Original Communication

Tribulus Terrestris may decrease muscle damage markers following a high-intensity resistance exercise: A pilot study

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Abstract: The purpose of this study was to investigate the effect of two weeks of *Tribulus Terrestris* (*TT*) on the responses of Interleukin-6 (IL-6), high sensitivity C-reactive protein (hs-CRP), and enzymes creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) following a single session of resistance exercise (RE). Eighteen healthy non-athlete males (age: 22.44 ± 2.54 years, BMI: 26.15 ± 1.62 kg/m^2) participated in this study and were divided randomly into two 9-person groups of supplementation or placebo. The participants consumed two 250-mg capsules of *TT* or placebo (maltodextrin) per day and performed six REs with the intensity 80, 85, and 90% of 1RM in three circles at the day after the end of supplementation period. Blood samples were collected before the initiation of supplementation, and before and after the RE session. Total changes of IL-6 (p<0.001) and LDH (p=0.005) were significant in both groups. Bonferroni post hoc test showed that increased values of IL-6 and CPK in both groups were significant after exercise compared with pre-exercise and baseline (p<0.001). There were no significant differences in relation to within- and between-group changes in hs-CRP (p>0.05). Moreover, differences between the groups regarding post-exercise IL-6 and CPK were not significant (p>0.05). However, post-exercise LDH in supplementation group were lower than placebo group (p=0.015). In conclusion, short-term supplementation with *TT* has no effect on IL-6 and hs-CRP, but may be effective on the reduction of muscle damage enzymes CPK and LDH following high-intensity circuit RE.

Keywords: Inflammation, Interleukin-6, high sensitivity C-reactive protein, creatine phosphokinase, lactate dehydrogenase, acute responses

Long-term exercise training is capable of inducing anti-inflammatory adaptations. However, an acute bout of physical activity may lead to inflammation [1]. Although immune responses to aerobic exercise have been well studied, few studies have been conducted on the acute inflammatory and immune responses induced by a session of resistance exercise (RE) [2]. High-intensity RE can damage muscle myofibril and thus trigger inflammatory responses. In fact, the secretion of cytokines increases in response to intense concentric and eccentric muscle actions, which is a characteristic of most RE programs [2, 3]. Cytokines are a kind of proteins that affect the survival, proliferation, differentiation, and function of immune cells. Apart from neutrophils, damaged muscle cells and endothelial cells, skeletal muscle can also secrete cytokines as a result of the contraction of motor units [4]. Skeletal muscle-derived interleukin-6 (IL-6) was the first cytokine to be identified and is known as a characteristic of exercise variables such that exercise-induced increase in IL-6 is more noticeable than other cytokines [3, 5]. As a pro-inflammatory cytokine, IL-6 has various effects on body tissues, including stimulation of acute-phase protein synthesis, activation of the hypothalamic-pituitary axis, and heat production [6]. The function of some cytokines, especially IL-6, increases the synthesis of certain proteins in the liver, one of which is high-sensitivity C-reactive protein (hs-CRP) [7]. hs-CRP level in body fluids and serum is low in healthy individuals, but during inflammatory reactions such as high-intensity exercise, it can increase up to three thousand times its normal level [8]. hs-CRP plays an important role in the acute response of the immune system to inflammatory conditions and is a sensitive indicator of systemic inflammation as well as an independent predictor of cardiovascular events [6].

High-intensity exercise is also associated with increased muscle damage indices such as the enzymes lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) [9],

which have been mostly studied during RE. In fact, changes in these enzymes are a type of muscle response, and during muscle damage, serum levels of these enzymes increase due to changes in the membrane of the muscle [10]. Thus, increased serum concentrations of CPK and LDH following physical exercise is due to increased permeability and disruption of the membrane of muscle fibers that may occur during or after exercise. Moreover, production of free radicals may also result in some of these damages. Reactive oxygen species (ROS) produced during physical activity can oxidize and degrade protein structures, resulting in damage to the muscle fibers and eventually lead to inflammation [11]. Systemic inflammation plays an important role in the progression of certain diseases, such as atherosclerosis and diabetes. For this reason, over the past decade, researchers have been focusing on some important and sensitive inflammatory markers such as IL-6 and hs-CRP [8, 12]. Therefore, in the field of human health, establishing strategies to minimize these damages can be of great importance.

