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PHYSICALLY ACTIVE INDIVIDUALS SUBMITTED TO TREATMENT WITH TRIBULUS TERRESTRIS VERSUS PLACEBO

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ABSTRACT

The purpose of the present study is to evaluate anthropometric, biochemical, hormonal and performance differences among physically active individuals who utilize (or not) Tribulus terrestris (TT) supplementation. The cohort is composed of 32 subjects divided into two groups: 1) 8 males and 8 females individuals who are physically active and utilize Tribulus terrestris; 2) 8 males and 8 females individuals who are physically active and utilize placebo. Inclusion criteria for all subjects consisted of age requirement within the range of 18 and 40 years old, a history of at least 6 months of physical activity and absence of any drug treatment/consumption. Analysis of body composition, physical performance test and blood collection for subsequent hormonal and biochemical tests were collected. Data were processed and analyzed based on descriptive statistical tests and one-way-ANOVA. Results were considered statistically significant when $p<0.05$. Results show significant increase in testosterone levels for both females and males treated with TT, decreased levels of body fat among the treated women, increased libido for treated individuals and enhanced strength among treated women. Biochemical parameters among all groups were within normal limits.

Key words: Physical activity. Body fat composition. Tribulus terrestris.

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RESUMO

Indivíduos fisicamente ativos submetidos ao tratamento com Tribulus terrestris versus placebo

O objetivo do presente estudo é avaliar as diferenças antropométricas, bioquímicas, hormonais e de desempenho entre indivíduos fisicamente ativos que utilizam (ou não) a suplementação com Tribulus terrestris (TT). A coorte é composta por 32 indivíduos divididos em dois grupos: 1) 8 indivíduos do sexo masculino e 8 do sexo feminino, fisicamente ativos e que utilizam Tribulus terrestres; 2) 8 indivíduos do sexo masculino e 8 do sexo feminino que são fisicamente ativos e utilizam placebo. Os critérios de inclusão para todos os sujeitos consistiram na exigência de idade entre 18 e 40 anos, histórico de pelo menos 6 meses de atividade física e ausência de qualquer tratamento / consumo de drogas. Análise da composição corporal, teste de desempenho físico e coleta de sangue para testes hormonais e bioquímicos subsequentes foram coletados. Os dados foram processados e analisados com base em testes estatísticos descritivos e ANOVA one-way. Os resultados foram considerados estatisticamente significantes quando $p<0,05$. Os resultados mostram aumento significativo nos níveis de testosterona para mulheres e homens tratados com TT, diminuição dos níveis de gordura corporal entre as mulheres tratadas, aumento da libido para indivíduos tratados e aumento da força entre mulheres tratadas. Parâmetros bioquímicos entre todos os grupos estavam dentro dos limites normais.

Palavras-chave: Atividade física. Composição da gordura corporal. Tribulus terrestris.

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INTRODUCTION

Tribulus terrestris plant (T. terrestris or TT), also known as “Puncture Vine,” is found in Africa and Europe, although it’s constantly cited as having Asian origins (Junior, Lemos, Lemos, 2011).

It is well known in traditional Chinese medicine and has been broadly used in many countries for thousands of years (Melnyk, Marcone, 2011).

According to Choi (2013), the plant contains many compounds, including steroid saponins, flavonoids, alkaloids and amino acids. The main constituents of T. terrestris are considered steroidal, flavonoid and alkaloid saponins. Of these components, saponins are considered as the most important and most active, having a wide range of biological effects, such as relieving sexual dysfunction and improving erectile function in rabbits (Do et al., 2013), thus demonstrating its testosterone activity among other effects. Saponins have also been reported to benefit the cardiovascular system by protecting the myocardium against ischemic injuries as well as improving overall hypertension and coronary disease (Qureshi, et al., 2014).

