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The Effect of Resistance Training with *Tribulus terrestris* on Liver Enzymes in Rats Exposed to Stanozolol

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Abstract

Introduction: Today, anabolic androgenic steroids (AAS) and growth hormone are widely used in men and women to increase strength and muscle mass in athletes. The aim of this study was to evaluate the effect of resistance training (R) and *Tribulus terrestris* (TT) on aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in rats exposed to stanozolol (S).

Methods: In this experimental study, 49 male Sprague Dawley rats with an age range of 8-10 weeks and an approximate weight of 180-200 g were randomly divided into 7 groups of seven animals, including: (1) sham, (2) stanozolol (5 mg/kg/day) (S), (3) 50 mg/kg *T. terrestris* extract (TT50), (4) 100 mg/kg *T. terrestris* extract (TT100), (5) resistance training (RT), (6) RT+TT50, and (7) RT+TT100. Resistance training was performed for eight weeks, three sessions per week. Data analysis was performed using one-way analysis of variance and Tukey's post hoc test in SPSS version 22.0 ($P \geq 0.05$).

Results: AST, ALT, and ALP levels in the RT, TT50, TT100, RT+TT50, and RT+TT100 groups were significantly lower compared to the S group ($P \geq 0.05$). AST and ALP levels in the TT100 group were lower compared to the TT50 group ($P \geq 0.05$). Moreover, ALT and ALP levels in the RT+TT100 group were lower compared to the RT+TT50 group ($P \geq 0.05$).

Conclusion: It seems that resistance training together with TT consumption synergistically improves liver enzymes in rats exposed to stanozolol. In addition, the effect of resistance training+100 mg/kg *T. terrestris* extract (RT+TT100) is much more favorable than RT+TT50, TT50, and TT100.

Keywords: Resistance training, *Tribulus terrestris*, Stanozolol, Liver

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Introduction

Today, anabolic androgenic steroids (AAS) and growth hormone are widely used in men and women to increase strength and muscle mass in athletes.[1] However, the use of these drugs at doses up to 100 times higher than the therapeutic range leads to irreversible effects on organs of the body, including the liver.[2,3] Data show that among the complications of AAS use, cardiovascular side effects have the highest mortality. In other words, approximately 33%-66% of mortality cases are due to these complications, with the remaining being caused by liver failure and liver cancer.[4] Oral 17-α alkylated androgens such as methyltestosterone, nandrolone, oxandrolone, and stanozolol (S) disrupt the process of absorption and clearance of the liver and disturb liver enzymes[5]. In this vein, hypoglycemia, insulin resistance, and dysfunction of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were observed in athletes who reported AAS abuse in a survey.[5] In some studies, the relationship between AAS abuse and hepatotoxicity was also reported.[6,7] Studies show that the abuse of AAS leads to liver cell dysfunction by increasing oxidative stress and inflammatory factors and impairing lipoprotein metabolism and aminotransferases.[8] In another study, ALT and AST levels were significantly higher in athletes who were taking the drug compared to athletes who had quit it in the last three months.[9] In another study, the results showed that AST

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and ALT levels were significantly higher in people who took steroids orally than in those who took steroids by injection.[8] Moreover, exposure to AAS at high doses for 12 weeks increased ALT and AST and impaired the fat profile of rats.[10] Studies show that resistance training has beneficial effects on liver function. In other words, these studies show that resistance training with or without AAS can improve metabolism and fat profile, reduce inflammatory factors, and improve liver enzymes.[8,9]

According to studies, recently many researchers have been attracted to the use of medicinal plants, which have been shown to have beneficial effects on many metabolic disorders and heart diseases. Among these medicinal plants, teasel with the scientific name of *Tribulus terrestris* (TT) is an annual plant that grows in hot and humid regions and Mediterranean climate.[11] This medicinal plant contains saponins, flavonoids, vitamins, and antioxidants that have anti-oxidant, anti-tumor, and anti-apoptotic effects on cells, especially liver cells.[11,12] Studies show that dose-dependent consumption of TT with the mechanism of the activation of protein kinases, as well as metabolic and anti-inflammatory factors leads to improved heart function and fat profile.[12] In addition, studies show that the use of TT in combination with resistance training reduces triglycerides, cholesterol, and low-density lipoprotein (LDL) and increases high-density lipoprotein (HDL) in rats exposed to stanozolol.[13] In addition, the consumption of TT at doses of 50 and 100 mg/kg along with resistance training leads to a decrease in S-induced neurotoxicity in rats.[14]

Given the increasing prevalence of AASs abuse and their irreversible side effects, it seems necessary to learn more about replacing natural supplements with similar effects, as well as finding the best complementary medicine to prevent or treat liver disorders in individuals. Therefore, the present study aimed to investigate the effect of resistance training (RT) along with TT on liver enzymes in rats exposed to stanozolol (S).

