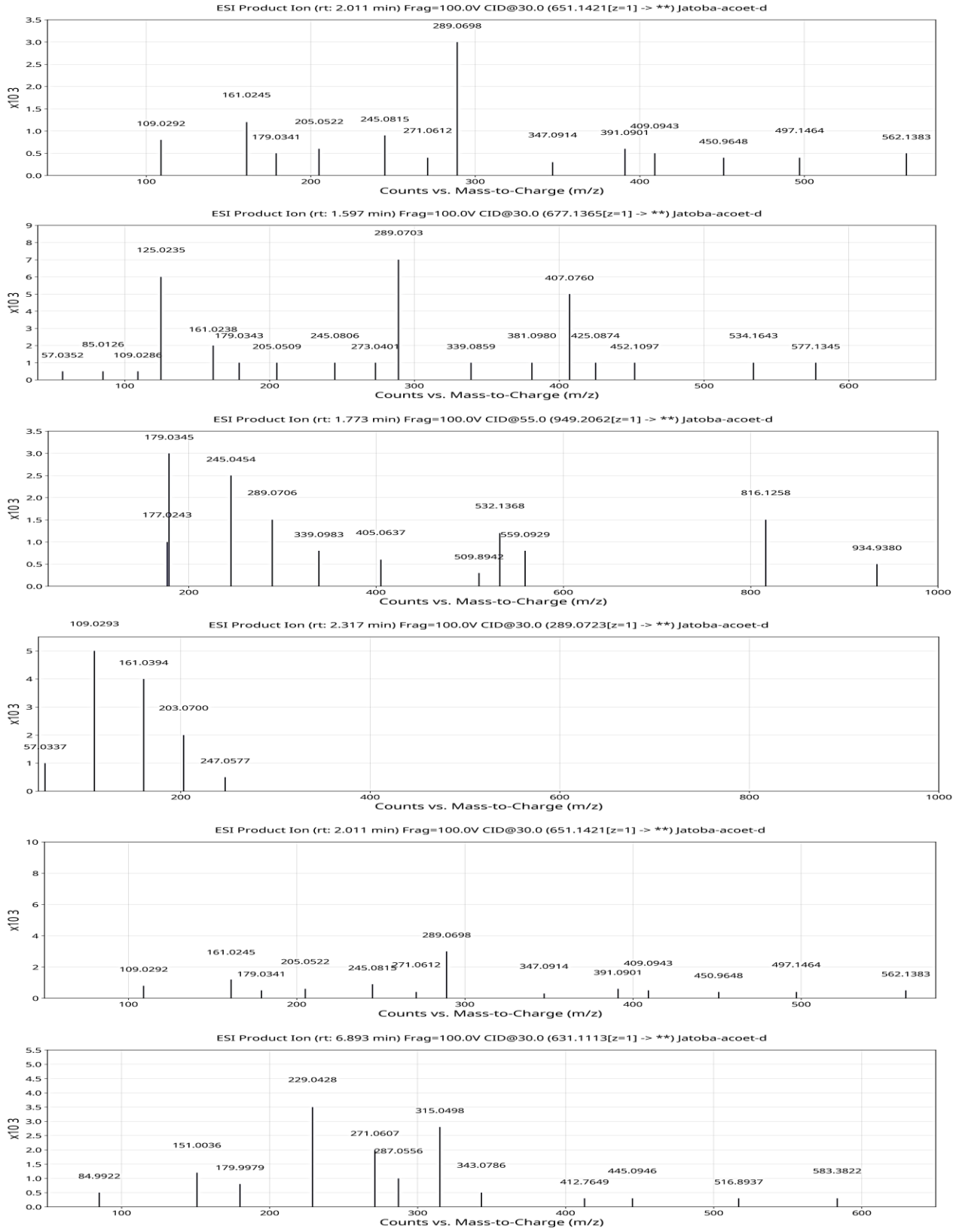


Figure 2. Phenolic compounds from Jatobá sap (*Hymenaea Courbaril L.*). (A) Properlagondin dimer; (B) Proanthocyanidin dimer; (C) Proanthocyanidin trimer; (D) Catechin; (E) Taxifolin; (F) Caffeoylquinic acid glycoside.