There are further reports that utilize the plant for the purpose of reducing hyperglycemia, for its vasodilating activity, for inducing increase in hormonal levels (testosterone) and for antimycotic activity, as in the treatment of candidiasis (Do et al., 2013).

Physically active individuals commonly use T. terrestris in order to gain muscle mass and strength, although there is no scientific evidence on the effectiveness of this biological activity. Hydrolyzation of saponins is a mechanism that supports such claims. Hydrolyzed saponins become steroidal sapogenins and these have antispasmodic and diuretic properties, increase the production of luteinizing hormone (LH), testosterone, estrogen and other steroids (Guazzelli, 2014).

The alleged purpose of using T. terrestris for these effects is to increase androgenic and anabolic pathways by enhancing endogenous testosterone, leading towards a safe augmentation of hormonal levels that is devoid of side effects (Brown et al., 2001) (El-Tantawy et al., 2007) (Gauthaman et al., 2002) (Singh et al., 2012).

It is noteworthy that there is currently no consensus and no standardization on the recommended dose for the use of this herbal medicine, although a study conducted suggests a daily dose of 3.21 mg per kg body weight (Antônio et al., 2000).

There are other studies that relate the effectiveness of the plant, however, most of them was performed in animals. The studies that were performed using human subjects generated more controversies than clarifications due to small numbers of subjects.

The use of medicinal plants, as well as their extracts, must be extensively studied so that their effectiveness and safety can be established.

Furthermore, concerns of generalized public health are intensified through broad and uncontrolled advertising via websites that encourage the use of such products. A diversity of plants and herbs have been used for thousands of years, however, without proper studies to ensure their safety.

The purpose of the present study is to evaluate the effectiveness of TT in individuals who are physically active, devoid of interactions with other drugs and/or ergogenic supplements.

MATERIALS AND METHODS

Experiments were performed at the Human Movement Laboratory of the São Judas Tadeu University and at the Marlin Academy, located at the same University.

Sample Size

Sample consisted of 32 subjects divided into two groups: Physically active group that used T. terrestris (GPAT) n = 16 (eight male and eight female participants), and physically active group that used placebo (GPAP) n = 16 (8 male and 8 female participants). Subjects were recruited via personal contacts of the research team.

Inclusion Criteria

The study included male and female bodybuilders who had a minimal training frequency of 3 workouts per week, within ages of 18 and 40 years old. Individuals who are bodybuilders but also practice some other form of physical exercise (gymnastics, dances, fights, street race, etc.) were excluded from the research.

Other exclusion criteria: individuals who developed illnesses that prevented the performance of one or more tests; individuals who used pharmacological and/or non-

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pharmacological ergogenic agents (supplements); individuals who did not maintain an adequate training frequency of at least three workouts per week over a one-month period.

This work was approved by the ethics committee of Universidade São Judas Tadeu - São Paulo - under opinion number 2,144,320 on June 28, 2017

Monitored Training

Research participants maintained a minimum training frequency of three workouts per week over a period of one month. Subjects trained at the facility of their choice and did not have to physically report to São Judas Tadeu University during this training period.

Monitoring of all training frequency was performed remotely via social media WhatsApp® application, through a group that was designed specifically for this purpose.

Participants were instructed not to modify their diet during the monitored training period, as well as not to consume any form of dietary supplements.

Tribulus Terrestris Supplementation

At the beginning of the study, participants received a thirty days supply of TT supplementation. Total daily dosage was designated as 375mg for women and 750mg for men. Subjects were weighted and TT supplementation was individually compounded at the USJT Pharmaceutical Sciences Laboratory.

The placebo, Peumus boldus capsules, was compounded in indistinguishable capsules, at the same dosage and in identical containers. Both supplement and placebo were prescribed by the pharmacist responsible for the study, who was qualified in accordance with CFF Resolution 586/2013. The study was performed according to the double-blind model.

Evaluations

Biochemical and anthropometric body composition as well as performance analyzes were conducted at the beginning and at the end of the study period.