Given the observed muscle damage and inflammatory responses after physical activity, an important question is raised that how these responses could be minimized in different types of activities. Previous studies have shown that some herbal supplements may be effective in this regard. *Tribulus Terrestris* (*TT*) is a plant widely used to enhance muscle mass in combination with RE [13]. It is native to the tropical and desert regions and its fruit has been widely used in traditional medicine to treat various chronic diseases such as cardiovascular disease, urinary tract complications and sexual function improvement in men [14]. However, there are divergent findings about the effectiveness of *TT* supplementation in athletes and healthy adults [15]. Rogerson et al. [16] concluded that a *TT* extract (450 mg per day) taken by rugby players over five weeks did not produce the large gains in strength or lean muscle mass. In contrast, a 20-day supplementation period with *TT* resulted in a significant rise of muscular power and blood testosterone among youth men [17].

In addition to mentioned effects, it has been reported that *TT* has anti-inflammatory and antioxidant properties [18, 19]. Daily consumption of 500 mg of *TT* for eight weeks improved the resistance-trained men's antioxidant defense system [13]. It has also been observed that the consumption of this herb can reduce muscle damage indices caused by intense and heavy exercise training in male boxers [20]. However, there is insufficient information on the effect of *TT* on muscle damage and inflammatory responses caused by intense RE. Since investigation of these responses and using strategies to minimize them can be of great importance, the aim of the present study was to evaluate the effect of short-term consumption of *TT* supplement on the muscle damage (CPK and LDH enzymes) as well as inflammatory (IL-6 and hs-CRP) responses following a session of high-intensity whole-body RE.

Subjects and methods

The study population included non-athlete male college students. Following a notification call at the university, a total of 21 individuals volunteered to participate in the study, and 18 of whom were further selected as samples based on inclusion criteria such as age (18 to 25 years), no history of injury or illness, no history of cardiovascular diseases and no engagement in regular physical activity within the six months prior to the study. Exclusion criteria were smoking and recent use of any type of supplements and medications. This study was conducted in accordance with the Declaration of Helsinki. All participants were informed about the risks and benefits of participating in this study and further information on how to perform the study procedures was also provided for them. Finally, informed consent forms approved by the Human Studies Review Committee of Islamic Azad University were obtained from all participants.

The preferred method of the present study was repeated measures design. The participants were divided into two groups of supplement and placebo such that nine participants were randomly assigned to each group. In the first exercise session, the descriptive characteristics of the participants were measured, and they were then familiarized with how to perform exercises correctly. During the second session, the first blood sampling was performed to measure baseline values of the study variables. Subsequently, the maximum strength of muscle groups targeted in the RE protocol was determined using the one-repetition maximum test (1RM) as well as the Brzycki equation [21]. At the end of this session, bottles containing *TT* supplement or placebo capsules were randomly distributed among participants such that all of the 18 bottles containing the capsules (nine supplement and nine placebo bottles) were placed in a non-transparent bag and participants were asked to take one bottle. In this way, participants could be categorized into the supplement or placebo group in a random manner. The bottles were coded in a double-blind manner by a third person. Participants were instructed to consume two 250-mg capsules of *TT* supplement (Fruit of the plant containing $\approx$ 100 mg furostanol saponins, $\approx$ 27 mg total phenolic content, and $\approx$ 34 mg total flavonoids) that was a blend of Tribula Terrestris powder and extract, or placebo (maltodextrin) every day in the morning and evening after meal for two weeks. To ensure that the supplement and placebo capsules were identical, they were made in the same color and size to minimize the differences between them.

Participants were not engage in any form of structured exercise training and were also instructed to maintain their routine daily physical activity. In order to control energy intake, all participants recorded their food intake for two working days and one weekend day throughout the week before supplementation. Written and verbal instructions with regard to the type and portion sizes of daily food were provided. After analyzing food diary data, all participants received some instructions to reduce or increase energy intake and were asked to maintain this diet during the supplementation period. Furthermore, food diary was also completed 48 h before the RE session.