Materials and Methods

Animal Care

In this experimental study, 49 male Sprague Dawley rats with an age range of 8-10 weeks and an approximate weight of 180-220 g were purchased and transferred to the Sports Physiology Laboratory of Islamic Azad University, Marvdasht Branch. It is noteworthy that during the entire research period, rats were maintained in standard conditions of 12-hour dark-light cycle, relative humidity of 55%-60%, standard temperature of 22-24°C in transparent polycarbonate cages with autoclave capability and ad libitum access to water and food for rats.

Grouping and Design

After seven days of adaptation to the environment, the rats were divided into 7 groups of seven animals, including: (1) sham control (sham), (2) stanozolol (S), (3) 50 mg/kg *T. terrestris* extract (TT50), (4) 100 mg/kg *T. terrestris* extract (TT100), (5) resistance training (RT), (6) resistance training + 50 mg/kg *T. terrestris* extract (RT + TT50), and (7) resistance training + 100 mg/kg *T. terrestris* extract (RT + TT100) by a simple random sampling method. Then, the stanozolol groups received stanozolol peritoneally five days a week at a dose of 5 mg/kg (cumulative weekly dose of 25 mg/kg).[15] The training groups performed resistance training 3 sessions per week for 8 weeks.[16] Additionally, the TT groups received the hydroalcoholic extract of TT peritoneally.[17]

Preparation of Tribulus terrestris Extract

To prepare the hydroalcoholic extract of TT, TT plant was prepared from Marvdasht Agricultural Jihad Center in the summer of 2020. Then, 100 g of TT was dried. After grinding, this powder was dissolved in 150 mL of 70% alcohol and kept in the laboratory for 3 days. After three days, the solution was first passed through a paper filter and the filtrate was purified using a vacuum rotatory evaporator to obtain extract. After mixing the extract with normal saline, it was injected into the rats at doses of 50 and 100 mg/kg/d peritoneally for eight weeks.[17] It should be noted that TT extract was injected into rats for 6 days a week at 11 am (2 hours after resistance training).

Resistance Training

To perform resistance training after one week of adaptation to the environment, first, the rats were trained to climb the ladder for five sessions. Based on previous studies, the ladder in this study was 1 m high, the distance between each step was 4 cm, and the slope was 85 degrees. At the beginning and end of the main training, the rats climbed the ladder without weights. For the main training, the weight of rats was measured weekly and the training protocol was designed based on the weight of the rats. In the first week, the rats lifted weights based on 30% of their body weight and in the last week, they climbed the ladder with attached weights weighing 100% of their body weight. Today, anabolic androgenic steroids (AAS) and growth hormone are widely used in men and women to increase strength and muscle mass in athletes. It should be noted that the interval between each repetition was 35-45 seconds and the interval between each set was considered to be 60-120 seconds.[16]

Description and Sampling

Forty-eight hours after the last training session and supplementation, the rats were anesthetized in a 12-hour fasting state using ketamine and xylazine (Alfasan, Woerden, the Netherlands) at a ratio of 3:1. After ensuring anesthesia with ketamine (50 mg/kg) and xylazine (20 mg/kg), blood samples were taken directly from the heart tissue with a 5-mL syringe (Supa Medical Devices, Tehran,

Iran). After keeping them in the room for 30 minutes, the samples were centrifuged at a speed of 12 000 rpm for 10 minutes to measure the values of variables in the serum. To measure the serum levels of AST, ALT, and ALP with a sensitivity of U/L, Pars Azmoon enzyme kits were used in accordance with the IFCC recommendations.

Statistical Analysis

The Kolmogorov-Smirnov test was used to evaluate the normality of the distribution of findings and one-way analysis of variance with Tukey's post hoc test in SPSS version 22.0 was used to analyze the results ($P \geq 0.05$).

