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Research Article

Effect of Eight Weeks of Resistance Training and Consumption of Tribulus Terrestris on Androgenic Receptor-1, Fas Ligand Gene Expression, and Lipid Profiles in Rats Exposed to Stanozolol

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Abstract

Background: Protective effect of medicinal plants on the heart has been reported, but the effect of resistance training (RT) and *Tribulus terrestris* (TT) on the heart exposed to anabolic-androgenic steroids (AAS) abuse is still unknown.

Objectives: The present study aimed to investigate the effect of RT and TT on androgen receptor-1 (*ar-1*), Fas ligand (*fasl*) gene expression and lipid profiles in rats exposed to stanozolol (S).

Methods: Thirty-five male rats were selected and divided into 7 groups as follows: (1) sham (normal saline/Sh), (2) stanozolol (S), (3) S+100 mg/kg TT (S+TT100), (4) S+ 50 mg/kg TT (S+TT50), (5) S+RT+TT, (6) S+RT+TT100, and (7) S+RT+TT50. Over a course of eight-week period, groups 3, 4, 6, and 7 received 50 and 100 mg/kg/d doses of TT peritoneally and groups 5-7 performed three sessions of increasing RT per week.

Results: RT decreased plasma cholesterol and low-density lipoprotein cholesterol (LDL-C) levels, as well as *ar-1* and *fasl* gene expression in S-exposed rats ($P<0.05$). TT50, TT100, SRTT100, and SRTT50 reduced *ar-1* and *fasl* gene expressions ($P<0.05$). TT50 reduced triglyceride (TG), cholesterol and increased high-density lipoprotein-cholesterol (HDL-C) ($P\leq0.01$), and TT100 decreased LDL-C levels ($P<0.05$). Additionally, SRTT100 reduced TG, cholesterol, and LDL-C levels and increased HDL-C level ($P<0.05$), and SRTT50 decreased cholesterol level and increased HDL-C level in S-exposed rats ($P<0.05$).

Conclusion: RT and consumption of TT appear to have protective effects on the improvement of apoptosis-dependent androgen receptor-1 and lipid profile in S-exposed rats.

Keywords: Resistance training, *Tribulus terrestris*, Lipid profile, Androgen receptors, Stanozolol

Introduction

Anabolic-androgenic steroids (AAS) include testosterone and a number of its derivatives that have beneficial effects on anabolism and are used to boost protein synthesis and muscle growth (1). Nandrolone, stanozolol (S), oxandrolone, methandrostenolone, and trenbolone are industrial derivatives of testosterone that are used to treat certain diseases such as genital weakness and osteoporosis (1). Studies have shown that AAS abuse is associated with cardiovascular disease such as stroke, sudden death, left ventricular hypertrophy, atherosclerosis, and high blood pressure in athletes and non-athletes (2,3). In addition, studies have shown that stanozolol abuse as one of the most widely used AASs leads to heart disorders; it also leads to increased pathological hypertrophy in the left ventricle via increasing inotropic activity and the expression of 5α-reductase, aromatase, and androgen receptors (ARs) (1). AASs reduce the level of high-density lipoproteins (HDL) and increase cholesterol (Chol) and low-density lipoprotein-cholesterol (LDL-C) levels by increasing the availability of free fatty acids in metabolic disorders (3). Excessive increase in the expression of *ARs* appears to be associated with increased expression of lipogenic enzymes. It also appears that an increase in ARs following AAS abuse leads to impairment in lipid profile by causing impairment in liver enzymes and lipogenic liver X receptors (4). Oxidative stress caused by lipid profile disorder activates Fas death receptors and by binding it to its ligands, which activate FasL caspases, ultimately induces apoptosis of cardiac tissue (5). Evidence also suggests that increased inflammation of the tumor necrosis factor-alpha (TNFα) pathway and degradation of Fas receptor inhibitors such as cFlip can cause cell death (5,6). In this regard, the researchers showed that AAS abuse leads to an increase in

AR expression and LDL level and a decrease in HDL-C level. It also leads to apoptosis in neuron cells by increasing levels of oxidative stress and caspases (7).

