Original Article

The Effect of Endurance Training and L-Carnitine Supplementation on Gene Expression of Hepatic Enzymes (AST,ALT,ALP) in Wistar Male Rats Toxicated by Boldenone

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Abstract

Background & Objective: The aim of this study was to investigate the effect of endurance training and L-carnitine supplementation on gene expression of hepatic enzymes (AST, ALT, ALP) in Wistar male rats toxicated by Boldenone.

Materials & Methods: In this experimental study, 30 male Wistar rats aged 12 weeks (weight 195±7.94g) were randomly divided into five groups: control, no-treatment, boldenone (5mg/kg), L-carnitine and aerobic training-L-carnitine. The endurance moderate intensity training program (50-55% of maximal oxygen consumption) was performed for 6 weeks and 5 times a week. Boldenone injection once a week, on an appointed day, and in the hamstring was conducted in depth. After anesthesia, autopsy was performed and the liver was isolated. The hepatic enzymes gene expression in the samples were measured by Real Time PCR. Data were analyzed by t-test, One-way ANOVA and post hoc Scheffe at the significant level $P<0.05$.

Results: The results showed that there was a significant difference between the mean expression of liver enzymes (AST, ALT, ALP) in male Wistar rats in different groups ($P<0.001$). The changes in liver enzymes gene expression (AST, ALT, ALP) in L-carnitine and Training-L-carnitine groups were significantly lower than the boldenone group ($P<0.001$).

Conclusion: According to the findings of this study, supplementation of L-carnitine with regular aerobic training reduces liver damage induced by anabolic androgenic steroids.

Keywords: Aerobic training, Boldenone, L-carnitine, Hepatic enzymes, Wistar rats

Introduction

Anabolic androgenic steroids are testosterone-derived compounds that have beneficial effects on some organs of the body. However, studies have shown that anabolic androgenic steroids have many side effects depending on the duration and dosage of administration. High physiologic doses of anabolic androgenic steroids cause toxicity and impaired liver function (1). The liver is the largest gland in the body and is involved in many metabolic functions, including protein synthesis and detoxification, and has various enzymes such as alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP). All three enzymes (AST, ALP, ALT) are widely distributed in the liver, and any damage to the liver cells causes the release of these enzymes into the bloodstream (2). Boldenone is a testosterone-derived steroid that exhibits strong anabolic and androgenic actions to improve growth (3). Various studies have shown that using boldenone causes liver damage (4-6). Abuse of boldenone may alter liver structure and functions as a major site of clearance of androgenic anabolic steroids (6). Also, vascular hypertension and hemorrhage, inflammation, degeneration, cell and nucleus deformation have been reported in liver tissue following abuse of boldenone (7,8).

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L-carnitine is a bioactive form of carnitine, an unnecessary endogenous branched amino acid found naturally in skeletal muscle and the heart, liver, kidney and plasma (9). L-carnitine plays an important role in energy production and has been shown to transport free fatty acids into the mitochondria, thereby increasing the preferred substrate for metabolic oxidation (10). Therefore, athletes utilize L-carnitine as an energetic substance in endurance activities to increase the release of free fatty acid into the mitochondria (11). Studies have shown that L-carnitine plays a potential therapeutic role in chronic diseases. In this regard, the effects of L-carnitine on liver enzymes in rats have been investigated. Studies have shown that L-carnitine may prevent liver damage by altering AST, ALT, and ALP levels (12).

On the other hand, it has been shown that the effects of anabolic androgenic steroids are moderated following exercise. Exercise has beneficial effects on various organs of the body, especially the liver by reducing the risk factors (13). The results of studies reporting the effect of exercise on liver enzymes are inconsistent, with a significant increase (13,14), decrease (15), and no change (16,17) in liver enzymes after exercise.

Health researchers have been interested in anabolic androgenic steroids since many athletes have used over-the-counter doses of anabolic androgenic steroids to increase muscle mass or improve performance (18), and these conditions lead to liver tissue toxicity and damage. Long-term administration of high doses of anabolic androgenic steroids reduces the protective mechanism of the liver. There have been numerous reports of research about the side effects of anabolic steroids on various organs of the body, including liver damage, which may endanger the life of hepatocytes (5, 19,20).

