

Tribullus Terrestris' Supplementation Improves the Antioxidant System of Resistance Trained Subjects

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ABSTRACT

Background: Numerous studies show the effect of herbal-based supplements on muscle mass and strength gain, associated with the prevention and/or reduction of oxidative stress damage. Association of Tribullus Terrestris supplementation with strength exercise has been widely used to increase muscle mass. However, there are few studies showing its effectiveness and biological action. **Purpose:** This study aimed to evaluate the effect Tribullus Terrestris' supplementation in the antioxidant system of resistance exercise trained subjects. **Methods:** Blood samples were collected from volunteers aged between 25 to 35, before and after eight weeks' supplementation with 500 mg per day of Tribullus Terrestris or talc (placebo). Blood was separated in plasma and erythrocytes for the following analysis: plasma TBARS and uric acid, and activity of superoxide dismutase, catalase and glutathione peroxidase. **Results:** Tribullus Terrestris supplementation induced an increase of antioxidant capacity (uric acid, SOD and CAT activity) and a decrease of TBARS level. **Conclusions:** Data obtained here conducted us to conclude that supplementation with Tribullus Terrestris may be beneficial to trained subjects' redox state, once it can protect body from the oxidative stress caused by the exercise practice, helping to improve their health and recovery.

Introduction

An alteration in the balance between Reactive Oxygen Species (ROS) production and antioxidant system can contribute to accumulation of oxidative damage. Antioxidant defense is composed by two distinct systems: non-enzymatic and enzymatic. An important non-enzymatic antioxidant compound is uric acid in plasma [1]. Uric acid is formed by the purine catabolism, where the enzyme xanthine oxidoreductase catalyzes a two steps reaction using hypoxanthine to form xanthine, and subsequently uric acid. This compound is considered a potent antioxidant and free radical scavenger, which can exert important physiological and biochemical functions acting as a first cellular defense line against plasma oxidative stress [2].

Under homeostatic physiological conditions, besides the amount of non-enzymatic compounds, the balance between ROS and antioxidant system is also maintained by some antioxidant enzymes (Superoxide Dismutase (SOD), catalase (CAT), and Glutathione Peroxidase (GPx)) [3,4]. It is well known that

moderate exercise training can improve the antioxidant system, which helps to maintain a stable redox status against a possible oxidative stress induced by exercise practice [5].

Table 1: Subjects baseline anthropometric characteristics.

Variable	Subjects baseline values (mean \pm SEM)
Age (years)	30 \pm 5
BMI (Kg/m ²)	25 \pm 3
Fat body (%)	22.5 \pm 3.46
Muscle mass (Kg)	66.7 \pm 6.2

All values are presented as mean \pm SEM.

Nowadays, in the attempt to increase performance and/or health, the use of supplements is becoming even more common. The use of formulations based on plant extracts, for example, is based on the goals of improving performance by induce hypertrophy, increase muscle strength, reduce body fat and improve recovery post-workout period [6]. Individuals looking to improve athletic performance, or even treating a health problem, may show interest in use medicinal plants. However, many of these formulations are used for these purposes with little or no scientific data to corroborate to the hypotheses mentioned above.

Tribulus Terrestris has been used in herbal therapy for treatment of sexual dysfunction, including erectile dysfunction and angina pectoris [7-9]. In addition, is suggested it can induce a lowering of cholesterol [10], and it is also considered as an energizer and vitalizing compound in Indian and Chinese medicine. Studies show Tribulus Terrestris mainly composed by alkaloids (harman and harmine), unsaturated fatty acids, phytosterols, flavonoids, nitrates, potassium, resins, saponins and tannins [11]. Furthermore, studies using castrated rats performed in New Zealand, has found a new constituent of Tribulus Terrestris, the protodioscin [12].