Results

The results of one-way ANOVA showed a significant difference in serum levels of AST ($P=0.001$, $F=90.43$), ALT ($P=0.001$, $F=67.64$), and ALP ($P=0.001$, $F=12.71$) between the research groups.

The results of Tukey's post hoc test which was used to determine the significance of differences between groups showed that the serum level of AST in the S group was significantly higher compared to the sham group ($P=0.001$), but in the RT ($P=0.001$), TT50 ($P=0.001$), TT100 ($P=0.001$), RT + TT50 ($P=0.001$), and RT + TT100 ($P=0.001$) groups, the levels were significantly lower compared to the S group. Besides, the levels in the TT100 ($P=0.001$), RT ($P=0.001$), RT + TT50 ($P=0.001$), and RT + TT100 ($P=0.001$) groups were significantly lower compared to the TT50 group, and the levels in the RT + TT100 group were significantly lower compared to the RT group ($P=0.001$) (Figure 1).

The serum level of ALT in the S group was significantly higher compared to the sham group ($P=0.001$), but in the TT50 ($P=0.015$), TT100 ($P=0.001$), RT ($P=0.001$), RT + TT50 ($P=0.001$), and RT + TT100 ($P=0.001$) groups, the levels were significantly lower compared to the S group. Moreover, the levels in the RT ($P=0.001$), RT + TT50 ($P=0.001$), and RT + TT100 ($P=0.001$) groups were significantly lower compared to the TT50 group. In addition, in the RT + TT100 group, the level of ALT was significantly lower compared to the TT100 group ($P=0.001$), RT ($P=0.002$), and RT + TT50 ($P=0.001$) groups (Figure 2).

The serum level of ALP in the S group was significantly higher compared to the sham group ($P=0.001$), but in the TT50 ($P=0.017$), RT ($P=0.001$), RT + TT50 ($P=0.03$), and RT + TT100 ($P=0.001$) groups, the levels were significantly lower compared to the S group. Additionally, in the RT + TT100 group, it was significantly lower compared to the TT100 ($P=0.002$) and RT + TT50 ($P=0.03$) groups (Figure 3).

Discussion

The results of the present study showed that resistance training reduced serum levels of AST, ALT, and ALP in

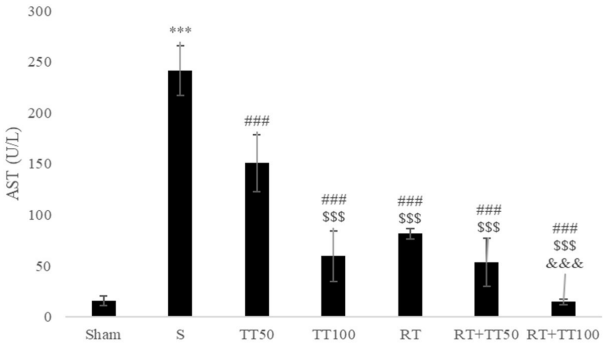

Figure 1. Serum Level of AST in Rats in the Research Groups. *** ($P=0.001$) Significant increase in the S group compared to the sham group. ### ($P=0.001$) Significant decrease in the TT50, TT100, RT, RT+TT50, and RT+TT100 groups compared to the S group. $$$ ($P=0.001$) Significant decrease in the TT100, RT, RT+TT50, and RT+TT100 groups compared to the TT50 group. &&& ($P=0.001$) Significant decrease in the RT+TT100 group compared to the TT100, RT, and RT+TT50 groups

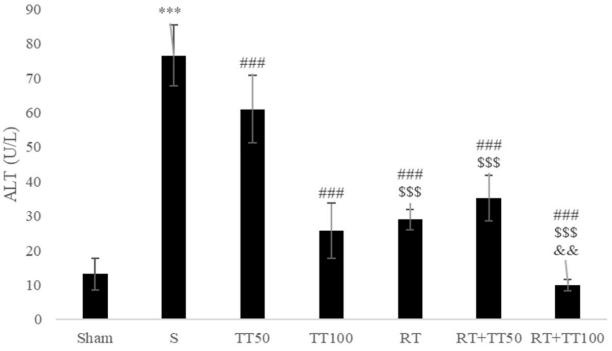

Figure 2. Serum level of ALT in Rats in the Research Groups. *** ($P=0.001$) Significant increase in the S group compared to the sham group. ### ($P=0.001$) Significant decrease in the TT50, TT100, RT, RT+TT50, and RT+TT100 groups compared to the S group. $$$ ($P=0.001$) Significant decrease in the RT, RT+TT50, and RT+TT100 groups compared to the TT50 group. && ($P=0.01$) Significant decrease in the RT+TT100 group compared to the RT group