At the final day of supplementation period, the participants ate their last meal at 10 pm and attended the gym to perform RE session at 10 am under fasted condition. After a resting period of 30 minutes, the blood samples were taken before exercise. The participants were warmed up for 10 to 15 minutes, which included low-intensity exercises and stretching, and then RE session was performed. Circuit RE including bench press with Smith machine, leg press, lat pull down, leg extension and biceps curl barbell were performed in three sets of 80, 85 and 90% of 1RM with 8 to 10 repetitions. The rest interval between sets and exercises were allowed to be 45 and 120 seconds, respectively [22].

All of the blood samples were collected into the standard tubes in the same room at a temperature range of 22 to 24 °C. Each time, 10 ml of blood was taken from the participants' left arm vein. After separating serum by centrifugation at 3000 rpm for 10 minutes, the samples were stored at –20 °C for subsequent analyses at the appropriate time. The ELISA kit (Biovendor, Germany) was used to measure serum IL-6 level with a sensitivity of 0.65 pg/ml. hs-CRP was also measured by ELISA method using a specific kit (Binding Sit Group Ltd, UK) with a sensitivity of 0.01 mg/l. Serum concentrations of CPK and LDH were measured by Hitachi auto-analyzer (Model 902, Japan) using Pars Azmun kits (Tehran, Iran), with a sensitivity of one and five units, respectively.

Shapiro-Wilk test was used to determine the normal distribution of data, where a *p*value more than 0.05 was considered normally distributed. Repeated measures analysis of variance (ANOVA) and post hoc Bonferroni tests were then used for statistical analysis of intra-group differences. Observed differences of study variables were also compared between the two groups at measured time points using the independent-samples t-test with Levene's test for equality of variances, and mean difference (MD) was also reported. Moreover, an effect size statistic for each variable was reported using partial eta squared (η^2). SPSS for WINDOWS software program (version 18®; SPSS Inc, Chicago, IL) was used for data analysis and $p<0.05$ was considered as statistically significant.

Results

Characteristics of the study participants such as age, height, weight, and body mass index (BMI) are presented in Table 1.

Table 2 shows that there were no significant differences in the approximate amount of energy intake, carbohydrate, protein, and fat between the groups 48 h before the RE session.

The repeated measures ANOVA showed that total alterations of IL-6 values were significant among the participants ($p < 0.001$, $F = 383.35$, $\eta^2 = 0.98$). As a result, Bonferroni post hoc test was used to identify the differences, which revealed that post-exercise level of IL-6 in both *TT* supplement and placebo groups was significantly higher than pre-exercise values (MD in supplement group = 4.47 mg/ml; MD in placebo group = 4.77 mg/ml) as well as baseline levels (MD in supplement group = 4.51 mg/ml; MD in placebo group = 4.82 mg/ml) ($p < 0.001$). However, as shown in Figure 1-a, there was no significant difference between the supplement and placebo groups ($t = -0.70$, $p = 0.48$). In addition, as shown in Figure 1-b, the intra-group ($F = 2.00$, $p = 0.17$, $\eta^2 = 0.2$) and inter-group ($t = -1.28$, $p = 0.21$) alterations of hs-CRP values were not statistically significant.

The changes in serum CPK and LDH are illustrated in Figure 2-c and Figure 2-d, respectively. Although the total alterations in serum levels of CPK were not statistically significant ($F = 1.22$, $p = 0.31$, $\eta^2 = 0.13$), post-exercise values of this enzyme in the placebo group was significantly higher compared to its pre-exercise levels (MD = 38.11 IU/L) and baseline (MD = 38.33 IU/L) ($p < 0.001$). Likewise, the total changes of LDH in participants were statistically significant ($F = 3.96$, $p = 0.005$, $\eta^2 = 0.33$), but the results of Bonferroni post-hoc test did not show any significant difference ($p > 0.05$). On the other hand, in relation to CPK concentrations, the independent t-test indicated no significant difference between the supplement and placebo groups after RE ($t = -0.65$, $p = 0.52$). However, LDH level in the supplement group was significantly lower than the placebo group in response to RE ($t = -2.73$, $p = 0.015$).