Therefore, due to the negative and uncontrolled effects of anabolic androgenic steroids on the body, and in particular their effect on the liver, it may be possible to control liver enzyme levels by investigating the effect of effective supplements such as L-carnitine. To the best of our knowledge, there are few studies on the harmful effects of boldenone on the liver and the defense mechanisms against boldenone-induced hepatotoxicity. Also, the effect of L-carnitine on the hepatotoxicity induced by androgenic anabolic steroids following exercise is not known. Therefore, the present study aimed to investigate the effect of a course of endurance training and L-carnitine administration on the expression of hepatic enzymes (AST, ALT, ALP) in Baldenone-toxicated male Wistar rats.

Materials & Methods

Animals

In this experimental study, 30 male wistar rats aged 12 weeks with the weight of 195±7/94 were selected. The sample was accomplished using targeted sampling method according to weight and age. Then, they were divided randomly into 5 groups, with six rats in each group. First, all groups began to use 7-week high-dose steroids (5 mg/kg). Groups were divided as follows, 1) Control group did not use any substance and did not perform any activity (C); 2) The group without treatment did not do any activities and did not take any extracts since the beginning of injection and training (WT); 3) One group that continued using steroids (boldenone group (B); 4) One of the groups used only carnitine (100 mg per kg) (C); 5) The other group used carnitine and did training (CT).

Intervention

Study groups were divided into rat's special cages for rat made of PVC with steel mesh cap and the floor was covered with clean wood chips. The room temperature was 22 ± 4/1 degrees with humidity of 65 to 75 percent. The sample animals had a 12-hour sleeping and awakening cycles with access to water and foods. They were fed by compressed special food made by Gorgan Factory and given refined civil water. For boldenone injection of insulin graduated syringes were used. The injections were done once a week, at 11 a.m and on an appointed day of the week. The injections were administered deeply in the posterior thigh muscles. The control group received the physiological solution of normal saline.

Procedure for Intake of L-Carnitine Supplementation

The experimental groups during the intervention period received 100 mg of L-carnitine as gavage per kilogram of body weight (21).

Aerobic training Protocol

In the present study, intermediate training intensity (50-55% of maximal oxygen consumption) and physiologically effective exercises were used. The training groups were given treadmill exercises with the average

intensity of 5 days a week for the duration of 6 weeks. Speed and duration of treadmill exercise gradually increased from 10 meters per minute for 10 minutes in the first week, 10 meters per minute for 20 minutes in the second week, 14-15 meters per minute for 20 minutes for the 3rd week, 14-15 meters per minute for 30 minutes in the fourth week finally to 17-18 meters per minutes for 30 minutes in the fifth week. In order to achieve consistency of results in uniform mode, all training variables were kept constant in the final week. To stimulate the rats to run, auditory stimuli (hitting the treadmill) were used. At the first session, electric low-voltage stimulus along with sound stimulus were used. After conditioning the rats to running, at the other sessions only auditory stimuli were used for ethical purposes (22).

Sampling Procedures and Measuring Changes in Gene Expression in Liver Tissue

At the end of the study, the animals were kept fasting for 12 hours. The samples were then weighed and anesthetized for sampling. Anesthesia was done using mixture Ketamine ™ (30-50mg/kg body wt, ip) and Xylazine (3-5mg/kg body wt, ip). For anesthesia the animal was fixed on the rodent surgery board, autopsy was performed and liver tissue was immediately removed. In this research, ethical issues about laboratory work on animals including the availability of water and food, proper maintenance and non-refoulement training were considered. All experiments were conducted in accordance with the Helsinki Contract Policies approved by the Ethics Committee of Ferdowsi University No. 19753/3.

Measuring the gene expression of AST, ALT, and ALP was assessed by Real time - PCR technique and analyzed after the quantification of gene expression values using the formula $2^{\Delta\Delta ct-}$. The considered Primer genes and GAPDH were designed and studied by Allele ID and MEGA 6 software. The specificity of the primers for the target genes was investigated by the BLAST program. In this study, GAPDH gene was used as an internal control. The sequences of primers used in this study are presented in the table 1.

Statistical analysis

After ensuring the normal weight distribution with the Kolmogorov - Smirnov test, Levene test was used to check homogeneity of variances. One-way analysis of variance test was used for changes within the group and Scheffe post hoc test was used to assess differences between groups. All statistical operations were done using SPSS version 23, the considered significance level was P< 0.05.