RESULTS

Phase 1. Phytochemical Characterization of Hymenaea Courbaril L.

The present study identified propelargonidin dimer (Figure 2A), a pigment that belongs to the anthocyanin category and is responsible for the orange-red coloration of plants. In addition, the flavonoids proanthocyanidin dimer (Figure 2B), proanthocyanidin trimer (Figure 2C), catechin (Figure 2D), taxifolin (Figure 2E), and caffeoylquinic acid glycoside (Figure 2F) were identified. High-performance liquid chromatography coupled with mass spectrometry was used to identify these compounds (HPLC-MS).

Phase 2. Study on the effects of using Jatobá sap for eight weeks on Soccer athletes

The anthropometric characteristics of the participants are presented in Table 1. Based on these data, it was possible to present descriptive statistics, and no differences were detected between the control group and the Jatobá group, all with $p > .05$. The data are presented as mean (position measurement), standard deviation and standard error (dispersion measurements), kurtosis and asymmetry (shape measurements).

Table 1. Subject characteristics (n = 30).

	Average		Standard Deviation		Standard Error		Kurtosis		Skewness	
	Placebo	Jatobá	Placebo	Jatobá	Placebo	Jatobá	Placebo	Jatobá	Placebo	Jatobá
Age (years)	24.8	27.5	3.43	3.65	1.03	0.98	0.47	-0.36	-0.79	0.20
Height (m)	1.81	1.77	0.09	0.06	0.03	0.02	2.07	-0.48	-1.24	-0.40
Body mass (Kg)	78.5	79.7	9.61	12.39	2.90	3.31	-0.84	2.39	-0.11	1.36
Muscle mass (Kg)	39.0	38.4	4.97	4.90	1.50	1.31	0.08	-0.82	-0.74	0.41
Fat mass (Kg)	10.1	13.4	2.56	6.83	0.77	1.82	0.31	3.14	0.62	1.49
BMI (Kg/m2)	24.0	25.3	1.90	3.28	0.57	0.88	-1.63	0.86	-0.13	0.74
Fat (%)	13.1	16.4	2.70	5.90	0.81	1.58	-0.82	2.48	-0.33	1.73

Note. The values presented do not indicate statistically significant results. Categorical variables were analysed with the Fisher test. The Shapiro-Wilk test was applied to verify the normality of the data, with p > .05. The paired t-test was applied to verify the difference between two means (Parametric).

For the study's dependent variables, a holistic and integrated analysis was presented through the equations indicated in the "*calculations*" field of the methods and presented in Table 2, comparing the pre-game (T1), post-game (T2), and 24-hour post-game (T3) times. The analytes urea, creatinine, AST, ALT, and GGT did not show any significant difference or effect, indicating renal and hepatic safety for the participants.

CRP showed a difference between the times T1 x T3 and T2 x T3, with a greater percentage variation observed for the Jatobá group. For the control group, CPK showed a significant difference between the times T1 x T3, with the Jatobá group showing a difference between the times T1 x T2 and T1 x T3. The percentage variations once again were greater for the supplemented group.

Regarding LDH, the groups showed similar behaviours, with an increase between the times T1 x T2 and a decrease between T2 x T3, both of which were significant.