However, studies have shown that AASs in athletes have been associated with improved athletic performance, and lipid profile (8). In another study, Boldenone supplementation, anabolic androgenic steroid, caused an increase in the hematocrit levels of rats, however, the damage in the training + Boldenone group was lower (9). Moreover, the consumption of AASs after a resistance training session (RT) increased the level of oxidative stress biomarkers such as malondialdehyde (MDA) and 8-hydroxy-2-deoxy guanosine (8-OHdG) compared to the AAS-free training group (10). It seems that exercise training in animal models of AAS abuse improves fat metabolism by improving the physiological hypertrophy and lipoprotein and apolipoprotein profiles (11). Consequently, in a study, the results showed that the exercise training for six weeks, five sessions per week, had far more favorable effects in mesterolone groups than in inactive and mesterolone groups (11). Although previous studies have shown that AASs cause DNA damage and increase oxidative stress in athletes (10), nevertheless, the mechanism of the effect of AASs on ARs and various pathways of apoptosis in cardiac tissue following exercise has not yet been fully understood.

Therefore, considering the detrimental effects of AASs on the heart metabolism and function, it seems that the use of interventions that prevent heart disease in AAS consumers is necessary. Given that the use of medicinal plants has long been considered in the treatment and prevention of many diseases in recent years, the number of studies on the effect of medicinal plants as an alternative to synthetic drugs has increased. Among medicinal plants, *Tribulus terrestris* L. (TT) which belongs to annual plants from the *Zygophyllaceae* family is rich in flavonoids, alkaloids, saponins, legins, amides, and glycosides, and its protective effects on the heart and arteries have been proven (12).

TT with its antioxidant and anti-inflammatory effects improves fat metabolism and appears to inhibit inflammatory and apoptotic activities of tumor necrosis factor-alpha ligand (TNFL) and FasL as well as cell death (13). In this regard, 250 and 500 mg/kg of TT extract by improving antioxidant and anti-apoptotic pathways led to an improvement in the cardiac function of rats with cardiac ischemia, although in some markers, a higher dose had more favorable effects (11). The researchers found that 85 and 50 mg/kg of hydroalcoholic extract of TT significantly improved lipid profile in rats with coronary heart disease induced by injecting isoproterenol (13). Moreover, 0.3, 7.1, and 8.7 µg/mL of TT significantly reduced TNFL and FasL levels in the cell and induced apoptosis in cancer cells (12). However, studies have shown that athletes also use the estrogenic effects of this herb, as Pokrywka et al showed that dose-dependent consumption of TT in athletes improved athletic performance and increased strength (14,15). Ma et al also showed that 1250 mg/kg of TT reduced serum creatinine kinase and insulin-like growth factor-binding protein 3 (IGFBP-3) levels and increased strength in the TT + exercise group compared to the exercise group (16). In a study by Wilk et al, 12 weeks of TT consumption in middle-aged men increased muscle mass as well as the plasma levels of DHT and IGF-1 and reduced body fat percentage; however, there was no significant change in their fat profile (17). Moreover, in a study by Van Eenoo et al, 20 mg/kg of TT had a greater effect than 10 mg/kg on the increase of male testosterone serum testosterone (18). However, Rogerson et al showed that taking 450 mg/kg of TT for five weeks did not have a significant effect on strength, body composition, and urinary testosterone-to-epitestosterone ratio during 5 weeks of endurance training (18). In this regard, researchers have shown that exercise has protective effects on the heart by improving the enzymes of fat metabolism, reducing oxidative stress and apoptosis, and increasing angiogenesis (19,20).

