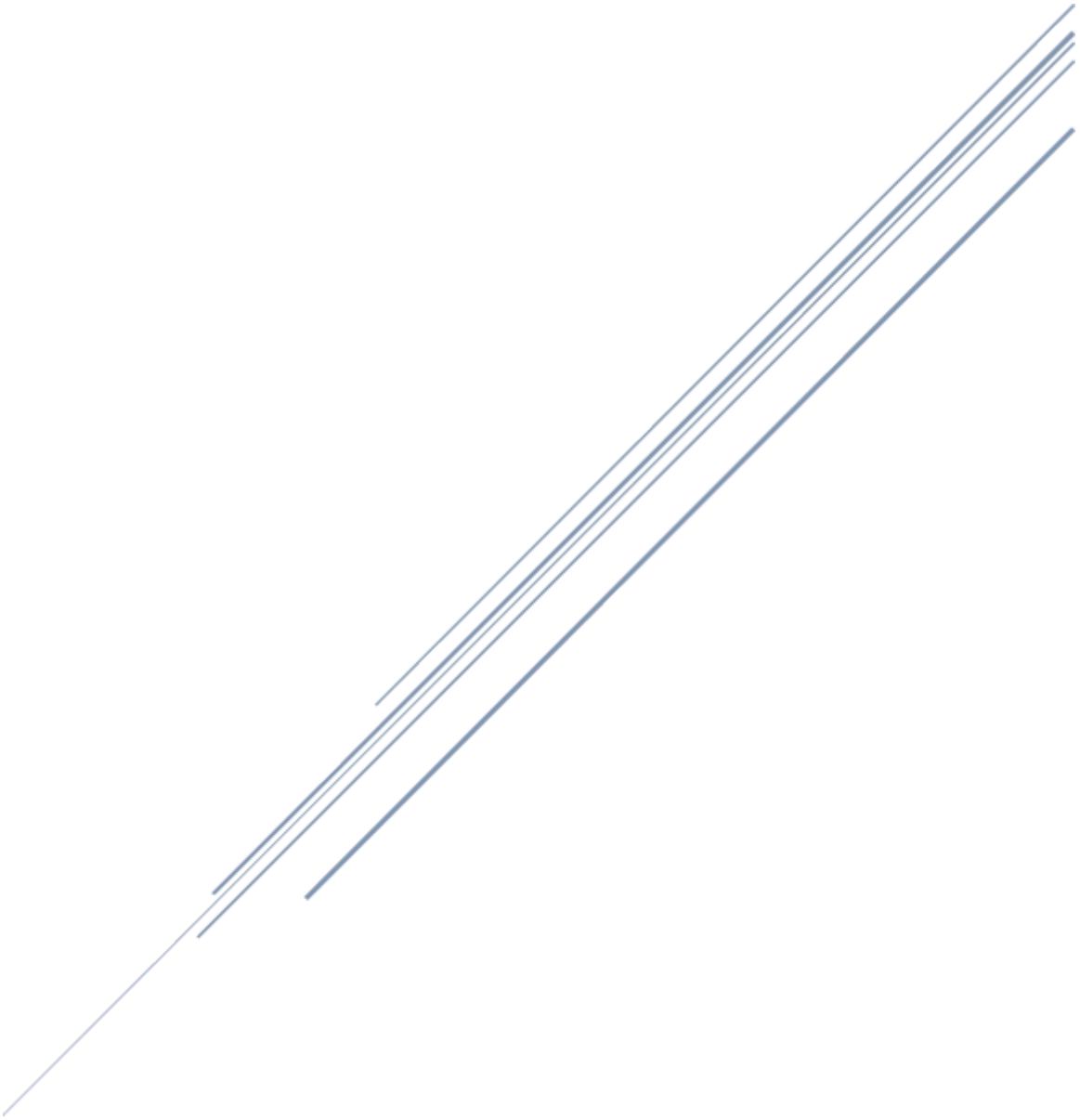
Official Title: Effect of supplementation with *Euterpe edulis* on the physical and mental performance of healthy men submitted to physical exercises

Ethical committee Protocol No: 6.202.888

Study protocol



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1. Type of study

This is a clinical study, double blind, with the purpose of offering the juice *of E. edulis*, daily for 4 weeks before the day of the final evaluation, compared to the control. There will be about 40 men, aged 19 to 30 years, who perform regular physical exercises, in total of 150 min per week, at a minimum of 3 months.