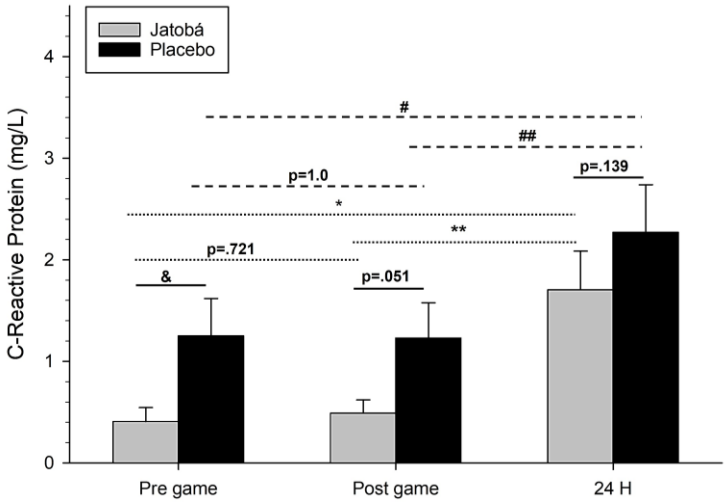
As for hormones, cortisol showed similar behaviour between the groups, increasing between T1 x T2 and decreasing between T2 x T3. However, it is noteworthy that the increase in cortisol in the control group was around 150%, while in the Jatobá group, it was only 58.3%, almost a third. In addition to the control group showing an increase of almost 300% in cortisol, the Jatobá group also showed a significant decrease in testosterone between the T1 and T2 times, which did not occur in the Jatobá group. Between the T1 x T3 times, while the control group still showed a reduction in testosterone levels (-2%), the Jatobá group already showed an increase (15.2%).

Table 2. Holistic and integrated view of dependent variables.

		Placebo			Jatobá		
		p - Value	Cohen d	Δ%	*p* - Value	Cohen d	Δ%
Urea	T1 x T2	.767	0.05	1.68	.906	0.04	-1.47
Creatinine	T1 x T2	.804	0.1	1.14	.857	0.07	-0.69
AST	T1 x T2	.921	0.02	-0.08	.835	0.03	-0.73
ALT	T1 x T2	.947	0.01	-0.73	.782	0.05	-2.72
GGT	T1 x T2	.974	0.001	-0.06	.909	0.01	-0.43
PCR	T1 x T2	1.0	0.02	-0.71	.721	0.16	183.2
	T1 x T3	**.030**	0.7	591.5	**.001**	1.2	1605.5
	T2 x T3	**.030**	0.8	590.3	**.001**	1,1	1274.4
CPK	T1 x T2	.067	0.8	50.7	**.004**	1.1	97.7
	T1 x T3	**.016**	1.1	96.2	**<.001**	1.6	149.9
	T2 x T3	.245	0.5	28.5	.373	0.3	24.7
LDH	T1 x T2	**.001**	2.3	38.4	**<.001**	2.8	53.7
	T1 x T3	.241	0.5	8.8	.073	0.7	16.0
	T2 x T3	**.004**	1.4	-20.2	**<.001**	1.7	-24.4
Cortisol	T1 x T2	**<.001**	3.7	150.5	**.026**	0.9	58.3
	T1 x T3	.288	0.5	-16.1	.325	0.4	-13.6
	T2 x T3	**<.001**	3.5	-65.0	**.006**	1.1	-42.1
Testosterone	T1 x T2	**.023**	1.0	-27.6	.251	0.3	-13.0
	T1 x T3	.922	0.03	-2.0	.434	0.3	15.2
	T2 x T3	.108	0.8	57.0	**.027**	0.5	40.2

Note. AST - Aspartate Aminotransferase; ALT - Alanine Aminotransferase; GGT - Gamma Glutamyl transferase; CRP - C-Reactive Protein; CPK - Creatine Phosphokinase; LDH - Lactate Dehydrogenase. The paired t-test was applied to variables with normal distribution and the Mann-Whitney test to those with non-normal distribution. Significant values are in bold.