Reviewing the results, it seems that the optimal dose of TT along with exercise activities is not yet fully known. Studies have also shown that the main pathways of pathological hypertrophy and apoptosis associated with metabolic disorders in cardiac tissue following AAS abuse depend on gene expression pathways of AR-1 and *fasl* receptor. It seems that the study of the simultaneous effect of RT and TT consumption provides more information about cardiac apoptosis and pathologic hypertrophy in athletes who turn to AAS abuse. Therefore, the present study aimed to investigate the effects of RT along with TT on the gene expression of cardiac apoptosis-dependent androgen receptor-1 and lipid profile of rats exposed to stanozolol.

Materials and Methods

Thirty-five Sprague-Dawley rats with the mean age of 8 weeks and mean weight of 150 to 200 g were purchased from the Center for Breeding and Reproduction of Laboratory Animals, Islamic Azad University, Marvdasht Branch. After a one-week period of adaptation to the laboratory environment, the rats were randomly assigned to 7 groups of 5 rats, as follows: (1) sham/injection of normal saline (Sh), (2) stanazolol (S), (3) S + 100 mg/kg of TT (S+TT100), (4) S+TT50, (5) S + RT, (6) S+RT+TT100, and (7) S+RT+TT50. It is noteworthy that during the present study, the rats were kept in standard conditions in terms of ambient temperature (22 to 24˚C), relative humidity of 55% to 60%, dark-light cycle, (12 hours), in polycarbonate cages with autoclave capacity, sterile straws for urine uptake and replacement after 2 days, and free access to water and food. Over the course of 8 weeks, groups 2-7 daily received 5 mg/kg of stanozolol (Sigma-Aldrich; CAS Number: 10418-03-8) peritoneally (21). Groups 5-7 attended RT sessions three times a week (22) and groups 3, 4, 6, and 7 daily received specific doses of TT peritoneally

(23). Forty-eight hours after the last exercise session as well as stanozolol and TT administration, the rats were anesthetized with ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg). Then, the blood collection was performed directly from the heart tissue of rats and heart tissue was removed to measure *ar-1* and *fasl* expression levels. To measure HDL-C, Chol and LDL-C, the enzymatic-photometric method (Pars Azmoun Company, Iran) was applied and to measure triglyceride, the enzymatic-colorimetric method (Pars Azmoun Company, Iran) was used. Moreover, *ar-1* and *fasl* gene expression levels were measured by quantitative reverse transcription polymerase chain reaction (PCR) method.

Resistance Training Protocol

The rats performed RT using a one-meter-high fence, with a distance of 4 cm between the stairs and a slope of 85°, so that the RT started from 30% of body weight in the first week and was completed with 100% of body weight of rats in the eighth week. It is noteworthy that to warm up at the beginning of the training, the rats climbed the training ladder four times without weights. Additionally, the training in each session included four sets (4 climbs with 50, 75, 90, and 100% of the previous maximum carrying load the animals were able to raise) with two repetitions (climbing the stairs twice). The interval between each set was 2 to 3 minutes and the interval between each repetition was considered to be 40 to 60 seconds (22).

Preparation of Tribulus Terrestris

In order to prepare TT extract, first, the fruit of this plant prepared from Jihad Keshavarzi of Marvdasht was ground, then 100 g of the powder was placed in 80 mL of 70% alcohol. Then, the solution was kept in the laboratory for 3 days. After three days, the solution was first passed through a paper filter and the liquid section was purified using a vacuum device and a dry extract of this plant was obtained. Subsequently, after concentrating the extract with normal saline, the rats received 50 and 100 mg/d doses of it peritoneally per day (23).

Quantitative q-RT PCR Assay

RNA Extraction

For molecular studies at gene expression level, RNA was first extracted from the heart tissue according to the manufacturer's protocol (CinnaGen Co, Iran), then using the light absorption property at a wavelength of 260 nm, the concentration and purity of the RNA sample were obtained quantitatively.

cDNA Synthesis

After extracting RNA from the studied samples with high purity and concentration, cDNA synthesis steps were performed according to the manufacturer's protocol (Fermentas Kit K1622) and then the synthesized cDNA was used to perform the reverse transcription reaction. The designed primers were first examined for genes, and then the expression of genes was investigated using the quantitative RT PCR method.