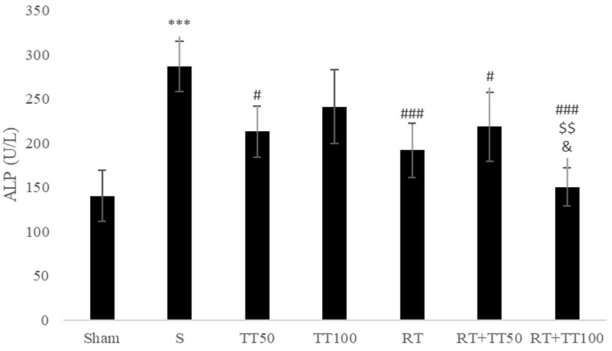

Figure 3. Serum level of ALP in Rats in the Research Groups. *** ($P=0.001$) Significant increase in the S group compared to the sham group. # ($P=0.05$) and ### ($P=0.001$) Significant decrease in the TT50, RT, RT+TT50, and RT+TT100 groups compared to the S group. $$ ($P=0.01$) Significant decrease in the RT+TT100 group compared to the TT100 group. & ($P=0.05$) Significant decrease in the RT+TT100 group compared to the RT+TT50 group

rats exposed to stanozolol. Studies have shown that the abuse of stanozolol with the mechanism of disrupting the fat profile and the metabolism of lipoproteins and increasing the oxidative stress in the liver tissue leads to the disruption of liver enzymes.[5] Studies show that exercise increases the transcription of antioxidant enzymes and enhances the resistance of liver cells to oxidative stress. In addition, increasing the metabolism of fats in muscle cells serves to reduce the levels of LDLs, cholesterol, and triglycerides in the blood, and thus helps the liver tissue to store and regulate fat metabolism and prevent an increase in liver enzymes.[18,19] In addition, researchers in previous studies have shown that increasing antioxidant capacity following resistance training improves the fat profile and decreases apoptotic markers in S-exposed rats.[13,20] Consistent with the present study, the results of a meta-analysis study show that aerobic training improves the fat profile and reduces ALT and AST levels in patients with non-alcoholic fatty liver. Resistance training improves fat profile and AST in these patients; in addition, high-intensity interval training reduces ALT.[21] In another study, 8 weeks of elastic band resistance training reduced AST, ALT, and ALP levels in obese middle-aged women.[22] However, inconsistent with the present study, the researchers showed that 8 weeks of endurance training had no significant effect on AST and ALT levels in diabetic rats.[19] It seems that differences in the statistical population and the type of disorder, as well as different mechanisms of exercise training are the reasons for differences in results. As stated in the study of Xiong et al, hepatic metabolic proteins have the greatest effect on the liver, while aerobic training has the utmost metabolic effect by improving the fat profile and metabolic markers.[21] In a study by Arazi et al, the results showed that Sustanon as an anabolic steroid caused an increase in oxidative stress levels and liver enzymes. Considering these values during 8 weeks of resistance training and consumption of Sustanon, a significant decrease was observed in oxidative markers; however, no significant change was observed in liver enzymes.[23] The difference in the results of these two studies can be attributed to the differences in the type of steroid, so as mentioned before, liver enzymes exposed to stanozolol are in the front line of change and damage.[4]