The number of scientific articles evaluating physiological effects of Tribulus Terrestris is very small and the parameters evaluated in majority of studies involve only plasma testosterone and muscle strength. Since this plant

is rich in flavonoids, saponins and other compounds is important to evaluate their effect on redox balance as well. Therefore, the aim of this study was to evaluate the effect of Tribulus Terrestris' supplementation in the antioxidant system of resistance trained male subjects. We hypothesized that the supplementation would be beneficial by inducing an augment of subjects' antioxidant system.

Material and Methods

Participated in this study 12 male volunteers who were already practicing resistance exercise (at least six months), aged between 25 and 35 years. We selected individuals who had no history of severe muscle damage, endocrine disorder, leukemia or use of hormonal or nutritional supplement. It was also excluded individuals who had cardiovascular problems, autoimmune diseases, who were using anti-inflammatory medications, doing hormonal replacement or those with a history of allergies to plant extracts.

Participants were randomly divided into two groups: Supplemented with Placebo (SGP) and Supplemented with Tribulus Terrestris (SGT). Baseline data of these subjects were used as control (CG) for all comparisons.

All participants signed an informed consent. The Ethics Committee of Cruzeiro do Sul University approved the study (CC/UCS-107/2012 Protocol).

1. Supplementation

Individuals were treated with 500 mg of Tribulus Terrestris extract per day (capsules) for 8 weeks. Placebo group was supplemented with 500 mg of encapsulated powder (talc) during the same period. The study was conducted following the double-blind crossover model.

2. Training

All volunteers underwent a resistance training for eight weeks (supplementation period), four times per week. Training consisted of working with three exercises for each muscle group, with an average of eight to ten repetitions and sixty to ninety seconds of rest [13].

Anthropometrics variables

The Body Mass Index (BMI) was calculated from the measured weight and height (BMI = weight (kg) /

height² (m²)). To estimate and validate the classification of groups, the percentage of body fat was determined using a tetrapolar bioimpedance (Biodynamics Corporation, 310, EUA). The bioimpedance measurements were performed as described by Lukaski [14]. The values are presented in Table 1.

3. Blood collection

Blood samples (10 ml) were collected in vacuum tubes containing EDTA as anticoagulant and maintained at 4 °C for about 30 minutes until processing. 16 mL of blood from each individual were collected. Blood was centrifuged at 200 g for 10 minutes and plasma and erythrocytes were separated.

4. Lipid peroxidation measurements

To perform the measurement of TBARS, we used the protocol proposed by Janero [15]. Briefly, for each assay was added 22.5 µL BHT (t-butyl hydroperoxide 2% in absolute ethanol) at 250 µL of plasma (centrifuged at 12,000 for 1 minute). Subsequently were added 250 µL of thiobarbituric acid (1% in 50 mM NaOH) and 250 µL of 25 % HCl, and the standard aliquots of 250 µL of 1,1,3,3-tetramethoxypropane were placed 10-1 mM. Tubes containing the mixture were incubated in a boiling bath (100° C) for 10 minutes and cooled in an ice bath. Then were added 730 µL of butanol to each tube and agitated by vortexing until there is a full transfer of the pink color of the lower layer to the upper layer. Then centrifugation was performed for 5 minutes at 12,000 rpm. Butanol phase was removed and absorbance measured at 532 nm in a spectrophotometer. TBARS concentration was then determined using the molar extinction coefficient of MDA ($\epsilon = 1.56 \times 10^{-5} \text{ M}^{-1} \cdot \text{mL}$) using the formula: = TBARS concentration ($\square_{\text{absorbance}} / 0.156$) x dilution = nm / mg protein.

5. Uric acid

Plasma uric acid content was evaluated using a biochemical kit from BioClin-Quibasa (Belo Horizonte, Brazil). In the assay mixture, H₂O₂ produced from uric acid in the presence of uricase (to form allantoin) is coupled with p-hydroxybenzoate and 4-aminoantipyrine, an oxidation catalyzed by peroxidase

to form a pinkish chromophore detected at $\lambda = 505 \text{ nm}$. Total uric acid released in plasma was calculated by determining area under curves within the time-span of t₀ and t₆₀ (AUC_{t0-t60}) [16].