In all experiments, an equal amount of cDNA of the internal control gene β2 microglobulin (B2M) was used in comparison with the target gene. After completing the operation of the device and observing the graphs based on the increase of the number of desired fragments and fluorescence propagation, the magnitude of change in the expression of the desired gene was measured using $\Delta\Delta C_t$ and compared to B2M and the control group. Then, the analyzable raw data were calculated using the $2^{-\Delta\Delta Ct}$ formula. The sequence of the primers used in the present study is reported in Table 1.

Data Analysis

The Shapiro-Wilk, one-way ANOVA with the Tukey's post hoc tests were used to analyze the data in SPSS software version 22.0 ($P<0.05$).

Results

Lipid Profile Analysis

In Table 2, the levels of the lipid profile are presented. The results of one-way ANOVA test showed that there were significant differences in triglyceride (TG), Chol, LDL-C, and HDL-C levels in the study groups ($P<0.05$).

The results of Tukey's post hoc test showed that TG levels in the Sh and S groups did not differ significantly ($P=0.35$), but TG levels in the S+TT50 and S+RT+TT100 groups were significantly lower compared to the S group. TG levels in the S+RT+TT100 group were significantly lower compared to S+TT50, S+TT100, S+RT, and S+RT+TT50 groups ($P<0.05$) (Table 2).

Chol levels in the Sh and S groups did not differ significantly ($P=0.76$), but in the S+RT and S+RT+TT100 groups, they were significantly lower compared to the S group. Moreover, in the S+RT+TT100 group, they were significantly lower compared to the S+TT50, S+TT100, S+RT, and S+RT+TT50 groups, and they were lower in the S+RT group than in the S+RT+TT50 group ($P<0.05$) (Table 2).

LDL-C levels in the S group were significantly higher compared to the Sh group, but in the S+TT100, S+RT,

Table 1 . Sequence of the Primers Used in the Present Study

Genes	Primer Sequences	Product Size (bp)
B2M	Forward: 5′- CGTGCTTGCCATTCAGAAA -3′ Reverse: 5′-ATATACATCGGTCTCGGTGG -3′	244
fasl	Forward: 5′- TGGCCCACTTAACAGGGAAC-3′ Reverse: 5′- CTCATTGATCACAAGGCCGC -3′	124
ar-1	Forward: 5′- CAAGGAACTTGATCGCATCATTG -3′ Reverse: 5′- CTTGCAATAGGCTGCACAGA -3′	89

ar-1: Androgen Receptor-1, *fasl*: Fas ligand, B2M: β2 microglobulin

Table 2. Lipid Profile Levels (Mean±SD) of Rats in the Study Groups

	TG (mg/dL)	Cholesterol (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Sh	75.28±3.63	57.30±1.85	25.81±3.34	36.08±3.88
S	81.80±3.22	60.36±8.26	31.44±4.96 *	26.15±1.43 ***
S+TT50	67.15±6.19 ##	56.08±2.62 #	27.93±3.91	35.69±2.10 ###
S+TT100	76.00±5.88	54.87±3.08	19.97±2.72 ###	26.81±2.76
S+RT	75.67±3.81	53.42±2.57 Ψ	21.10±1.48 ###	26.61±1.54
S+RT+TT50	72.69±2.24	60.64±2.97	31.79±2.93	35.35±3.30 ###, εεε
S+RT+TT100	53.37±10.56 ###, φφφ	42.78±1.99 ###, φφφ	20.60±0.85 ###	32.64±3.12 ###, εεε

Analysis at the significance level of 0.05.
* ($P<0.05$) and *** ($P<0.001$) Significant difference compared to the Sh group.
# ($P<0.05$), ## ($P<0.01$) and ### ($P<0.001$) Significant difference compared to the S group.
φφφ ($P<0.001$) Significant decrease compared to the S+TT50, S+TT100, S+RT, and S+RT+TT50 groups.
Ψ ($P<0.05$) Significant decrease compared to the S+RT+TT50 group.
εεε ($P<0.001$) Significant increase compared to the S+RT group.