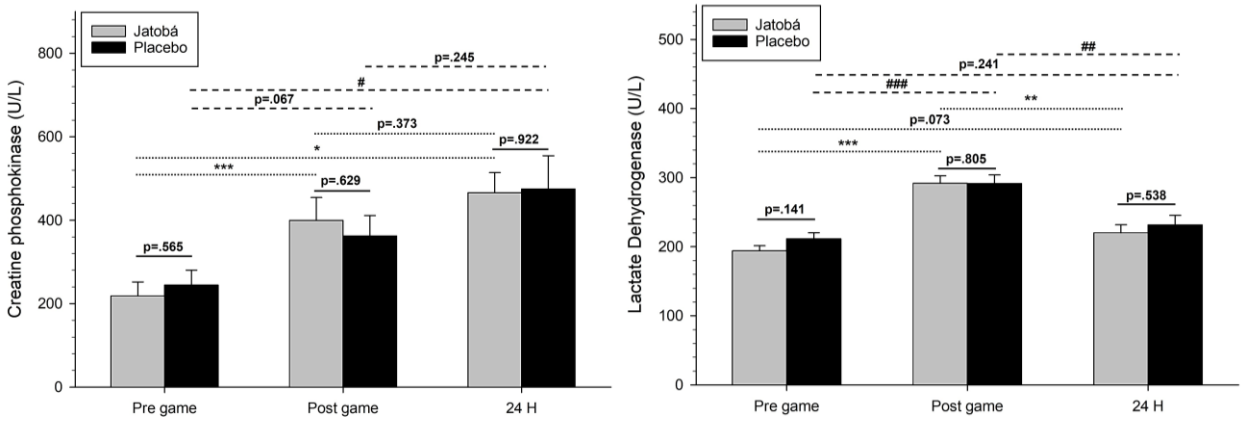
Regarding the observation of the absolute values of each biomarker, the PCR analysis (Figure 3) shows that professional soccer players, after 8 weeks of using Jatobá extract, presented a profile with less inflammation than the group that used the placebo (maltodextrin).



*Note. Data are presented with mean and SEM. The paired t-test was applied for parametric variables and the Mann-Whitney test for nonparametric variables. Differences were considered significant when p < .05. * for the Jatobá group, # for the control group and & between groups.*

Figure 3. Effect of treatment with Jatobá on the biochemical profile of CRP before, after and 24h after the game, reference value 0.3 to 1 (mg/dl).

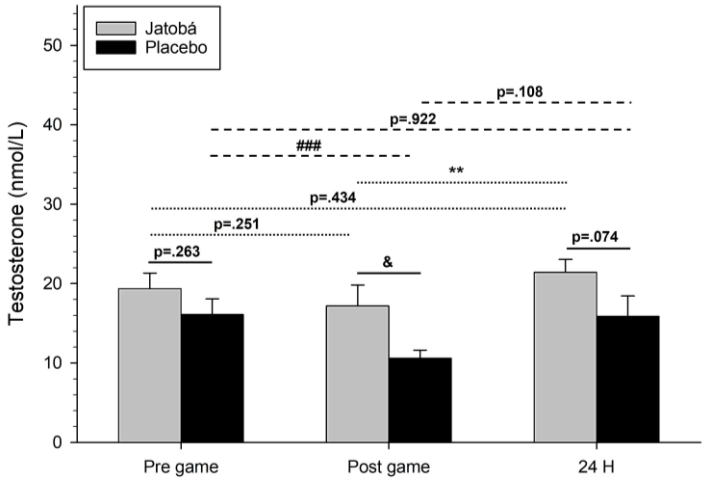
Still observing the absolute values, the muscle injury markers CPK and LDH had similar behaviour (Figure 4A and 4B), indicating that there was no differentiated impact on muscle tissue and that supplementation did not induce rhabdomyolysis.



*Note. Data are presented with mean and SEM. Paired t-test was applied for parametric variables and Mann-Whitney test for nonparametric variables. Differences were considered significant when p < .05. * for Jatobá group, # for control group and & between groups.*

Figure 4. Effect of treatment with Jatobá on the biochemical profile of muscle injury markers, namely (A) CPK and (B) LDH, pre-match, post-match and 24h post-match.