Discussion

The aim of the present study was to investigate the effect of two-week intake of *TT* as an herbal supplement on serum concentrations of muscle injury and inflammatory factors in response to a session of high-intensity circuit RE. Based on comprehensive literature review, few studies have investigated the effect of this herbal supplement on these responses in human. Regarding the response of inflammatory factors to RE, the present findings revealed that the

Table 1. Mean (SD) of descriptive characteristics of the groups

	TT group (n=9)	Control group (n=9)	*P* value
Age (year)	22.6 (2.29)	22.2 (2.9)	0.72
Height (cm)	173.7 (5.35)	171.1 (8.7)	0.44
Weight (kg)	75.7 (5.71)	80 (9.91)	0.29
BMI (kg/m^2)	25 (1.39)	27.2 (1.06)	0.002**

TT = *Tribulus Terrestris*; BMI: body mass index. **significant differences between the groups.

Table 2. Mean (SD) of dietary intake of each group

	TT group	Control group	*P* value
Energy intake (Cal)	2516.2 (246.2)	2474.9 (333.9)	0.76
Carbohydrate (g)	435.8 (43.6)	425.3 (63.7)	0.69
Protein (g)	115.4 (12.4)	118.5 (19.9)	0.7
Fat (g)	41.5 (5.8)	38.6 (9.22)	0.44

TT = *Tribulus Terrestris*.

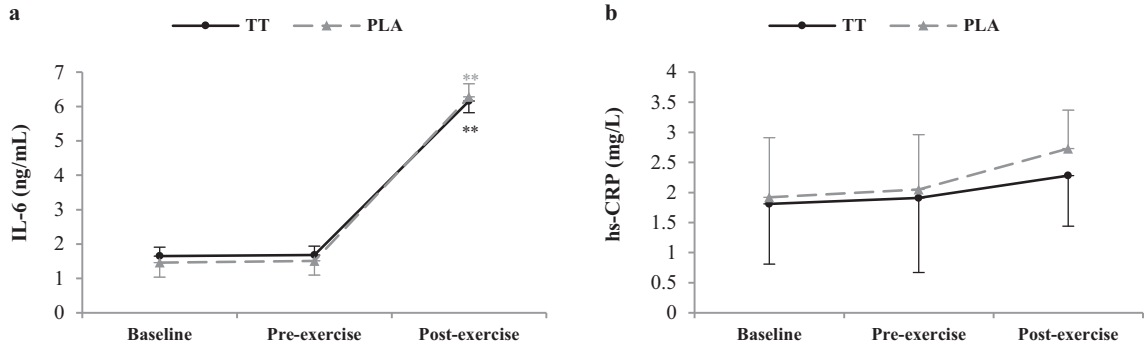

Figure 1. Serum levels of Interleukin-6 (a) and high sensitivity C-reactive protein (b) before a two-week supplementation (Baseline), and before (Pre-exercise) and following (Post-exercise) a session of high-intensity resistance exercise at the end of the supplementation period in *Tribulus Terrestris* (*TT*, n = 9) and placebo (PLA, n = 9) groups. Data are shown as means (solid circles and triangles) ± SD (error bars). **$p < 0.01$ vs. baseline and pre-exercise.

mean concentration of IL-6 in the supplement and placebo group increased by approximately 274% and 346%, respectively, which was statistically significant ($p < 0.001$); however, there was no difference between the groups ($p > 0.05$). Also, post-exercise hs-CRP levels in the supplement and placebo group increased by approximately 64% and 46%, respectively, which was not statistically significant (p> 0.05) and there was also no difference between the two groups ($p > 0.05$).

The responses and adaptations of RE to inflammatory factors have been studied in various experiments [1, 23–26]. RE protocols are diverse in terms of the performed exercises. Moreover, intensity, duration, number of sets, and repetitions of RE can have an impact on the responses to this type of exercise. In the present study, a session of intense circuit RE was performed and the resting interval was adjusted to 45 seconds between each set so that we were able to investigate alterations in muscle injury and inflammatory factors in the participants. Additionally, in order to determine the net effect of *TT* supplement on the desired responses, non-athlete participants were recruited to exclude the effects of exercise experience or previous consumption of supplements from the results.