and S+RT+TT100 groups, they were significantly lower compared to the S group. In the S+TT100 group, they were significantly lower compared to the S+RT+TT50 group. In the S+RT group, they were significantly lower compared to the S+RT+TT50 group. Moreover, in the S+RT+TT100 group, they were significantly lower compared to the S+RT+TT50 ($P<0.05$) (Table 2).

HDL-C levels in S group were significantly lower compared to the Sh group. However, in the S+TT50, S+RT+TT50, and S+RT+TT100 groups, they were significantly higher compared to the S group. In the S+TT50 group, they were significantly higher compared to the S+TT100 and S+RT groups. In the S+RT+TT50 and S+RT+TT100 groups, they were significantly higher compared to the S+TT100 group. Additionally, in the S+RT+TT50 and S+RT+TT100 groups, they were significantly higher compared to the S+RT group ($P<0.05$) (Table 2).

Cardiac Gene Expression Analysis

In Figures 1 and 2, the levels of the research variables are presented. The results of one-way ANOVA test showed that there were significant differences in *ar-1* and *fasl* levels in the research groups ($P<0.05$).

The results of Tukey's post hoc test showed that *ar-1* gene expression levels in the S group were significantly higher compared to the Sh group. However, in the S+TT50, S+TT100, S+RT, S+RT+TT50, and S+RT+TT100 groups, they were significantly lower compared to the S group. In S+RT+TT100 group, they were significantly lower compared to S+TT50. In the S+RT, S+TT50, S+RT+TT50, and S+RT+TT100 groups, they were significantly lower compared to the S+TT100 group and in the S+RT+TT100 group, they were significantly lower compared to the S+RT+TT50 group ($P<0.05$; Figure 1).

Fasl gene expression levels in the S group were significantly higher compared to the Sh group. However, in the S+TT50, S+TT100, S+RT, S+RT+TT50, and S+RT+TT100 groups, they were significantly lower compared to the S group. In the S+RT+TT100 group, they were significantly lower compared to the S+TT50 group and in the S+RT+TT100 group, they were significantly lower compared to the S+TT100 and S+RT groups ($P<0.05$; Figure 2).

Discussion

The results showed that peritoneal injection of stanozolol increased LDL-C level, as well as *ar-1* and *fasl* gene expression levels and decreased HDL-C levels. The abnormality of lipoprotein metabolism in atherosclerotic pathology is associated with increased levels of circulating androgens following endocrine disorders or anabolic androgenic steroid abuse (24). AAS abuse leads to increased oxidative stress and inflammatory factors, impaired endothelial vascular function, and increased levels of some liver enzymes such as lipase TG enzyme, which result in increased plasma TG and HDL-C levels and impaired fat profile (24,25). In addition, AAS abuse affects the fat profile by impairing the hypothalamic-pituitary-gonadal axis and affecting metabolic hormones of the adrenal cortex (25). Additionally, the reduction of LDL receptor levels in liver tissue and increase of the expression of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) reductase increase Chol in the liver tissue following AAS abuse (26).

However, RT reduced Chol and LDL-C levels, as well as *ar-1* and *fasl* gene expression levels in S-exposed rats. The effect of RT on lipid profile is not yet fully understood, and there are conflicting results. For example, eight weeks of RT did not change lipid profile in patients with coronary artery disease (19). In another study, 4 weeks of RT did not change lipid profile of rats (27). Moreover, eight weeks of RT had no significant effect on the fat profile of rats with coronary artery disease (19). Eight weeks of exercise inhibited TNF-α, FasL, Fas receptor, caspase-3, decreased Bax, and increased Bcl-2 in the heart tissue of obese rats (20). In addition, eight weeks of exercise improved vascular endothelial growth factor (VEGF) levels and reduced apoptosis as well as fat weight in rats exposed to AAS (28). It seems that the exercise intensity and amount of calories consumed after RT, as well as the length of the