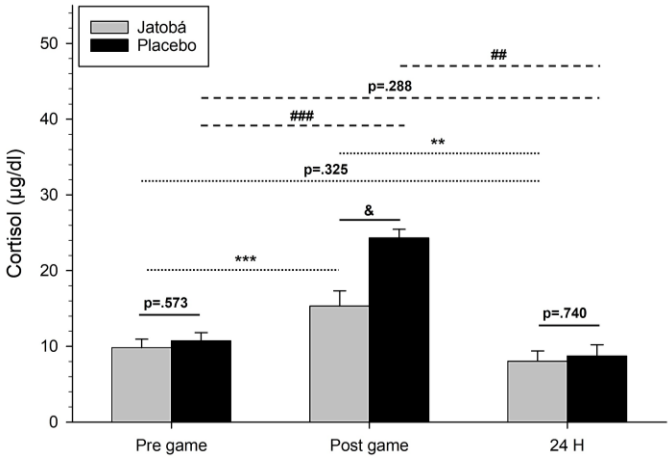
Moving on to the hormonal profile, the group of variables that drew the most attention started testosterone, which for the placebo group showed a significant decrease (p = .023) in the post-game period about the pre-game period, a fact that did not occur in the supplemented group. Also, the supplemented group showed a significant increase in the levels of this variable in the 24 hours of the post-game period (p = .027), which was not observed in the placebo group. A difference was also observed between the groups in the post-game period (p = .021), indicating that supplementation with Jatobá sap presented a positive modulation in the levels of this hormone (Figure 5).



*Note. Data are presented with mean and SEM. Paired t-test was applied for parametric variables and Mann-Whitney test for nonparametric variables. Differences were considered significant when p < .05. * for Jatobá group, # for control group and & between groups.*

Figure 5. Effect of treatment with Jatobá on the biochemical profile of testosterone before, after and 24 hours after the game, reference value 2.62 to 16.7 (ng/dl).

Regarding the other hormone observed, cortisol, it was possible to observe that both groups showed growth after the game with a return to baseline levels in 24 h, which was significant for both. However, the stress of the game caused the placebo group to increase the blood concentration of this hormone by 150.5% (effect of 3.5), while the growth of the supplemented group was 59.3% (effect of 0.9). The data revealed that supplementation modulated the testosterone x cortisol hormonal balance, with significant anabolic control (p = .002) between the groups after the game (Figure 6).



*Note. Data are presented with mean and SEM. Paired t-test was applied for parametric variables and Mann-Whitney test for nonparametric variables. Differences were considered significant when p < .05. * for Jatobá group, # for control group and & between groups.*

Figure 6. Effect of treatment with Jatobá on the biochemical profile of Cortisol before, after and 24h after the game.

What was stated in the previous paragraphs regarding testosterone (Figure 5) and cortisol (Figure 6) becomes even clearer when observing the testosterone x cortisol relationship since higher values indicate a more anabolic behaviour (more testosterone about cortisol), and lower values indicate greater catabolic stress (more cortisol about testosterone). In the pre-game period, the placebo group presented a difference from the Jatobá group in the order of -24%, a difference that was even greater between the groups in the post-game period (-61%) and remained 24 hours after the end of the match (-32%). These findings indicate better hormonal control modulated by Jatobá sap (Figure 7).

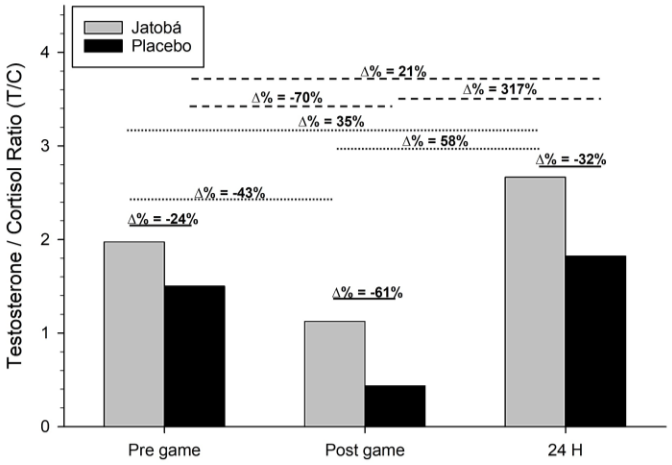


Figure 7. Effect of treatment with Jatobá on the Testosterone x Cortisol ratio before, after and 24 hours after the game. Δ% was used to demonstrate variation at all times of the study.