2. Experimental Protocol

The men will be randomized into two groups, called GJ who will receive the sample and GP who will receive the placebo. The sample consists of 250 mL of juçara juice and the placebo which is 250 mL of water with pectin and natural dye. Research subjects will maintain their exercise routine, as well as fill out a food diary that will be sent electronically. The research will be for 11 days, and on day 1 (D1) of the research he will have the blood collected. On day 11 of the D11 survey, at time zero he will have the blood collected, and then consume the sample or the placebo. Two hours after he will be subjected to the battery of exercises, following 1. Dorsal dynamometer, 2. shoulder dynamometer, 3. manual dynamometer, 4. ergospirometry + electrocardiogram + digital lactate, 5. jump test for 1 min (amounts of jumps), 6. Pollock abdominal test and 7. functional mobility test. At the end, the blood will be collected, and a psychological form will be applied. After 2 and 7 days of physical exercise will be applied visual pain test.

3. The size of the sample

The calculation of the sample of patients for this study was performed using the software G*Power3.1.9.7 (Public domain). Considering the alpha value equal to 0.05 and the power of test $1 - \beta$ as 95% (0.95, that is, determining that the probability of type II error (β) should not be greater than 5%), the value of 20 patients was reached.

The selection will be simple random probabilistic from a total population of patients who fit the inclusion criteria. Among the inclusion criteria are: being male, aged between 19 and 30 years and practicing physical exercises regularly for at least 3 months. Among the exclusion criteria are people who have motor problems, cardiovascular changes, have type I and II diabetes, smokers, athletes, cognitive deficits, and psychomotor disorders. Each patient will be evaluated at the beginning of the exercise and at the end of the exercise. Patients were randomly divided into 2 groups: treatment with placebo product and with the supplement of E. edulis.

4. Ethical issues

The study will be conducted after approval by the Ethics Committee on Research with Human Beings of the Vila Velha University and conducted in accordance with the Declaration of Helsinki. The clinical study will also be registered with REBEC, and will only be initiated after all ethical approvals. All participants will sign a written agreement (ICF).

As for the risks, in a previous study with 39 participants, none presented pictures of allergy or gastric or intestinal intolerance to JFJ and as a benefit, such a study demonstrated a positive effect on the acute adaptive inflammatory response (MENDES et al., 2021).

5. Inclusion criteria

Amon the inclusion criteria are:

- Being male
- Aged between 19 and 30 years old
- Practicing physical exercises regularly for at least 3 months

6. Exclusion Criteria

Men who use:

- Steroids
- Anabolics
- Taurine
- BCCA
- Exogenous supplementation

7. Removal criteria

Among the exclusion criteria are people who have:

- Motor problems
- Cardiovascular changes
- Have type i and ii diabetes
- Smokers
- Athletes
- Cognitive deficits
- Psychomotor disorders.

Each patient will be evaluated at the beginning of the exercise and at the end of the exercise. Patients will be randomly divided into 2 groups: treatment with a placebo product and with an E. edulis supplement.

8. Intervention

On day 11 of the D11 survey, at time zero he will have the blood collected, and then consume the sample or the placebo. Two hours after he will be subjected to the battery of exercises, following 1. Dorsal dynamometer, 2. shoulder dynamometer, 3. manual dynamometer, 4. ergospirometry + electrocardiogram + digital lactate, 5. jump test for 1 min (amounts of jumps), 6. Pollock abdominal test and 7. functional mobility test. At the end, the blood will be collected, and a psychological form will be applied. After 2 and 7 days of physical exercise will be applied visual pain test.

9. Preparation of Juçara juice

The preparation will be obtained with the same methods already well established in previous studies (SCHULZ et al., 2016.; MENDES et al., 2021). Participants will receive 250 ml of water + purple dye + pectin (control) or commercial samples of juçara (E.edulis) refrigerated to 4°C to 10°C, per day, for 10 days, and on the 11th will be the exercise and on this day will be administered the juice of the juçara 2 h before on the day of the monitored exercise. The chemical characterization of Euterpe edulis will be with at least 350.0 + or - 17.5 mg of total phenolics (gallic acid equivalent), 186.0 + or - 7.5 mg of total monomeric anthocyanins (cyanidin 3-glycoside), 0.73 + or less 0.01 g of proteins, and 2.75 + or - 0.03 g of lipids (ether extract). During the 10 days of previous treatment, maintain the routine of physical exercises.

10. Values evaluated

10.1 Evaluation: Simple reaction psychomotor test

The examined individual should sit in front of the monitor with the index finger of the dominant hand positioned on the sensor (called the "stand-by key") located on the control panel. The individual will be instructed to hold their finger on the "stand-by key" and move it to the "reaction key" as soon as the stimulus (yellow light) appears.

The tested parameters are:

A1 - median reaction time (interval between the start of a given stimulus and the release of the "stand by key", in ms):

A2 - median movement time,

A3 - median total response time.

10.2 Evaluation: Psychomotor test of reaction of choice

One individual was instructed to respond appropriately and as quickly as possible to stimuli appearing on the screen using the upper and lower limbs.