Anamnesis

At the beginning of the study, all participants agreed and signed the Informed Consent Form (ICF), which contained all printed information relevant to the study.

Subsequently, participants filled out the clinical history questionnaire, food history questionnaire, 24-hour recall and food frequency questionnaire in order to obtain a qualitative profile of the subject population.

Nutritional Report

This report was obtained in order to define and quantify all foods and beverages ingested in the pre-interview period, which consists of the preceding 24 hours or, more commonly, the day before starting the study (Gibson, 1990).

The report consisted of a personal interview conducted by the nutritionist during the anamnesis. To aid in the process, the professional may have used photo albums displaying food types, three-dimensional food models or common measurements. Food could be recorded in non-specific units, such as a slice of a certain food, a medium-sized fruit, a piece of candy or a cookie bag.

Biochemical Analyzes

Measurement and serial biochemical analysis of testosterone hormone was performed using an ELISA kit. Collection and analysis of blood composition was performed using the Complete Blood Count kit.

Serum biochemistry (fasting glucose, glycosylated hemoglobin, total cholesterol and fractions, triacyclglycerols) was analyzed using appropriated kits.

The hormonal profile was evaluated in partnership with the Laboratory of Toxicological Analysis - Division of Pharmaceutical Sciences of the University of São Paulo (LAT-FCF / USP) by using the ELISA technique, as mentioned above. The hormone evaluated was testosterone.

1RM Ascending test (for maximum repetition)

The 1 RM test was performed as described by Brown and Weir (2001). The test consisted of the horizontal free straight bench press exercise, which is representative for upper limb strength.

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Body Composition

For body composition, 4 analyzes were performed: body weight, height, skinfold measurements and bioimpedance testing.

Body Weight

Body weight was measured using an InBody brand digital scale, model Inbody 120®. The same scale was used in all body weight evaluations. Measurements were performed by each subject standing with his/her back to the scale and with reasonable lateral clearance of the feet, which were placed at the center of the platform. The subject was to stand tall and look forward. Only one measurement was taken (Pedersen, Gore, 2000).

Height

Height was measured using a wooden stadiometer, graduated in centimeters and decimeters. Measurements were taken with the subject standing barefoot and with feet together. The back of the heels, pelvic waist, shoulder girdle and occipital region were placed against the wooden stadiometer. The measurement was performed in inspiratory apnea, with the head in the Frankfurt Plane (parallel to the ground) and the cursor at right angles to the scale. Three measurements were taken, from which their mean was considered a real value of height (Pedersen, Gore, 2000).

Skin Folds

These data were obtained according to established protocol of 7 (seven) cutaneous skinfold measurements for women 18 to 55 years (Jackson, Pollock, Ward, 1980) and for men 18 to 61 years (Jackson, Pollock, 1978).

The skinfolds analyzed were triceps, middle axillary, thigh, subscapular, supra iliac, abdominal and pectoral. The value obtained for analysis was the arithmetic mean of 3 measurements. All measurements were obtained using a Lange® adipometer instrument.

Bio Impedance Examination

In order to obtain data on bioimpedance, we have used the Inbody® brand portable model InBody120, which has 8 contact electrodes, allowing the analysis of the whole body as well as of segmented portions. (Source: Use of Bioimpedance for Body Mass Assessment - Federal Council of Medicine, ABRAN and SBNPE (2009) and manufacturer's instructions).

Data Analysis

Statistical analysis was performed using GraphPadPrism 6® software (GraphPad software, Inc., San Diego, California, USA). Bartlett's test was employed to verify the homoscedasticity of the data. For parametric data, one-way ANOVA was used. The significance level for all analyzes was considered for $p < 0.05$. Data were expressed as mean ± standard error.

RESULTS

Twenty-eight (28) physically active volunteers, who train at a regular basis (at least three times a week) were evaluated.