Since the study involves different variables, we investigated similarities and dissimilarities based on the groupings of all these study variables. Initially, we applied the Z Score to homogenize the measurement units of the variables. Then, we sought the most appropriate number of behaviour clusters using the elbow test (Figure 8A), indicating that three clusters were observed. Finally, we applied the Euclidean Similarity Index, with the distances presented by the agglomerative hierarchical dendrogram (Figure 8B). In this, a large cluster was clear, containing the variables CRP, type of supplementation, creatinine, testosterone/cortisol ratio, lactate, cortisol, testosterone, urea, GGT, AST, and ALT, leaving two other more dissimilar clusters, one for CPK and the other for LDH, which were more individualized in behaviour, showing no difference according to the supplementation (Figures 4 and 8B).

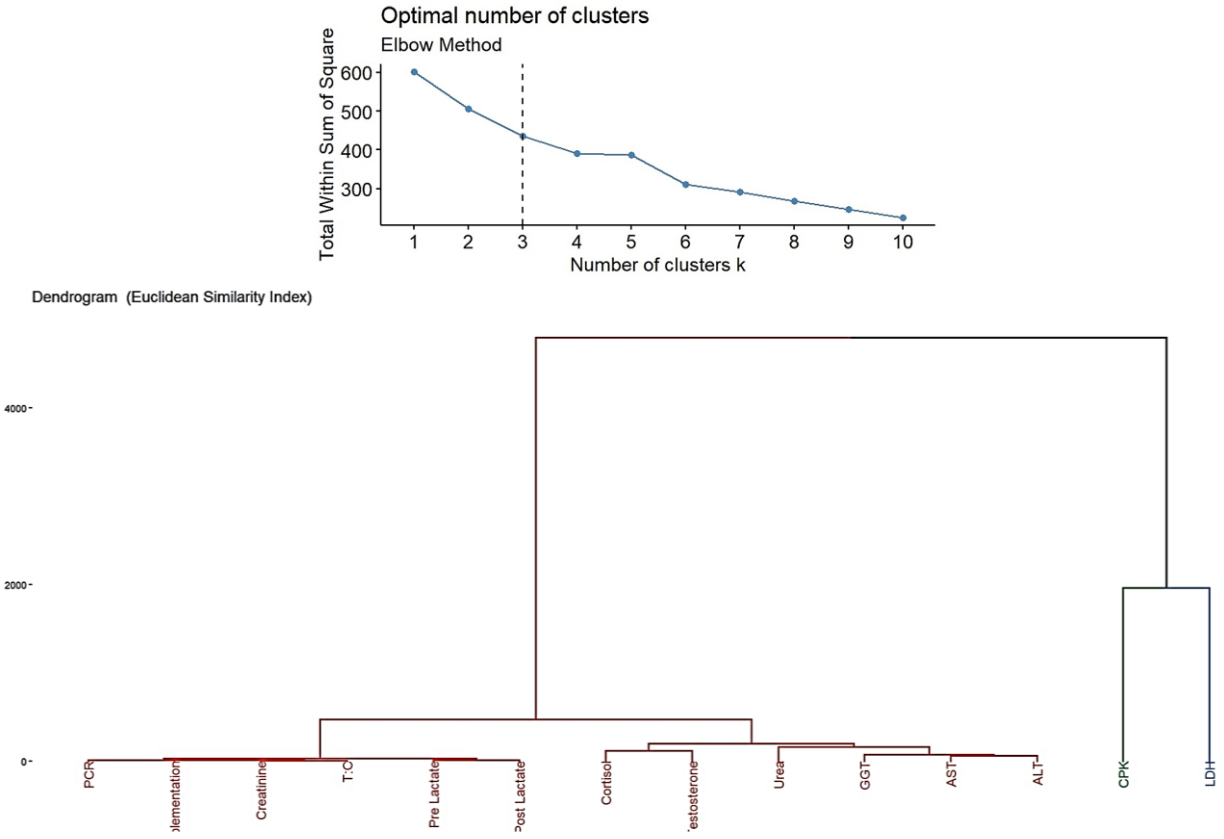


Figure 8. Elbow method to identify the optimal number of clusters (Figure 8A). Dendrogram using the agglomerative hierarchical method (Figure 8B).