6. Determination of total superoxide dismutase activity

Erythrocytes were washed in extraction solution containing 10 mM Na₂PO₄ pH 7.5. After homogenization, was added 1% Triton-X-100 and samples were incubated for 30 minutes at 4°C, and then centrifuged at 13.000 g for 20 minutes at 4°C. Aliquots of supernatant (5 µL) were used for measurement of total protein as described by Bradford [17].

SOD activity was monitored by overall reduction of cytochrome C (0.15 g/L) by superoxide radicals generated by the xanthine-xanthine oxidase system [18]. Firstly, background was determined by finding the appropriate amount of xanthine oxidase and assay buffer (10 mM cytochrome C, 100 mM xanthine in a 50 mM sodium phosphate buffer (pH 10.0)) to reach an initial absorbance nearest to 0.025. Then, it was added to each assay, 12 µL of sample and measurement was performed at 25 °C and 550 nm.

7. Determination of catalase activity

Catalase activity was performed according to the method previously described by Aebi [19]. The enzyme activity was determined by the consumption of H₂O₂ at 230 nm, 30 °C and pH 8.0. Reaction was monitored for 8 minutes using air as reference. In each assay were added 5 µL of sample, 15 µL of Tris-Base and 180 µL of 30 mM H₂O₂. To calculate catalase activity it was used the following equation: ($\square_{\text{absorbance}} / 0.071$) x dilution / mg protein.

8. Determination of glutathione peroxidase activity

Activity of glutathione peroxidase was determined by the method described by Wendel [20]. This determination occurs by monitoring the consumption of NADPH at 340 nm. Each assay was performed with addition of 10 µL of sample and 185 µL of stock buffer (143 mM sodium phosphate and 6.3 mM EDTA - pH 7.5) containing 0.25 mM NaN₃, 0.25 U/ml glutathione reductase, 1 mM GSH and 0.12 mM NADPH. After

incubation at 37 ° C for about 30 seconds, were added 5 µL of TBHP (t-butylhydroperoxide) and reaction was monitored for 10 minutes. 200 µL of stock buffer was used as reference. Results were expressed as nmol of TBHP reduced per minute per mg protein, considering the extinction coefficient factor of NADPH (ϵ NADPH) of 6.22 nM⁻¹cm⁻¹. Thus, activity of GPx was obtained according to the equation: GPx activity $\Delta = (\text{absorbance} / 6.22) \times \text{dilutions} / \text{mg protein}$.

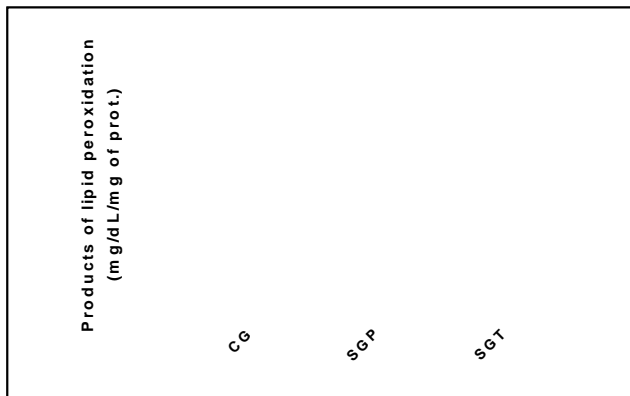


Figure 1: Plasma lipid peroxidation in individuals supplemented or with Tribullus Terrestrisora placebo. Results are presented as mean \pm SEM and considered statistically significant for $P < 0.05$. CG = baseline values; SGP = placebo group; SGT = Tribullus Terrestris group.

