The Effect of Tribulus Terrestris Supplementation on Inflammation, Oxidative Stress and Performance of Recreational Runners: Study Protocol for a Double-Blind Randomized Controlled Trial

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Research Article

Keywords: Tribulus terrestris, Recreational runners, EIOS, Oxidative stress, Inflammation, EIMD

Posted Date: September 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-507103/v1

Abstract

Background: High intensity and endurance exercises lead to exercise-induced oxidative stress (EIOS), exercise-induced muscle damage (EIMD), and inflammation, which are the influencing factors on muscle soreness, localized swelling, and sport performance. Therefore, the purpose of this study is to determine the effectiveness of Tribulus terrestris (TT) as an herbal supplement with antioxidant and anti-inflammatory properties on the nutritional, oxidative stress, and anti/inflammatory status, as well as the sport performance of recreational runners.

Methods/design: This study is a double-blind, randomized, placebo-controlled trial, which will be conducted among recreational runners of Tabriz stadiums, Iran. Thirty-four recreational runners will be selected, and participants will be assigned randomly to two groups: to receive 500 mg TT supplement or placebo capsules twice daily for two weeks. Both groups will do the high-intensity interval training (HIIT) workouts during the study. Baseline and post-intervention body composition, muscle fatigue, and soreness parameters will be assessed. In addition, assessment of malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), interleukin-10 (IL-10), creatine kinase (CK), lactate dehydrogenase (LDH), insulin-like growth factor-1 (IGF-1) and brain-derived neurotrophic factor (BDNF) will be done during three blood samplings.

Discussion: This study will be the first to assess the potential effects of TT in recreational runners. Our results will contribute to the growing body of knowledge regarding TT supplementation on the nutritional, oxidative stress, anti/inflammatory status and sport performance in recreational runners.

Trial registration: Iranian Registry of Clinical Trials (www.irct.ir) (ID: IRCT20150205020965N8). Registration date: 13 February 2021.

Background

The World Health Organization indicates that regular physical activity is well-known for providing essential health benefits and preventing chronic and non-communicable diseases, including heart disease, stroke, diabetes, and several cancers (1). Even so, several studies have proposed that intensive and unaccustomed exercises can lead to impaired muscle function, athletic performance, and recovery (2, 3). High intensity and endurance exercises lead to an imbalance between oxidants and antioxidants in favor of the oxidants defined as exercise-induced oxidative stress (EIOS) (4). EIOS and the high level of reactive oxygen species can contribute to muscle damage (2, 4). Besides, it should be noted that prolonged muscle contractions can result in a condition called exercise-induced muscle damage (EIMD). EIOS and EIMD are the influencing factors that result in muscle soreness, localized swelling, increased levels of creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin (MYO), and inflammatory markers including C-reactive protein (CRP), Interleukin 1 (IL-1), Interleukin 6 (IL-6), TNF-α (3, 5) which lead to poor athletic performance. Considering the point that oxidative stress is the leading factor of the

mentioned phenomenon, there is a growing interest in the use of antioxidant supplements by physically active individuals. Also, it seems that supplements with antioxidant and anti-inflammatory properties will provide more favorable effects. Tribulus terrestris (TT) is one of the herbal supplements with mentioned properties.

TT is a native plant of Iran classified in the family Zygophyllaceae (6, 7). It can be found in a wide range of warm and humid regions such as the Mediterranean, Asia, Australia, Africa, and the warm areas of Europe (8) and contains a high concentration of active ingredients such as sterol saponins, flavonoids, tannins, terpenoids, phenol carboxylic acids, and alkaloids. The leaves, seeds, and fruits of TT are used for therapeutic purposes; however, studies indicate that the highest amount of active ingredients are found in its fruit (6, 8). Studies concentrating on the therapeutic effects of TT have assessed its potential effects regarding sexual enhancement (9), fertility (10), urinary tract stones (11, 12), diabetes (13, 14), cardiovascular disease (15, 16), antioxidant properties (17, 18), anti-inflammatory properties (19, 20). Therefore, a wide range of clinical studies has assessed TT effects. Still, considering the point that there are few randomized clinical trials on the effect of TT in athletes (21) and physically active individuals (22) and also there is no randomized clinical trial in recreational runners; this study has been designed to assess the efficacy of TT on the nutritional, oxidative, inflammatory, and anti-inflammatory status and sport performance of recreational runners.