The results showed that TT consumption at doses of 50 and 100 mg/kg reduced AST, ALT, and ALP levels in rats exposed to stanozolol. Besides, the effect of the 100 mg/kg dose on reducing AST and ALP levels was far better than the effect of the 50 mg/kg dose. In this vein, it seems that the molecular and cellular nature and mechanism of this medicinal plant trigger protective effects on the liver. Studies have shown that T with its abundant isoflavones and saponins can activate the mechanism of cyclic adenosine monophosphate and lead to increased oxidative phosphorylation and improved fat metabolism via this pathway. Moreover, the mechanism of activation of insulin-like growth hormone-1 following TT consumption, increased angiogenesis due to increased vascular endothelial growth factor, and activation of nuclear transcription mechanisms and mitochondrial biogenesis are some of the pathways that TT can improve cell metabolism.[13] In addition, researchers have noted that saponins in TT improve liver function by lowering blood sugar, increasing insulin secretion from pancreatic cells, and improving liver metabolism.[24] In this regard, researchers have shown that consumption of 10 mg/kg of T hydroalcoholic extract of TT leads to an increase in superoxide dismutase and glutathione peroxidase, a decrease in malondialdehyde, as well as a reduction in necrosis and apoptosis in the liver tissue of type 2 diabetic rats.[25] In another study, researchers showed that consumption of methanolic extract of TT at doses of 0.5, 1, and 1.5 g/kg diet was associated with improved hepatic and digestive enzymes and fat profile, but higher doses had more favorable effects on hepatic and digestive enzymes.[26] In another study, researchers showed that the consumption of 500, 750, and 1000 mg/kg of hydroalcoholic extract of TT was associated with reduced levels of ALT, ALP, and AST and improved levels of necrosis and liver fat in rats with non-alcoholic fatty liver.[12] However, no research was found to be inconsistent with the present study.

The results of the present study showed that RT + TT50 and RT + TT100 reduced AST, ALT, and ALP levels in rats exposed to stanozolol. In addition, the interactive effect of training and TT supplementation was dose-dependent; therefore, the effect of RT + TT100 on the reduction of some markers was far more favorable than that of RT + TT50, TT50, and TT100. In this regard, the researchers showed that resistance training combined with 50 and 100 mg/kg of hydroalcoholic extract of TT reduced cholesterol, serum LDL, and apoptotic markers in the heart tissue of rats exposed to stanozolol.[13] Besides, 8 weeks of resistance training along with TT consumption at a dose of 100 mg/kg caused a decrease in caspase-3 and Bax and an increase in BCL2 levels in the heart tissue of rats exposed to stanozolol.[27] In another study, daily consumption of 770 mg of TT improved body composition, increased protein mass, improved hormonal response, and increased serum levels of vitamins B, E, A, and C in CrossFit athletes.[28] The results of studies show that exercise training, especially resistance training with a mechanism of increasing antioxidant capacity, weight loss, and improving fat profile leads to improved liver enzymes.[19,21] The consumption of TT also leads to improved liver function by improving antioxidant enzymes and fat profile and increasing transcription pathways of metabolic genes and mitochondrial biogenesis.[12,25,26] Therefore, in line with the studies conducted before, higher doses of TT combined

with resistance training have more favorable effects on cell biological markers.

Due to the effect of oxidative stress on liver dysfunction, it seems that the lack of measurement of antioxidant enzymes and oxidative stress is one of the limitations of the present study; therefore, it is recommended that future studies should investigate the role of the antioxidant-oxidative stress system in liver tissue. Given the importance of examining histopathological changes in liver tissue in achieving a more favorable result, it seems that the lack of measurement of necrosis and oxidative stress at the tissue level is another limitation of this study. Therefore, it is suggested that hematoxylin-eosin procedures in hepatic studies be examined in future studies.

Conclusion

Resistance training along with TT consumption appears to synergistically improve liver enzymes in stanozolol-exposed rats. Besides, the effect of training + 100 mg/kg of TT is much more favorable than the effect of training + 50 mg/kg of TT, as well as TT alone.

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Authors' Contribution

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Funding Acquisition: Najmeh Kiani.
Investigation: Najmeh Kiani, Saeed Keshavarz, Jamshid Banai;
Methodology: Najmeh Kiani And Seyed Ali Hosseini.
Project Administration: Najmeh Kiani, Saeed Keshavarz.
Resources: Najmeh Kiani,Jamshid Banai.
Software: Najmeh Kiani.
Supervision: Saeed Keshavarz; Validation: Najmeh Kiani, Jamshid Banai.
Visualization: Najmeh Kiani, Saeed Keshavarz, Jamshid Banai.
Writing – Original Draft: Najmeh Kiani;
Writing – Review & Editing: Saeed Keshavarz, Seyed Ali Hosseini, Jamshid Banai.

Competing Interests

The authors have not reported any conflict of interest.

Ethical Approval

Ethical principles of working with animals in this study were observed under the supervision of the Research Ethics Committee of the Islamic Azad University, Marvdasht Branch.

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