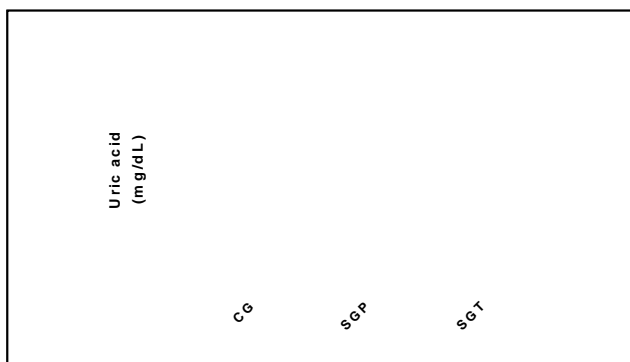


Figure 2: Plasma uric acid levels in individuals supplemented with Tribullus Terrestrisora placebo. Results are presented as mean \pm SEM statistically significant for $P < 0.05$. CG = baseline values; SGP = placebo group; SGT = Tribullus Terrestris group.

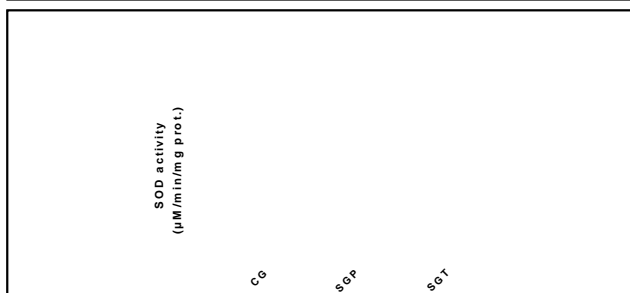


Figure 3: Erythrocytes' Superoxide Dismutase (SOD) activity of individuals supplemented with Tribullus Terrestrisora placebo. Results are presented as mean \pm SEM and considered statistically significant for $P < 0.05$. CG = baseline values; SGP = placebo group; SGT = Tribullus Terrestris group.

9. Statistical analysis

Data were presented as mean \pm standard error. Statistical analysis was performed using one-way ANOVA and Newman post hoc test. Results were considered statistically significant at $p < 0.05$. The software used was GraphPad Prism 5 (GraphPad software, inc., San Diego, CA, USA).

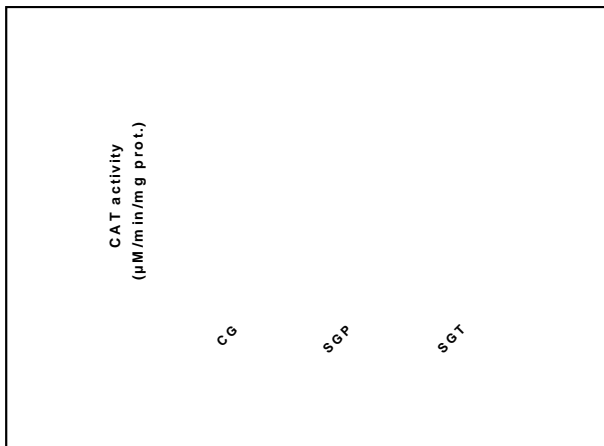


Figure 4: Erythrocytes' catalase (CAT) activity of individuals supplemented with Tribullus Terrestrisora placebo. Results are presented as mean \pm SEM and considered statistically significant for $P < 0.05$. CG = baseline values; SGP = placebo group; SGT = Tribullus Terrestris group.

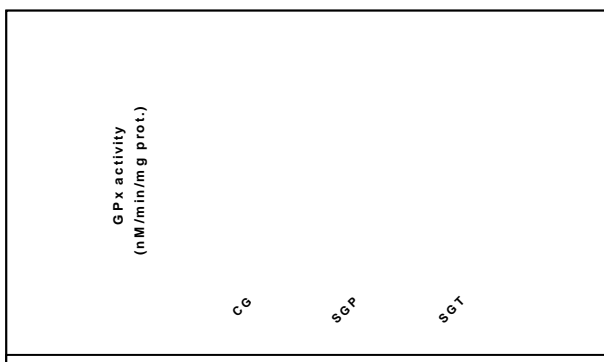


Figure 5: Erythrocytes' glutathione peroxidase (GPx) activity of individuals supplemented with Tribullus Terrestrisora placebo. Results are presented as mean \pm SEM and considered statistically significant for $P < 0.05$. CG = baseline values; SGP = placebo group; SGT = Tribullus Terrestris group.