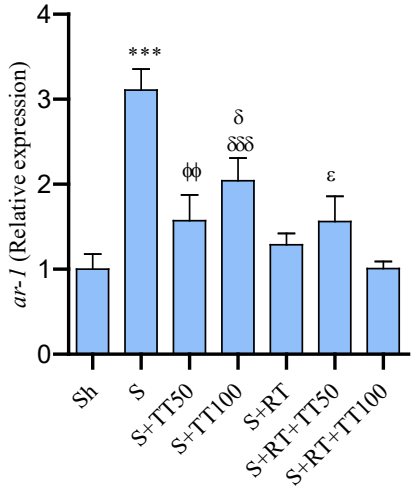

Figure 1. *ar-1* Gene Expression Levels in the 7 Study Groups.
*** ($P \leq 0.001$) Significant increase compared to the Sh, S+TT50, S+TT100, S+RT, S+RT+TT50, and S+RT+TT100 groups.
δδδ ($P < 0.001$), δ ($P < 0.05$) Significant increase compared to the S+TT50, S+RT, S+RT+TT50, andS+RT+TT100 groups.
φφ ($P < 0.01$) Significant increase compared to the S+TT100 group.
ε ($P < 0.05$) Significant increase compared to the S+RT+TT100 group.

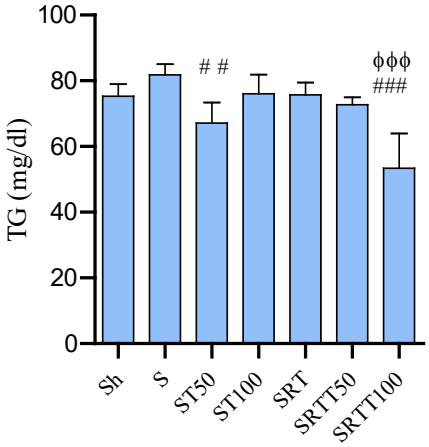

Figure 2. *Fasl* Gene Expression Levels in the 7 Study Groups.
*** ($P < 0.001$) Significant increase compared to the Sh, S+TT50, S+TT100, S+RT, S+RT+TT50, and S+RT+TT100 groups.
++ ($P < 0.01$) Significant increase compared to the S+RT+TT100 group.
δδδ ($P < 0.001$) Significant increase compared to the S+RT+TT100 group.
ε ($P < 0.05$) Significant increase compared to the S+RT+TT100 group.

training period, are factors affecting fat profile. Because some sources have suggested that the amount of calories consumed during exercise is important for changes in circulating levels of HDL-C and TG; HDL-C changes are also more dependent on exercise volume (27). Therefore, a non-significant increase in HDL-C levels and a non-significant decrease in LDL and Chol following RT can be dependent on these factors; however, 16 weeks of RT increased HDL-C and decreased plasma LDL in the elderly with Alzheimer's disease (29). However, studies on athletes with AAS abuse have also shown abnormalities in the lipid profile and Apo-A1 and Apo-B apolipoproteins in AAS-consuming bodybuilders (8). Exercise training for 6 weeks, five sessions per week, had far more favorable effects in the mesterolone groups than in the sedentary groups (11). RT, with the mechanism of increasing the oxidation of fatty acids in the muscle tissue, increased LDL receptor expression, insulin sensitivity, vasodilation, and activation of gluconeogenesis and decreased HMG-COA reductase expression in the liver tissue, inflammatory factors, intracellular adhesion molecules, and vascular cell adhesion molecule. In addition, it increased Akt/PKB phosphorylation in the adipose tissue which leads to the regulation of lipid profile homeostasis (19,26,27). Although studies have shown a positive effect of exercise training on the improvement of fat metabolism and atherosclerosis markers, the irreversible risks of using AAS at unconventional doses should always be considered by athletes (30). In addition, studies show that lowering lipid profile after exercise by increasing VEGF, angiogenesis in the heart tissue, and nitric oxide, as well as activating the cAMP pathway which activates metabolic pathways, such as transcription pathways of mitochondrial genes, improves lipid metabolism, increases antioxidants, and subsequently reduces the activity of caspases, especially caspase-3, and the apoptotic mechanism. It has also been shown that exercise with a mechanism of improving lipid profile in people with S abuse converts the pathological hypertrophy into physiological hypertrophy via improvement in beta-adrenergic receptors, angiogenesis, and mitochondrial biogenesis (20,28). Therefore, it seems that exercise can inhibit TNFL and FasL expressions in the heart tissue through these mechanisms (20).