DISCUSSION

Phytochemical research conducted with Jatobá (*Hymenaea Courbaril L.*) identified several phenolic compounds (Costa et al., 2021A). The present study identified propelargonidin dimer, a pigment that belongs to the anthocyanin category and is responsible for the orange-red coloration of the plants. In addition, several flavonoids were identified, including proanthocyanidin dimer, proanthocyanidin trimer, catechin, taxifolin, and caffeoylquinic acid glycoside. High-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) was used to identify these compounds.

Our group had already demonstrated the antioxidant effect of Jatobá sap in mice (Costa et al., 2021A) and in vitro (Costa et al., 2021B). The present study is a pioneer in investigating the effects of Jatobá sap from the Brazilian Amazon on biochemical and hormonal indicators in male professional soccer players.

Previous studies analysed biochemical parameters in soccer players without the use of ergogenic resources, indicating an increase in CRP levels (Aldelkovic *et al.*, 2015; SILVA, J. R. *et al.*, 2014), CPK (Aldelkovic *et al.*, 2015; Pimenta *et al.*, 2016; Silva, J. R. *et al.*, 2014), and LDH (Coppale *et al.*, 2019; Aldelkovic *et al.*, 2015). In contrast, Requena et al. (2017) found that CRP and CPK levels remained stable after a break between seasons in a professional soccer team, with a significant reduction in LDH, which can be attributed to considerable drops in training volume and intensity (Raquena *et al.*, 2017).

In the present study, CRP showed differences between times T1 x T3 and T2 x T3, with a greater percentage variation observed for the Jatobá group. For the control group, CPK showed a significant difference between times T1 x T3, with the Jatobá group showing differences between times T1 x T2 and T1 x T3, with the percentage variations once again being greater for the supplemented group. Regarding LDH, the groups showed similar behaviours, with an increase between times T1 x T2 and a decrease between T2 x T3, both being significant.

In top-level soccer, one to three matches may be played over a week (Julian; Page; Harper, 2021). A wide range of physiological indicators have been employed to monitor the condition of athletes over prolonged periods (Heisterberg *et al.*, 2013; Silva, J. R. *et al.*, 2014). Specifically, hormonal markers (such as cortisol and testosterone) have been recognized as indicators of training-induced stress, making them relevant in the biochemical analysis of athletes (Hackney, 2020). Furthermore, the testosterone to cortisol (T/C) ratio has been used to assess the balance between anabolic and catabolic states in players (Urhausen; Gabriel; Kindermann, 1995).

Moving on to the hormonal profile, the group of variables that drew the most attention started testosterone, which for the placebo group showed a significant decrease (p = .023) in the post-game period about the pre-game period, a fact that did not occur in the supplemented group. Also, the supplemented group showed a significant increase in the levels of this variable in the 24 hours of the post-game period (p = .027), which was not observed in the placebo group. A difference was also observed between the groups in the post-game period (p = .021), indicating that supplementation with Jatobá sap presented a positive modulation in the levels of this hormone.

Regarding the other hormone observed, cortisol, it was possible to observe that both groups showed growth after the game, returning to baseline levels within 24 h, which was significant for both. However, the stress of the game caused the placebo group to increase the blood concentration of this hormone by 150.5% (effect of 3.5), while the supplemented group increased by 59.3% (effect of 0.9). The data revealed that supplementation modulated the testosterone x cortisol hormonal balance, with significant anabolic control (p = .002) between the groups after the game.