Of those 28 subjects, 50% were male (n = 14) and 50% female (n = 14). The age group of subjects was between 18 (eighteen) and 40 (forty) years prior to the administration of Tribulus terrestris and after the 30-day period.

Results show no significant increase of urea levels within the participants treated with herbal medicine (Figure 1).

There was an increase in serum creatinine levels for both men and women treated and not treated with herbal medicine (Figure 2).

In the group that included men, there was an increase in alkaline phosphatase for both treated and untreated participants, but in the group that included women, there was a decrease in the concentrations of alkaline phosphatase between treated and untreated participants (Figure 3).

Regarding average values of serum alanine aminotransferase (ALT), in the group including men, there was no significant change. In the group that included women, there were observed increases in mean ALT levels among treated and non-treated subgroups (Figure 4).

Considering the cohort mean serum Aspartate Aminotransferase values, no relevant changes were observed in the studied population (Figure 5).

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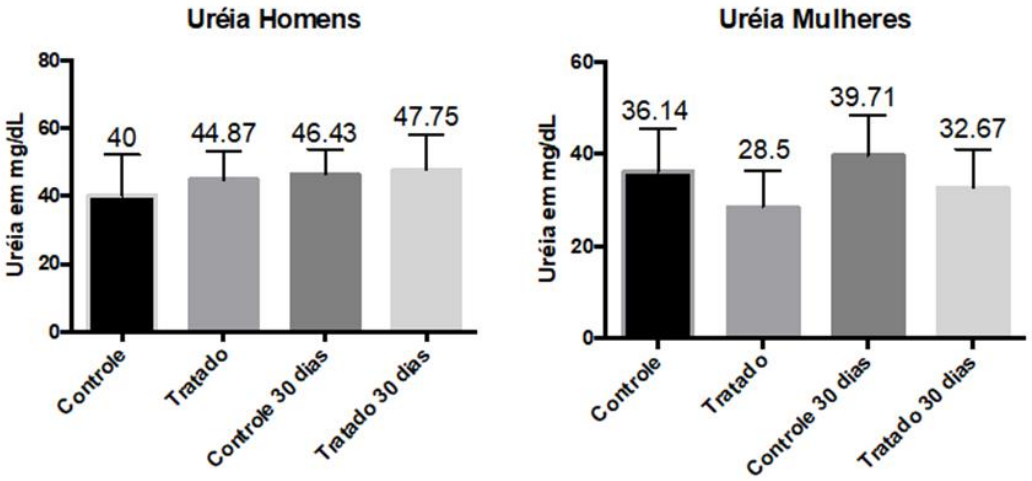

Figure 1 - Mean urea values obtained from both (A) men and (B) women who were included in the studies.

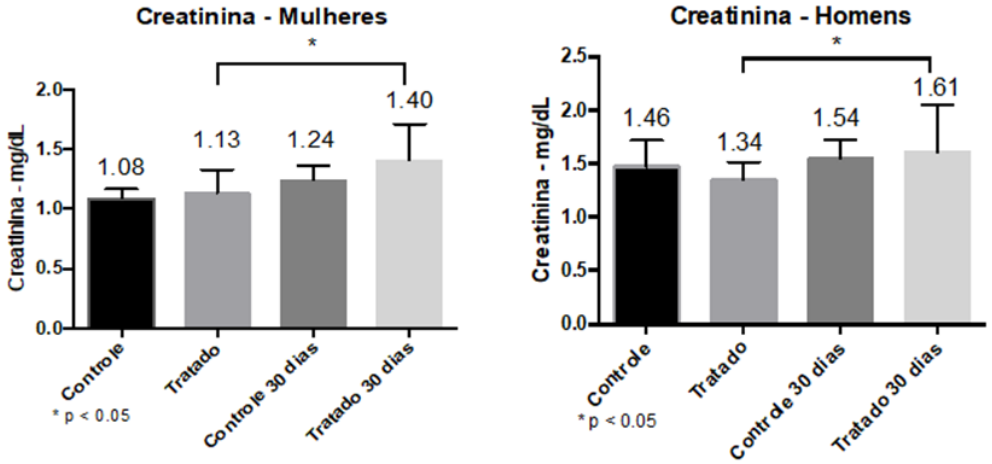

Figure 2 - Average creatinine values obtained from (A) men and (B) women subjects.