Tribulus terrestris extract at a dose of 50 reduced TG and increased HDL-C, while TT100 reduced LDL-C levels. Moreover, TT50 and TT100 reduced cardiac *ar-1* and *fasl* gene expressions in S-exposed rats. In this regard, the consumption of TT extract for 12 weeks increased HDL-C and decreased LDL, TG, and cholesterol in rats fed with a high-cholesterol diet (31). Additionally, the consumption of 50 and 85 mg/kg of TT improved fat profile and increased antioxidant enzymes in rats with ischemic heart disease induced by isopropanol; however, the effect of 50 mg/kg dose was more favorable than 85 (14). The optimal effects of TT on different diseases are dose-dependent. For example, receiving TT at 250 and 500 mg/kg doses for 21 days inhibited apoptotic pathways and reduced the risk of ischemia but had no significant effect on fat profile in rats with an isoproterenol-induced heart attack (14). Furthermore, the consumption of 120 mg/kg of TT for 8 weeks had a significant effect on AR-1 in rats (32). Additionally, TT consumption at different doses for 8 weeks improved the signaling pathways of p38αMAPK, JNK, and Akt, decreased Bax and Bad and increased Bcl-2 in cardiac tissue in vivo and in vitro (12). The use of

medicinal plants such as TT, by increasing the expression of catecholamines and cAMP, leads to hormone-sensitive lipase phosphorylation, the increase of fat phosphorylation and the expression of antioxidant enzymes, and inhibition of oxidative stress in the heart tissue (14). Moreover, saponins in TT are found to improve the function of human endothelial cells by increasing vasodilation and reducing inflammatory factors (33). In addition, the consumption of TT by activating the pathways of insulin-like growth factor-1 (IGF-1), IGF-related protein-3 and growth hormone increases nitric oxide synthase expression and the expression of *pparγ* and leads to an increase in mitochondrial biogenesis and improvement in fat metabolism (34). Moreover, by reducing MDA and oxidants, and increasing SOD and cardiac ATP, TT decreases inflammatory factors and their receptors such as TNF-α and TNFR and TNF-α ligand. It modulates Ca2+, improves ERK1/2 signaling pathways, and inhibits the expression of caspases and *fasl*. The inhibition of c-fos, c-jun, and pkc-α via angiogenesis also plays a significant role in inhibiting apoptosis of cardiac tissue (35). Studies on the effect of the consumption of TT along with S on fat profile have been limited, but high doses of this medicinal plant appear to have different effects on fat metabolism due to its androgen-like properties along with the effects of S.