Table 1. the primer sequences of the variables under study

Gene name	primers	Sequence	Length amplicon
AST	Forward Reverse	5′-GTCATGGGAAAGATGAGGAAG-3′ 5′-TCTCTGCTTTCCGGACACTGG-3′	109 bp
ALP	Forward Reverse	5'-GACCCTCCCCACTCCTGCCTG-3' 5'-TGGCAAGATCATGGTGTACC-3'	101 bp
ALT	Forward Reverse	5′-AGCGTGCCTTGGAGCTGGAGC-3′ 5′-TAACCTGGCGGAAGAAGGTGA-3′	120 bp
GAPDH	Forward Reverse	5′-AAGTTCAACGGCACAGTCAAGG-3′ 5′-CATACTCAGCACCAGCATCACC-3′	94 bp

Results

Table 2 shows the mean and standard deviation of variables in different groups. Data analysis showed that there was a difference between the average of ALP gene expression in the male Wistar rats in groups of research (P<0.001). The results of scheffe test showed that the gene expression of ALP in boldenone supplement group significantly increased compared with control and no-treatment groups (P<0.001). ALP gene expression in L-carnitine and training- L-carnitine group was significantly lower compared with the boldenone group (P<0.001). However, ALP gene expression did not change

significantly in the L-carnitine group compared to the L-carnitine-training group (P=0.421) (Chart 1).

significantly in the L-carnitine group compared to the L-carnitine-training group (P=0.756) (Chart 2).

Table 2. Mean and standard deviation of study variables in different groups

Groups Variables		Control	No-reatment	Boldenone (5mg / kg)	L-carnitine	Training-L-carnitine
Body Weight (g)	First week	244.0±19.5	244.0±4.0	228.6±32.0	232.1±26.8	228.3±25.8
	Sixth Week	317.2±31.5	283±33.5	308.2±26.2	341.6±37.0	325.3±28.9
Liver tissue weight (g)		11.0±0.8	8.4±1.2	9.3±1.0	11.5±1.2	10.1±0.71
ALP(ΔΔCT)		98.0±0.008	1.1±0.081	5.10±0.158	0.7±0.008	0.6±0.008
ALT(ΔΔCT)		0.96±0.54	1.2±0.115	4.2±0.200	0.8±0.070	0.7±0.070
AST(ΔΔCT)		1.1±0.070	1.2±0.050	6.3±0.273	0.8±0.000	0.8±0.000