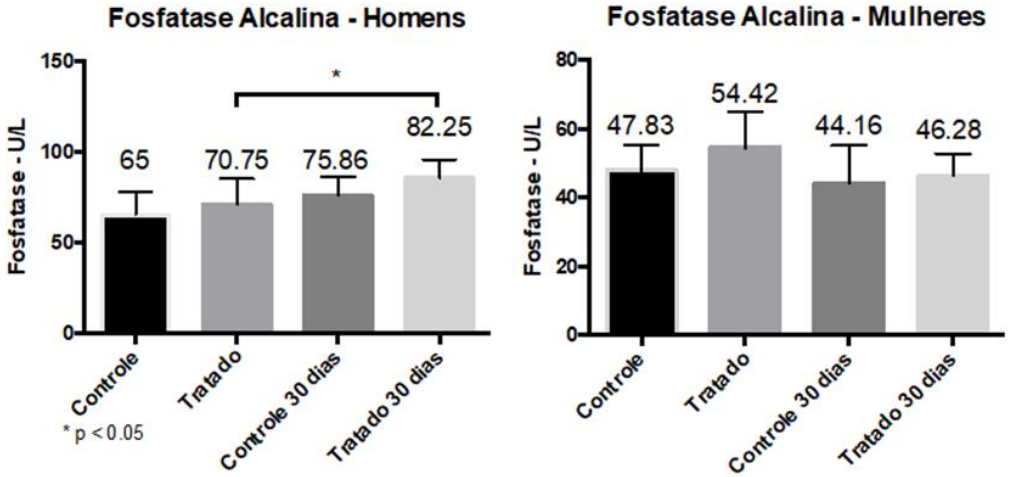

Figure 3 - Alkaline phosphatase average values observed in (A) men and (B) women subjects.

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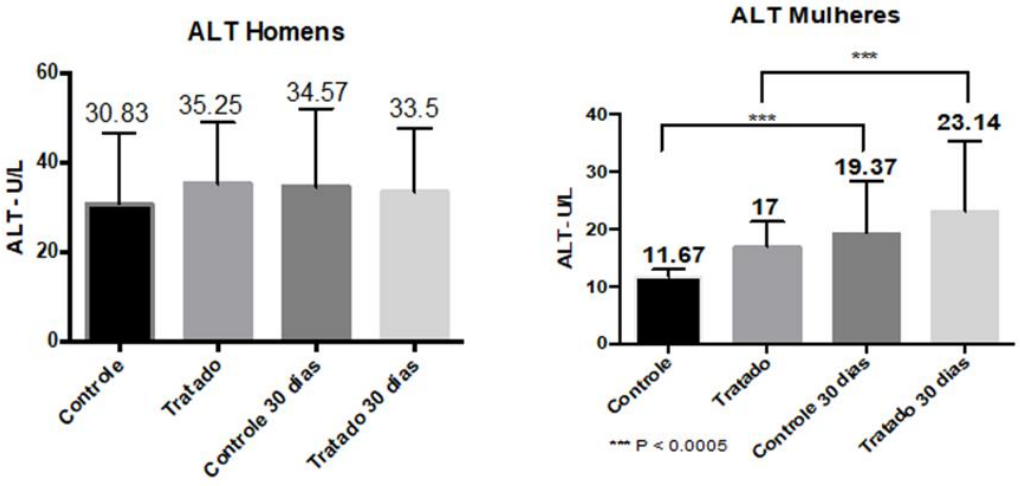

Figure 4 - Alanine Aminotransferase Mean Values Obtained from (A) Men and (B) Women Subjects.