S+RT+TT100 also reduced serum levels of TG, Chol, and LDL-C and increased HDL-C serum levels in S-exposed rats. Additionally, SRTT50 increased HDL-C in S-exposed rats. Moreover, SRTT100 and SRTT50 reduced the expression of *ar-1* and *fasl*. Exercise, by increasing the oxidation of fatty acids, increased the expression of LDL receptors and reduced the expression of HMG-COA reductase in the liver tissue (26). It also improved gluconeogenesis and increased insulin sensitivity, vasodilation, and Akt/PKB phosphorylation in the adipose tissue leading to improved fat metabolism (27). The consumption of TT, by increasing catecholamines, increased cAMP expression, hormone-sensitive lipase phosphorylation (14), vasodilation (33), expression of *pparγ*, mitochondrial biogenesis, and insulin sensitivity and improved fat metabolism and heart protection (34). Increased VEGF, cardiac angiogenesis, nitric oxide, activation of the cAMP pathway, transcription of mitochondrial genes and antioxidants can reduce caspase-3 activity and inhibit apoptosis (20,28). Therefore, it seems that exercise can improve lipid profile and reduce the FasL-dependent apoptosis via noted mechanisms (20). The testosterone-like effects of TT during exercise increase strength, muscle mass, and lean body mass and reduce the percentage of body fat in athletes; therefore, taking TT along with RT with synergistic effects increases energy expenditure in the muscle cells (26,27,34). In a study by Pokrywka et al, it was stated that TT consumption at higher doses had more favorable effects on athletic performance so that the dose of 3.2 mg/kg along with exercise did not have a significant effect on the increase of strength; 20 mg/kg was more desirable than 10 mg/kg. Moreover, 100 and 200 mg/kg had a positive effect on the increase of strength and muscle volume, but doses higher than 750 mg/kg had no significant effect on the improvement of performance (15). A study showed that exercise and consumption of 125 mg/kg of TT kg decreased muscle damage in athletes (16). Moreover, in a meta-analysis study, it was shown that TT consumption and RT with synergistic effects increase muscle mass and strength in male athletes (36). Five weeks of consuming TT at 400 mg/kg dose decreased fat mass and improved performance in elite rugby players (37). Additionally, 8 weeks of RT combined with daily consumption of 1000 mg/kg of TT improved sex hormones in men with type 2 diabetes (38). Previous studies have reported the anti-inflammatory and antioxidant effects of TT by modulating levels of nuclear factor kappa B (NF-κB), TNF-α, and IL-1β and increasing *PPAR* gene expression; however, these antioxidant effects appear to be dose-dependent in decreasing apoptosis and oxidative stress in the brain tissue (35). In a study, 50 and 100 mg/kg doses of TT had beneficial effects on sex hormone function and weight; however, higher doses had more favorable effects (35). However, in circumstances similar to the present study in which rats appear to have been affected by anabolic steroid abuse, the consumption of TT extract itself, partly due to estrogen-like compounds (15) at higher doses, could have different effects on *fasl* and *ar-1* expression levels as well as lipid profile in rats exposed to stanozolol.

Considering the favorable effects of RT along with a higher dose of TT on fat profile, RT and consumption of 100 mg/kg of TT can be suggested as a solution to the impairment of lipid profile and reduction of *ar-1* and *fasl* expression levels following AASs abuse. However, according to the complex and multifaceted mechanism of fat metabolism, and the dependence of RT on the severity and duration of the training period, the inability to investigate the effect of RT with different intensities on metabolic changes in the muscle, liver, and heart tissue following injury induced by S was one of the limitations of the present study. Therefore, it is recommended that in future studies, different intensities of training and more molecular cellular mechanisms should be evaluated. Due to the need for greater confidence in the optimal effects of training and TT, it seems that the lack of evaluation of the type of hypertrophy, oxidative stress, and apoptosis in the cardiac tissue was another limitation of the present study; therefore, it is recommended that pathological and morphological changes be measured in future studies.

Conclusion

Resistance exercise and consumption of TT appear to have positive effects on lipid profile, as well as *fasl* and *ar-1* gene

expression in S-exposed rats, but further studies are needed to investigate the tissues involved in fat metabolism using molecular cellular approach.

Authors' Contributions

Laboratory studies and tests: MD; study and review: FT and SA H; analysis and interpretation of data: MD, FT, KJD and SAH.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Ethical Issues

This study was approved by the Ethics Committee of Isfahan (Khorasgan) Branch of Islamic Azad University with code IR.IAU.KHUISF.REC.1398.264.

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