Results

Body composition analysis showed to be no different among groups (data not shown).

TBARS and concentration of uric acid products were evaluated as oxidative stress biomarkers. An increase (16.68%) of TBARS level was observed in SGP group, when compared to baseline data (CG). However, SGT group presented a decrease (15.26%) on TBARS amount, comparing to CG. When compared

only supplemented groups, TBARS level on SGP showed to be higher (27.38%) than SGT (figure 1).

When evaluated plasma uric acid levels, an increase of 44% on SGT group was observed when compared to CG and SGP groups. No difference was found between SGP and CG groups (figure 2).

Antioxidant enzymes activities (superoxide dismutase, catalase and glutathione peroxidase) were also carried out. As observed in figure 3, superoxide dismutase activity was higher in SGT group when compared to baseline values and SGP group (60% and 64%, respectively). No difference between SGP and CG was found.

Catalase activity observed in the SGT group was positively modulated when compared to CG (70%) and SGP (100%). No difference was found when compared results obtained on SGP group versus baseline values (CG) (figure 4).

Glutathione peroxidase activity has presented lower values in SGT (43.86%) and SGP (23.56%) groups compared to baseline data (figure 5).

Discussion

We found in the present study that supplementation with a placebo did not induced any change comparing to baseline values, and that Tribullus Terrestris supplementation was shown to be effective by increasing antioxidant enzymes activities, uric acid concentration and reducing TBARS levels of subjects enrolled in a resistance exercise training.

Firstly, anthropometrics variables were evaluated, but no differences were detected between groups. After this sample characterization analysis, we evaluated oxidative stress biomarkers to access the possible antioxidant effect of Tribullus Terrestris during the practice of resistance exercise.

In the present study, it was found that supplementation with Tribullus Terrestris was effective in reducing TBARS level. Accordingly, McBride et al [21]. found an increase of MDA in plasma, after 6 and 24 hours of practice of a resistance exercise in a circuit with two-minute intervals in the first series and one minute and thirty seconds in the second (high intensity protocol). However, an increase of

MDA, immediately after the end of exercise practice, was found in a group that received supplementation with vitamin E for two weeks, similar to the results found in our study.

In addition to TBARS levels, uric acid content (an important antioxidant) was also evaluated to better analyze the redox status of individuals supplemented or not with Tribullus Terrestris. Supplementation with Tribullus Terrestris increased plasma uric acid levels. In a study performed by Huang [22], it was verified that use of flavonoids caused a significant increase in uric acid levels, explained by the decrease of xanthine oxidase enzyme activity. And it is known that Tribullus Terrestris has significant amount of flavonoids, which can, therefore, be associated with such results.

As previously described, the main defense enzymes that protect cells from damage caused by ROS are SOD, CAT and GPx. These three enzymes were evaluated in the present study. We found that SOD activity was greater in SGT group when compared to SGP and to baseline results (CG), suggesting a higher antioxidant capacity for this group. SOD is responsible to dismutate superoxide into hydrogen peroxide, and this reactive specie must be eliminated to avoid hydroxyl radical formation.

The enzymes responsible for this elimination of hydrogen peroxide are CAT and GPx [25]. We observed an increase of catalase activity in the SGT group when compared to the other groups. Thus, it is possible that supplementation with Tribullus Terrestris was capable to induce an efficient elimination of H₂O₂. However, these data also show that SGP group should be more exposed to ROS which may be increasingly produced during the resistance exercise.

However, GPx activity was lower in the supplemented groups (SGT and SGP) when compared to baseline values. This process may be related to the characteristics of GPx that shows a higher contribution at larger production of H₂O₂. Apparently, the increase of CAT activity was enough to control hydrogen peroxide generation induced by resistance exercise.

In summary, as we firstly hypothesized, supplementation with Tribullus Terrestris showed to be effective by

modulating the redox state of subjects enrolled in a practice of resistance exercise, which can favor practitioners' health and/or performance.

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