Five colored optical stimuli (white, yellow, blue, green, and red) in the form of a circle appeared on the screen, and each circle received its own "reaction key" on the control panel that corresponded to the color of the stimulus.

The examined individual was asked to respond to the stimulus by pressing the corresponding "reaction key" with the right or left hand, and the foot was also instructed to press the right or left pedal whenever a white rectangular light appeared on the black background of the screen. Finally, the test included reaction to acoustic stimuli.

The tested parameters are:

B1 - number of correct answers (n),

B2 - number of incorrect and missed answers (n),

B3 - median response time(s)

10.3 Collection of blood samples and analysis of biomarkers

In the blood collection will be collected samples of 8 ml of the cubital vein of the forearm of the patients according to the method used in Mendes et al. (2021). The samples will be stored in tubes containing ethylenediamine tetraacetic acid (EDTA) 10 or without anticoagulants. The serum should be acquired by the centrifugation method (1000 x g for 10 minutes at 4°C) and stored at – 80°C in polypropylene microcentrifuge tubes until analysis.

Pro-inflammatory biomarkers will contain acute phase proteins such as the cytokines TNF-alpha, IL-6, IL-1 beta, monocyte chemoattractant protein 1, and adhesion molecules. Anti-inflammatory markers: cytokines such as IL-10 and adiponectin that acts on muscles as anti-inflammatory cytokines (FANG et al., 2023).

The serum levels of the biomarkers will be determined by cytometry (flow cytometer FACSverse, BD Bioscienses), using a commercial kit Cytometric Bead Array Human Inflammation kit, according to the manufacturer's instructions. Data will be analyzed using FCAP arrayTM (MENDES et al., 2021; SCHULZ et al., 2016)

Blood samples will be collected 1 hour before the high-intensity strength exercise, in the condition of muscle rest. From that moment the participants, randomly divided into two groups, will be selected to drink the extract of juçara (Euterpe edulis) or the placebo. New blood samples will be collected after the completion of the muscular effort, specifically 3 minutes after the exercise performed, in addition to the 3rd sample after 24 hours of rest.

Will be evaluated in the blood samples collected: plasma biomarkers, which include lactate concentration (LA). In plasma, creatine kinase (CK), total antioxidant capacity (TAC) and concentrations of testosterone (T), cortisol © and growth hormone (GH) will be analyzed. In addition to serum concentrations of inflammatory biomarkers such as interleukins (IL-6, IL-8, IL10) and TNF-alpha.

10.4 Force markers

10.4.1 Dynamometer

The dynamometer is an instrument used to measure muscle strength objectively. In this study will be used the static dynamometer that demonstrated measurement reliability in other clinical trials (HIRANO et al., 2020; MARTINS et al., 2018).

10.4.2 Jump tests

The jump tests are used as a measure of explosive strength and muscle power and this methodology will also be used in this research. The effectiveness of strength assessment

in the countermovement jump test has already been validated in other clinical studies (CARBAKAPA et al., 2023).

10.4.3 Functional tests

Functional tests assess strength in more complex movements related to day-to-day activities. In these studies, the 6-minute step test will be applied, as determined its reliability for the evaluation of muscle strength in a previous study (ARCURI et al., 2016).

10.4.4 Muscular endurance test

Muscle endurance tests assess the ability of muscles to sustain a contraction for a prolonged period. In this study will be used the abdominal resistance test, through the plank test, already reproduced effectively in another study (KOUMANTAKIS et al., 2021).

10.5 Aminogram

A sample of 250 ml of juçara juice will be sent to the laboratory C.B.O. laboratory analyzes Ltda, for analysis of Total Amino Acids: Aspartic Acid, Glutamic Acid, Alanine, Arginine, Cystine, Phenylalanine, Glycine, Hydroxyproline, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Serine, Taurine, Tyrosine, Threonine, Valine, Tryptophan and Total Proteins. Of all these amino acids the ones that are essential and the body does not produce, will be those that will make the difference compared to other foods in age-induced bone loss (ZIQUAN et al, 2022).

10.6 Subjective analysis of fatigue

A questionnaire will be developed about the subjective perception of patients about the effects of the treatment employed in the recovery of muscle fatigue. The questions will be based on the Chalder scale and the International Short-Form Physical Activity Questionnaire (IPAQ-SF) (LEE et al., 2011). However, the feeling of improvement or not of physical fatigue after exercise will be the focus of the preparation of a new and unprecedented questionnaire

11. Statistical analysis

The results will be expressed as mean ± SEM (standard error of the mean). Statistical analysis will be performed by one-way analysis of variance (ANOVA) (repeated measures), followed by Tukey's post hoc test, using the Prisma software (Prism 6.0, GraphPad Software, Inc., San Diego, CA, USA). The differences will be considered significant when $p<0.05$.

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