In the present work, it was observed that one session of high-intensity circuit RE could induce an increase in serum IL-6 levels and a two-week intake of the *TT* supplement had no effect on the magnitude of this increase (Figure 1). Our results are consistent with the findings of Phillips et al. [23] and Mendham et al. [24]. In the study conducted by Phillips et al. [23], it was reported that IL-6 levels were higher after low-intensity (65% of 1RM) and high-intensity (85% of 1RM) exercise compared to the control condition. This value was also reported to be higher after low-intensity exercise compared to high-intensity exercise. The authors concluded that in comparison with the intensity of the exercise, the overall volume of lifted weights was more

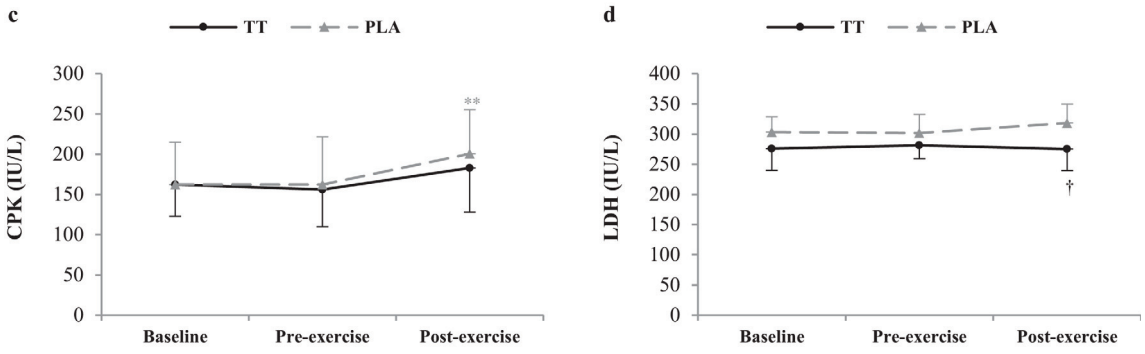

Figure 2. Serum levels of creatine phosphokinase (a) and lactate dehydrogenase (b) before a two-week supplementation (Baseline), and before (Pre-exercise) and following (Post-exercise) a session of high-intensity resistance exercise at the end of the supplementation period in *Tribulus Terrestris* (*TT*, n = 9) and placebo (PLA, n = 9) groups. Data are shown as means (solid circles and triangles) ± SD (error bars). $^{**}p < 0.01$ vs. baseline and pre-exercise; $^{\dagger}< 0.05$ vs. PLA group.

correlated with the release of IL-6. On the other hand, in another study carried out by Mendham et al. [24], it has been shown that moderate-to-intense protocols lead to a significant increase in IL-6 compared to low-intensity protocols. In the present study, the different exercise intensities were not compared, but the total IL-6 levels in both groups increased in response to high-intensity RE (80 to 90% of 1RM).

The obtained results of the present study regarding serum hs-CRP revealed that its concentration did not change following a high-intensity RE session in both supplement and placebo groups. These results are not in line with the findings of Mendham et al. [24] who reported that plasma CRP levels increased in response to a RE protocol at 80% of 1RM compared to the protocols at 60% of 1RM. The primary cause of such discrepancy could be attributed to differences in subject characteristics (non-athlete versus athlete men) and RE protocol. In the Mendham et al. experiment [24], there were seven exercises performed with a 90-second rest interval; whereas, in the present study, six exercises with a two-minute rest interval were performed. According to the previous studies, it seems that low-intensity resistance training (40 to 60% 1RM) does not significantly increase the acute response of CRP following exercise [27]. On the other hand, resistance training for one month has been reported to decrease the levels of CRP in healthy diabetic rats [28]. Reduced levels of CRP and IL-6 due to long-term resistance training (eight weeks) in sedentary middle-aged men have also been reported [29]. Taken together, these results indicate the effects of long-term RE on inflammatory factors (hs-CRP and IL-6) are favorable.