Main Aim

The present study is designed to determine the effect of TT supplementation on the nutritional, oxidative, inflammatory, and anti-inflammatory status and sport performance in recreational runners.

Primary objective

To assess the effect of TT supplementation on malondialdehyde (MDA), Total antioxidant capacity (TAC), Superoxide dismutase (SOD), hs-CRP, IL-6, IL-10, CK, LDH, and sport performance of recreational runners with TT supplementation and high-intensity interval training (HIIT) training.

Secondary objectives

To assess the effect of TT supplementation on nutritional status (energy and macronutrient intake), insulin-like growth factor-1 (IGF-1), brain-derived neurotrophic factor (BDNF), and body composition of recreational runners with TT supplementation and HIIT training.

Method

Study design and setting

This study is a double-blind, randomized, placebo-controlled trial, which will be conducted among recreational runners of Tabriz stadiums, Iran. The study protocol followed the Standard Protocol Items:

Recommendations for Clinical Interventional Trials (SPIRIT) guidelines (Additional file 1, SPIRIT Checklist), and the study protocol diagram is shown in Fig. 1 (23). Subjects will undergo two body composition analyses and three blood samplings. Also, written informed consent will be obtained before initiating any research procedures (Additional file 2). An identification code will be given to each participant, and all collected data will be identified by that in order to maintain participant confidentiality. The flowchart of the trial is presented in Fig. 2. The research protocol is approved by the "Ethical Committee of the Tabriz University of Medical Sciences" and registered on the "Iranian Registry of Clinical Trials" website (www.irct.ir/, IRCT20150205020965N8) and is in compliance with the declaration of Helsinki ethical principles.

Participants recruitment

After a 2-week run-in period, a total of 34 recreational runners (each group 17) will be recruited by advertisements from Tabriz stadiums, Iran. The volunteers will be screened by an initial face-to-face screening visit. In an organized meeting, participants will be provided with information, including study procedure, requirements, possible risks, and benefits. A participant information sheet detailing study aims and requirements will be provided too. The study population will include volunteer participants who have the eligible criteria.

Eligibility

The volunteers will be included in the study if they (1) are healthy (confirmed by PAR-Q questionnaire under the supervision of a physician); (2) aged 18 to 35 years; (3) do running workouts for at least three days a week (240 minutes per week) during the last two years; (4) have a stable body mass during the last five months (changes less than 3 kg); (5) not receiving TT supplement, other antioxidant supplements in the last three months; (6) abstained from any high-intensity interval training during the last three months and (7) are willing to cooperate in the study. Exclusion criteria include (1) musculoskeletal injuries; (2) smoking; (3) alcohol consumption; (4) hormone therapy; (5) long-term use of drugs and dietary supplements; (6) pregnancy; (7) lactation; (8) diabetes; (9) anemia (Hb < 13g / dl); (10) cardiovascular disease, (11) infectious diseases; (12) malignancies; and (13) cognitive disorders.

Sample size

Sample size was estimated using PASS software (version 15). The sample size calculation was done according to CK changes in Ma et al. study (21). A total of 17 recreational runners were calculated in each group (1) (TT supplementation and HIIT training) and (2) (maltodextrin supplementation and HIIT training) based on CK changes, with a 95% confidence level, 90% power, and the additional drop-out rate of 15%.

Exercise protocol

This trial will use a HIIT exercise protocol as a high-intensity exercise activity in recreational runners. The participants will do the HIIT program for two weeks (5 training sessions per week; a total of 10 sessions

during the study period). A 15-minute warm-up (with a variety of stretching, flexibility, walking, and running) will include each session. The main activity of both groups consists of two sessions with 3–4 repetitions and 15–30 seconds of running with an intensity of 80–95% of the reserve heart rate (pressure perception 16 to 19) in each repetition. After each repetition and after each period, there will be 90–180 seconds of active rest and 2.5-4 minutes of active rest, respectively (active rest in the range of 40–50% of heart rate reserve) (24).

Supplementation protocol

Participants will consume the TT fruit supplement (TT, Dayan Pharma Co, Iran) or placebo (maltodextrin, Jiujiang Hurirong Trade Co, China) in random order. The supplement and placebo capsules will have perfectly the same shape, color, odor, and size. Both the TT and placebo capsules will be provided to participants weekly for two weeks. Subjects will be randomly assigned to two groups to receive 500 mg supplement or placebo capsules, twice daily, for two weeks. Participants of both groups will be required to consume capsules twice daily (oral administration after breakfast and lunch).