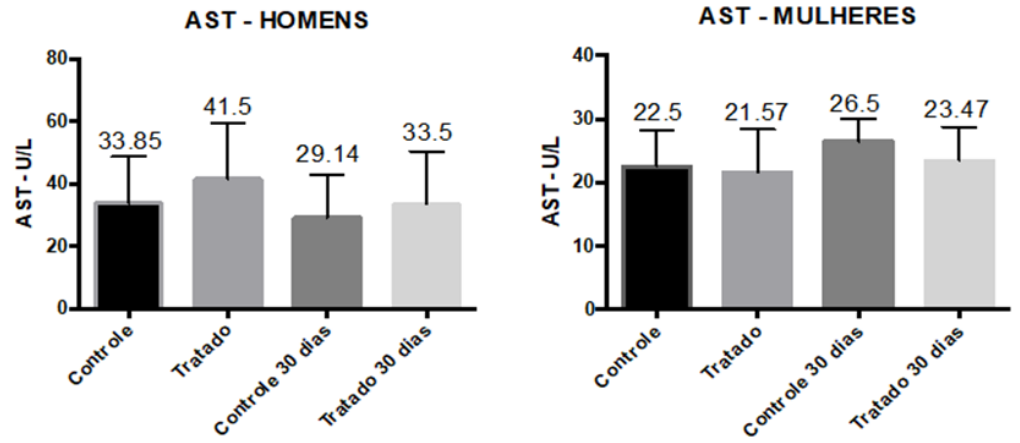

Figure 5 - Aspartate aminotransferase mean values observed in (A) men and (B) women studied.

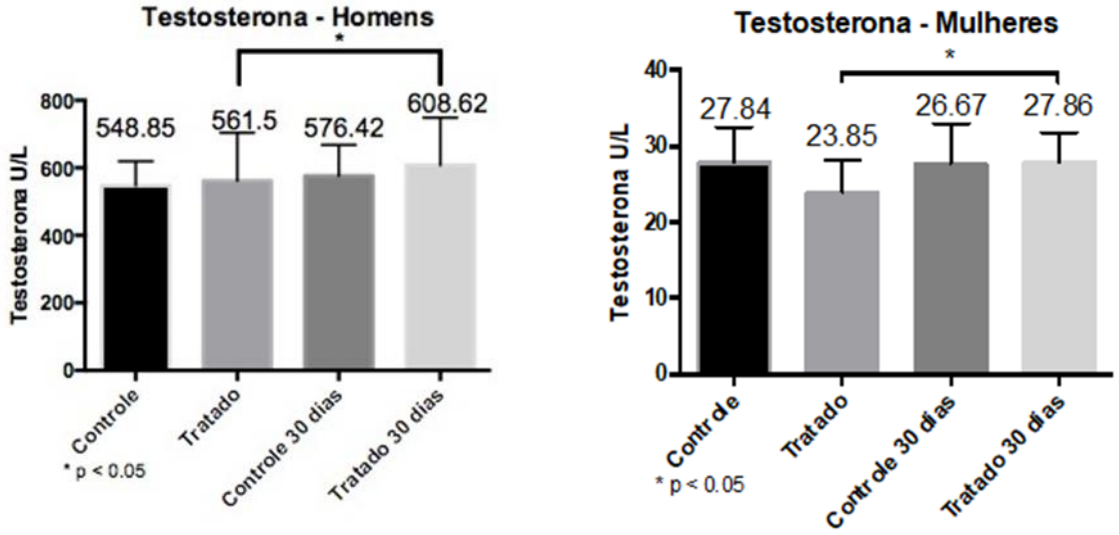

Figure 6 - Increase in mean testosterone values in both (A) men and (B) women treated with TT.