Concerning muscle damage indices, our findings showed that the mean level of CPK after exercise was increased by approximately 19% following *TT* supplementation for two weeks. Likewise, the mean level of post-exercise CPK was increased by approximately 27% in the placebo group. The alterations were significant in the placebo group ($p < 0.001$), but there was no difference between the two groups ($p > 0.05$). Furthermore, the mean concentration of post-exercise LDH was decreased by approximately 2% in the supplement group and was increased by approximately 6% in the placebo group. These differences between the two groups were statistically significant ($p = 0.015$). CPK is an intramuscular protein that is released into the bloodstream during muscle damage and is an accurate indicator of cellular damage. This enzyme is associated with damage caused by mechanical stimuli and many factors affect its concentration in blood [30]. In this study, it was observed that after a session of RE, the serum levels of CPK of the participants in the placebo group were significantly higher than those of the baseline and pre-exercise levels. However, this result was not observed in the supplement group. Given that exercise intensity does not affect serum CPK in response to one session of RE [10, 31], it seems plausible that the intake of *TT* supplement is the possible reason for this finding. However, it should also be noted that the peak of CPK accumulation is reported 24 to 72 hours after the exercise. Given the limitations of the present study, we did not measure the concentration of this enzyme in the aforementioned time periods.

Our findings indicating an increase in CPK levels in response to RE are consistent with the results of other studies [31, 32]. For instance, Rodrigues et al. [32] investigated the serum concentrations of CPK and LDH in 20 non-athlete men, with a mean age of 19 years, in multiple time periods after a RE session. RE protocol consisted of five upper-body exercises in three sets with an intensity of 80% of 1RM. Serum CPK and LDH levels were measured 24, 48 and 72 hours after each session. The results revealed that the concentration of CPK in response to relatively intense RE was increased, reaching its peak 48 hours after exercise and this response was independent of the intended rest interval during exercise.

The findings of the present study regarding LDH enzyme support those of other researchers [31–33]. Subjects who participated in the study conducted by Sheikholeslami Vatani et al. [33] were female active college students and the RE protocol consisted of seven exercise that were performed in five sets with 12–15 repetitions and 50% of 1RM. They investigated the effect of branched-chain amino acid supplementation on the responses of muscle damage markers caused by a session of RE and the results showed a significant increase in LDH following exercise. It is worthwhile noting that despite the relatively low-intensity of RE (50% 1RM), levels of muscle damage indices were increased significantly up to 24 hours after exercise. Therefore, gender may be an influencing factor in the severity of muscle damage responses in human subjects. In the current experiment, the CPK and LDH levels of the participants in the supplement group were lower than those of the placebo group before and after the exercise, but this decrease was statistically significant only for the LDH enzyme. It is possible that consumption of *TT* supplement at higher doses or for a longer period of time leads to a significant improvement in cellular damage responses to a session of RE, which could be due to the natural antioxidant and anti-inflammatory properties of *TT*. Thus, future studies in this area would be helpful.

Limitations and strengths

The novelty of our study is assessing the effect of a popular herbal supplement among strength/power athletes on the most important inflammatory and muscle damage responses after exercise. Strengths of this study include its double-blind placebo-controlled randomized design, inclusion of non-athlete subjects to overcome the possible effects of previous exercise experience, and control of dietary intake during the days before the exercise.

Despite these strengths, this pilot study has some limitations which need to be taken into account when interpreting our findings. The main limitation is the small sample size that limits the generalizability of the results. Therefore, more research with larger sample size is warranted. Although all participants were instructed to maintain their routine daily physical activity throughout the trial, the exact amount of this factor was not controlled separately.

Conclusions

The findings of the present study showed that the intake of *TT* supplement did not affect the inflammatory responses of IL-6 and hs-CRP in non-athlete young men following a session of intense circuit RE. However, short-term consumption of *TT* supplement may have favorable effects on serum CPK and LDH responses following exercise, which was not observed in the placebo group.

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History
Received December 17, 2019
Accepted March 9, 2020
Published online May 7, 2020

Acknowledgement
The authors would like to thank the participants in this study.

Conflict of interest
The authors declare that there are no conflicts of interest

Ethical approval
This study was conducted in accordance with the Declaration of Helsinki. All participants were informed about the risks and benefits of participating in this study and further information on how to perform the study procedures was also provided for them. Finally, informed consent forms approved by the Human Studies Review Committee of Islamic Azad University were obtained from all participants.

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