Randomization and blinding

The recreational runners meeting the inclusion criteria will be randomized to receive either the TT or the maltodextrin capsules. Participants will be assigned to a 1:1 ratio to one of the groups (1): TT supplementation and HIIT training and (2): maltodextrin supplementation and HIIT training. Randomizations will be conducted by a research assistant using random allocation software (RAS) via randomization blocks. To achieve a balanced distribution, stratified randomization will match participants based on VO_{2max} and gender distribution. Investigators, participants, and the statistical consultant will be blinded to the assignment until the end of the study too.

Adherence and compliance

Participants will be asked to return any unused capsules to assess their level of compliance and adherence. Subjects will receive supplements every week and will be asked to bring all remaining ones of their last visit. If less than 90% of the capsules were used, the person would be excluded from the analysis. Besides, the adherence to the training program will be assessed by the number of sessions attended.

Questionnaires

Four types of questionnaires will be completed by each subject at the beginning and end of the study: socio-demographic, 24-h food recall, the Visual Analogue Scale (VAS) (25), and the physical activity readiness questionnaires (PAR-Q) (26). Initially, PAR-Q will be used as a pre-study screening questionnaire that assesses a person's eating habits, lifestyle, medical history, and physical fitness in several items; answer yes to any of the items in the questionnaire means that participant will not enter to study. The demographic data (name, age, gender, marital status, occupation, and income level) will be collected. A 3-day 24-h dietary recall questionnaire will be obtained on two workdays and one weekend to assess the recreational runner's dietary intake. The collected data will be analyzed using N4 software

(NUTRITIONIST 4, First Data Bank, San Bruno, CA, USA). Also, a VAS will be used to determine the recreational runner's muscle fatigue and soreness (25).

Participant safety and withdrawal

The study team will monitor participants daily during the trial, and any occurrence of adverse events will be reported. Participants will be withdrawn from the study under the following conditions:

1. If less than 90% of the capsules were used each week, the person would be excluded from the analysis.
2. If less than 90% of training sessions participated each week, the person would be excluded from the analysis.

Any adverse events in the current study will be reported, regardless of the possibility of a causal relationship.

Outcome Measurements

Muscle fatigue and soreness assessment

A VAS will be used to determine the recreational runner's muscle fatigue and soreness (25).

Body composition analysis

The body composition of the participants will be measured using bioelectrical impedance analysis (Tanita BC-418, Tanita Corp., Tokyo, Japan).

Laboratory investigations

Blood sampling

Blood sampling will be done during the three stages; before the intervention, immediately after the last training session on the fourteenth day, and 24 hours after the previous training session. A 10-mL blood sample will be obtained during the three stages; before the intervention, immediately after the last training session on the fourteenth day, and 24 hours after the last training session by a lab technician and stored in a heparinized tube. Plasma centrifugation will be carried out at a speed of 1500 g for 20 min. Then, the plasma will be collected into separate micro-tubes and placed in a freezer at a temperature of -80°C before laboratory analysis.

Protocol amendments

Any protocol changes or amendments will be reviewed and approved by the all study authors. Any changes or amendments in protocol will be reviewed by the principal investigator and approved by the other study investigators. Any modifications, will be eventually reported.

Data collection and management

MN will collect data from questionnaires. After completion of questionnaires, MN will assess the collected data and, if there were any discrepancies in answers, the questioner will be requested to answer more clearly to reduce bias.

Statistical analysis

Statistical analysis will be done using SPSS version-24 software (SPSS, Inc. Chicago, IL, USA). The Kolmogorov-Smirnov test will be used to examine the normal distribution of variables. The independent t-test and analysis of covariance (ANCOVA) will be used for comparison of quantitative variables with normal distribution between the two groups at the beginning and the end of the study, respectively. Mann-Whitney test will be used for quantitative non-normal variables. In order to compare the mean changes of the baseline and end of the trial, the paired-sample *t*-test and Wilcoxon test will be used for normal and non-normal data, respectively. The two-way analysis of variance (ANOVA) with repeated measures and Sidak post-hoc test will be used for repeated measured variables. $P < 0.05$ will be considered statistically significant in all tests.