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A significant increase in serum testosterone levels was observed for both men and women within the groups treated with phytotherapy (Figure 6).

There was an increase in the percentage of body fat pertaining to the group of men not treated with herbal medicine and a decrease in the percentage of body fat in the group of women treated with TT according to bioimpedance method of analysis (Figure 7).

The Figures 8 and 9 show, respectively, the results related to average weight lifted in kg during the maximum resistant test (1RM) and average of skeletal muscle mass, in kg, for men and women groups.

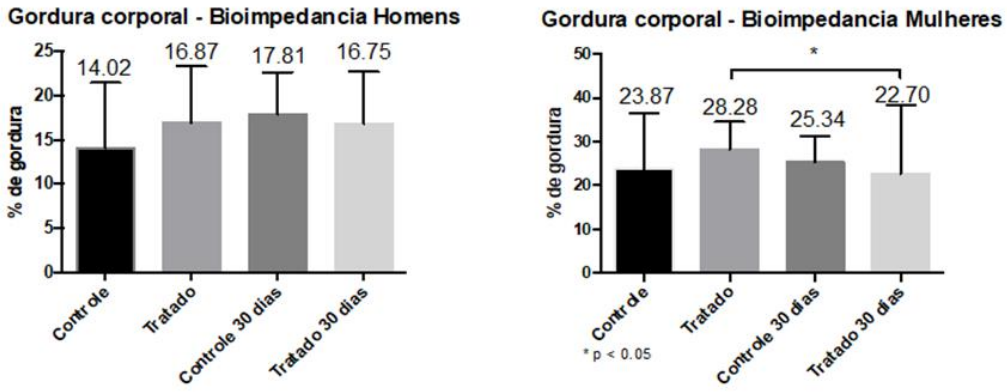

Figure 7 - Bio impedance analysis of body fat composition among treated and non-treated groups in both (A) men and (B) women.

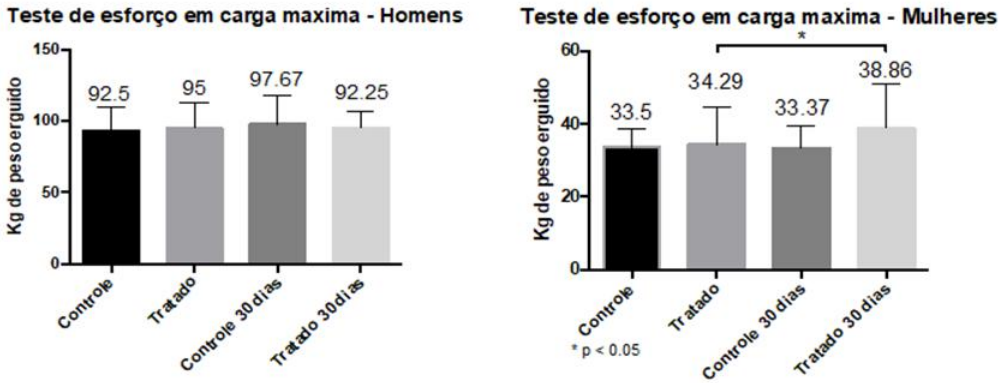

Figure 8 - Average weight lifted in kg during the Maximum Resistant (1RM) test for both (A) males and (B) women participants.