What was stated in the previous paragraphs regarding testosterone and cortisol becomes even clearer when observing the testosterone x cortisol relationship since higher values indicate more anabolic behaviour (more testosterone about cortisol), and lower values indicate greater catabolic stress (more cortisol about testosterone). In the pre-game period, the placebo group showed a difference from the Jatobá group of -24%, a difference that was even greater between the groups in the post-game period (-61%) and remained

24 hours after the end of the match (-32%). These findings indicate better hormonal control modulated by Jatobá sap.

The increase in cortisol levels for the placebo group can be attributed to the greater stress resulting from a more intense physical load, as well as the psychological pressure on the players (Santos *et al.*, 2012), which was prevented by Jatobá. Another study that used a 12-week training period with professional soccer players showed similar results (Silva *et al.*, 2011). Furthermore, (Michaidilis, 2014) examined cortisol fluctuations throughout the soccer season, revealing a significant increase in cortisol at the end of the season.

This study identified an increase in the T/C ratio after eight weeks of training with professional soccer players in the group that received the Jatobá extract. In part, this increase in the T/C ratio can be attributed to the estrogenic/antiestrogenic activity of isoflavones; it may also be due to an alteration in the biosynthesis and subsequent secretion of luteinizing hormone (LH), which acts to stimulate testosterone production in males (Kinahan; Budgett; Mazzoni, 2017).

Recent studies that did not use ergogenic aids demonstrated a significant decrease in the T/C ratio in professional soccer players (Andrzejewski *et al.*, 2021; Michaidilis, 2014; Saidi *et al.*, 2020; Silva *et al.*, 2011). The authors of these studies explain this decrease as resulting from neuromuscular fatigue, which can occur due to increased training intensity. This fatigue may be associated with a decline in physical performance (Saidi *et al.*, 2020; Silva *et al.*, 2014). Similar results were observed in the study presented here in the placebo group when we analysed the biomarkers of the T/C ratio. However, since this was not the focus of the study, we cannot establish a link with the decline in physical performance.

In part, these findings can be explained by the antioxidant properties of this extract due to its high content of flavonoids previously linked to anti-inflammatory effects (Costa *et al.*, 2021A; Costa *et al.*, 2021B).

Creatinine and urea analysis was performed as they are indicators of renal function (Maltai *et al.*, 2019). Previous studies have examined the effects of a dietary supplement containing a combination of flavonoids extracted from grape seeds and pine bark over 14 days in half-marathon runners, resulting in the prevention of increases in biomarkers associated with kidney damage (Semen *et al.*, 2020). There was no difference between groups or times for these markers, indicating the renal safety of their use.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) are enzymes frequently synthesized in the liver and are commonly evaluated as part of liver function tests (Giannini; Testa; Savarino, 2005). Elevated levels of these enzymes may signal inflammation or injury to liver cells (Benedict; Zhang, 2017). A recent study on molecular mechanisms related to curcumin, a polyphenol found in turmeric, indicated that the ingestion of this substance has several biological effects due to its antioxidant and anti-inflammatory characteristics, including liver protection (Xu *et al.*, 2018). At present, there was no difference between groups or times for these markers, indicating the hepatic safety of their use.

CONCLUSION

The study presented here examined the effects of Jatobá extract (*Hymenaea Courbaril L.*) for 8 weeks in professional soccer players, exploring a variety of biochemical and hormonal markers. In the phytochemical characterization phase by (HPLC-MS), the present study identified propelargonidin dimer, and the flavonoids proanthocyanidin dimer, proanthocyanidin trimer, catechin, taxifolin and caffeoylquinic acid glycoside. The

main findings of the primary phase point to the hormonal profile, where supplementation increased testosterone levels, reduced cortisol levels, and positively modulated the testosterone/cortisol ratio.

AUTHOR CONTRIBUTIONS

ABS, MJBF, KMS, ESL, RSLC, and RPMS planned and designed the study; ABS, MJBF, KMS, ESL, RSLC, and RPMS collected the data; ABS, JBC, RPMS, and LCOG processed and analysed the data; All authors participated in writing, discussing, and approving the final version of the study.

SUPPORTING AGENCIES

No funding agencies were reported by the authors.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article. The corresponding author can provide any other information by email.

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