Ancillary and post-trial care

The trial is not expected to cause any specific side effect. All participants will be guided to personally make decisions about the supplement intake during high-intensity exercise training.

Discussion

The interest in natural supplements to promote athletic performance has increased these days among athletes. Recently, TT supplementation has also been raised in various exercises due to its beneficial consequence, which was seen in strength athletes (21). In the study of Ma *et al.* conducted on boxers, it has been indicated that TT lowered IGF-1 binding protein-3 levels, which may improve IGF-1 bioactivity, as well as increase muscle strength and decrease post-exercise CK levels when used in conjunction with training (21). In a recent study pilot study, Talemi *et al.* showed that TT might be effective in the reduction of CK and LDH following the high-intensity resistance exercise in non-athletes (27). According to a review done by Zhu *et al.*, it has been shown that the TT has a wide range of healthful properties, including antioxidant activity, anti-inflammatory activity, antitumor activity, antibacterial activity, hepatoprotective activity, anthelmintic and larvicidal activity, anticaries activity, antiaging and memory improvement activity (28). Also, it is worth mentioning that the chemical constituents steroidal saponins and flavonoids, which have prominent anti-inflammatory and antiaging activities, were found to be the key contributors to conventional pharmacological activities (28). TT can promote athletic activity and decrease fatigue due to its antioxidant activity and anti-inflammatory activity; In addition, it has been shown that TT supplementation may result in decreased IGF-1 binding protein-3 levels and increased IGF-1 bioactivity. IGF-1 is the upregulator of muscle growth and muscle strength which leads to muscle hypertrophy, repair of muscle damage, and eventually the promoted performance (21, 29, 30).

To the best of our knowledge, this study will be the first to assess the potential effects of TT in recreational runners. The present protocol is based on a randomized placebo-controlled clinical trial design, which presents the strongest empirical evidence regarding the TT supplementation and determines the causality. Besides, clinical control over participants' diet and repeated blood collection in this study will provide more precise details.

Of course, it should be noted that this is a short-term study that will necessitate additional research in the long run. Due to financial constraints, we did not analyze the other inflammatory and oxidative parameters. Furthermore, our protocol is designed for recreational runners and cannot be applied to elite runners. The small sample size and sample size calculation based on one parameter in this study should be considered too.

Trial Status

The present protocol is version 1, dated 25 January 2021. The trial has not yet begun, and the process of recruiting participants is ongoing.

Abbreviations

TT
Tribulus terrestris; EIOS:exercise-induced oxidative stress; EIMD:exercise-induced muscle damage; HIIT:high-intensity interval training; MDA:malondialdehyde; TAC:total antioxidant capacity; SOD:superoxide dismutase; hs-CRP:high-sensitivity C-reactive protein; IL-6:interleukin-6; IL-10:interleukin-10; CK:creatine kinase; LDH:lactate dehydrogenase; IGF-1:insulin-like growth factor-1; BDNF:brain-derived neurotrophic factor; VAS:Visual Analogue Scale; PAR-Q:physical activity readiness questionnaires

Declarations

Ethics approval and consent to participate

This trial has been approved by the ethical committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1399.1010). In addition, it has been registered on the "Iranian Registry of Clinical Trials" website (www.irct.ir/, IRCT20150205020965N8). All informed consents will be obtained by M.N from all participants.

Consent for publication

There will be no personal identifying information published.

Availability of data and materials

By the end of the trial, all primary and secondary outcome data will be published; thus, the participant-level data will be available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

Funding

This trial has been supported by Tabriz University of Medical Sciences (grant number 66795). The funding party had no role in study design, analysis, interpretation of data, and reporting the results.

Authors' contributions

P.D created the initial concept of this work, which was further developed by M.N and M.K. M.N is conducting this trial as part of her MS.C thesis under the supervision of P.D and M.K. The submitted manuscript has been approved by all authors.

Acknowledgments

The authors would like to express their gratitude to Tabriz University of Medical Sciences.

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Figures

	Enrolment	Allocation	Close-out		
TIMEPOINT**	$-t_1$	0	t_1	t_2	t_x
ENROLMENT					
Eligibility screen	X				
Informed consent	X				
General characteristics	X				
Allocation		X			
INTERVENTIONS					
TT group + HIIT			←	→	
Placebo group + HIIT			←	→	
ASSESSMENTS					
Dietary recall	X		X	X	
VO_{2max}		X			
Body composition	X		X		X
VAS	X		X	X	
Biochemical assessments	X		X	X	X

Abbreviations: TT: Tribulus terrestris, VAS: Visual Analogue Scale, HIIT: high-intensity interval training

Figure 1

Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) chart for study process. The “X” is indicating what is done in the given period.