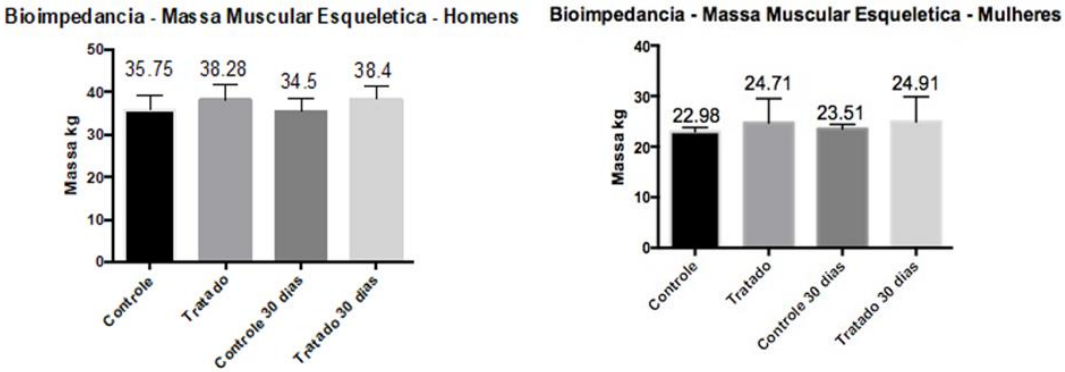

Figure 9 - Average of skeletal muscle mass, in kilograms, of the studied groups.

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DISCUSSION

Thirty-two participants were recruited for this study, Of the original subjects, 28 completed the treatment as designed, indicating a retention of 87.5%. For the total number of participating subjects who completed the study, all proposed tests were performed and data were obtained which represented significant differences in some of the evaluated parameters.

There was a relevant increase in creatinine levels for both men and women in the TT-treated group. However, it was observed that one individual in the group of treated men had a higher than average increase in creatinine levels, which skewed the statistical mean, but in average, all evaluated parameters remained within reference values.

According to Mraj (2016), TT increases the expression of antioxidant enzymes genes, reduces hyperoxaluria (urinary excretion greater than 40-45 mg / day of oxalate) and restores antioxidant enzymes in renal profile expression, which shows that the treatment diminished renal inflammatory epithelial damage and restores regular morphological glomerular function.

This fact is supported by observation of normal urea levels (within the reference value) before and after treatment.

According to González and Silva (2006), alkaline phosphatase taken together with measurements of other enzymes, enables analysis of liver function. Results of the present study showed a significant increase in alkaline phosphatase in treated men, but an increase in the levels of alkaline phosphatase was also observed among untreated men.

This increase may be due to increases in overall serum testosterone levels as well as possible changes in eating habits.

Regarding liver profile, alanine aminotransferase, another liver function marker enzyme, showed increase levels among the group of treated women, which may be related to increases in testosterone levels.

Here we evaluated total testosterone levels by employing the ELISA method. Results showed a significant increase in testosterone levels for both men and women who were treated with TT. This increase in overall testosterone levels is due to the fact that TT has 45% composition of a saponin called protodioscin (PTN). PTN is steroidal in nature, thus facilitating the steroidal pathway for production of androgens as intermediate molecules (Do et al., 2012).

Subjects reported 62% increased libido among the group of men treated with TT compared to 16% of men in the control group. In the female group there was an increase in libido in 28.5% of women in the TT-treated group, in comparison to 14.2% of women in the control group.

According to Qureshi et al., (2014), TT appears to have aphrodisiac side effects, probably due to its androgen-enhancing property.

There was a significant reduction in the percentage of body fat among women in the treated group followed by a slight increase in lean mass, however, in the placebo group there was a slight increase in the percentage of body fat as well as muscle mass.

In the exercise test, 1RM, there was an observed increase of 4.5kg ($p < 0.05$) in the group of women treated with TT. This increase may probably be linked to the increase in testosterone levels, which were evaluated in the preceding tests. Other results analyzed did not show significant differences.

CONCLUSION

Our results show that after the 30-day study period, it is appropriated to conclude that there was a significant increase in testosterone levels for both women and men in the TT-treated groups. There was also a decrease in body fat in the group of TT-treated women, and increased libido in both groups.

There were observable increases in strength levels for the treated women group. In regard to biochemical parameters, there was not much change observed, even when considering measurements that were considered as significant, since those fell within reference values. It should be noted, however, that further studies on the use and effects of Tribulus terrestris are needed in order to properly assess its safety.

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