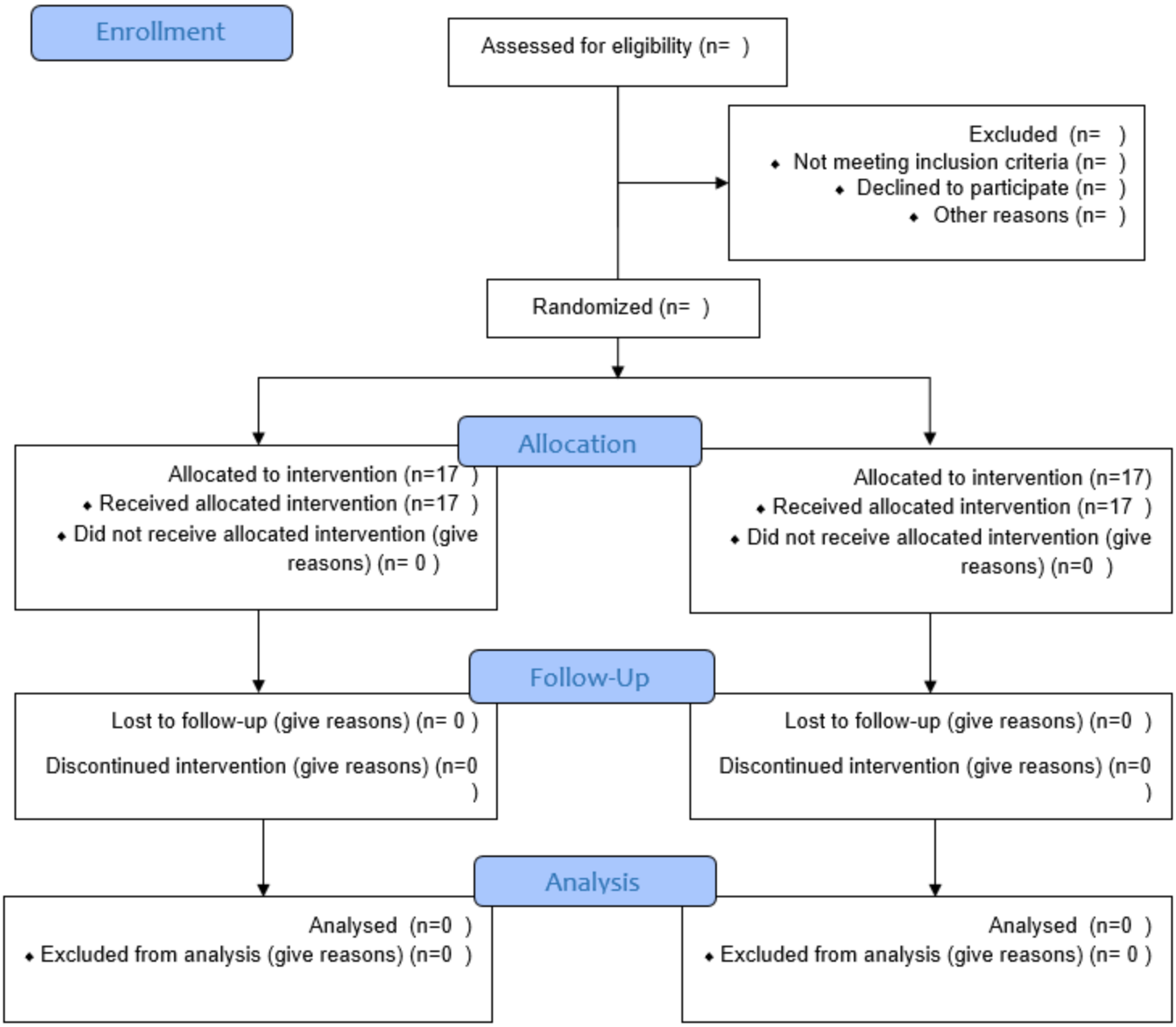

Figure 2

Consolidated Standards of Reporting Trials (CONSORT) diagram.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SPIRITchecklist.docx