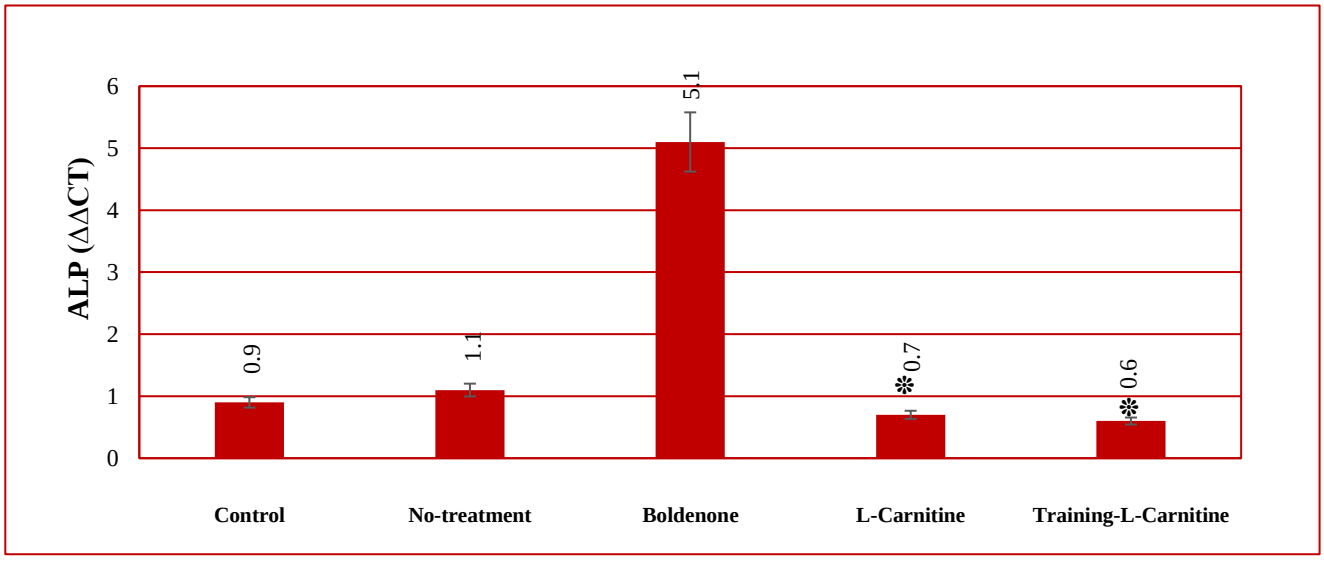


Chart 1. changes in the expression of ALP gene in male wistar rats in different groups
*Significant difference compared to Boldenone group (P<0.05)

Data analysis showed that there was a difference between the average of ALT gene expression in the male Wistar rats in groups of research (P<0.001). The results of scheffe test showed that the gene expression of ALT in boldenone supplement group significantly increased compared with control and no-treatment groups (P<0.001). Changes in ALT gene expression in the group of L-carnitine and L-carnitine-training was significantly lower than that of the boldenone group (P<0.001). However, ALT gene expression did not change

Also, the results showed that there is a difference between the average of AST gene expression in the male Wistar rats in groups of research (P<0.001). the results of scheffe test showed that the gene expression of AST in boldenone supplement group significantly increase compared with control and no-treatment groups (P<0.001). Changes in AST gene expression in the group of L-carnitine and L-carnitine-training was significantly lower than the boldenone group (P<0.001). However, AST gene expression did not change significantly in

the L-carnitine group compared to the L-carnitine-training group (P=0.1000) (Chart 3).

observed the increase in these enzymes following the boldenone administration periods

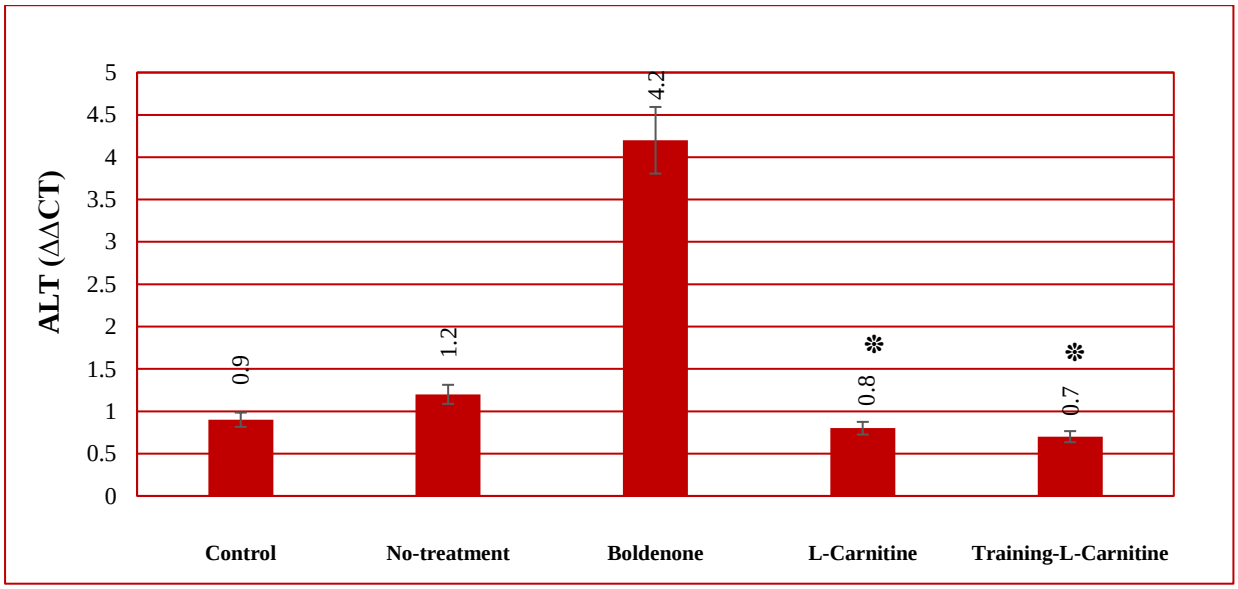


Chart 2. changes in the expression of ALT gene in male wistar rats in different groups
*Significant difference compared to Boldenone group (P<0.05)

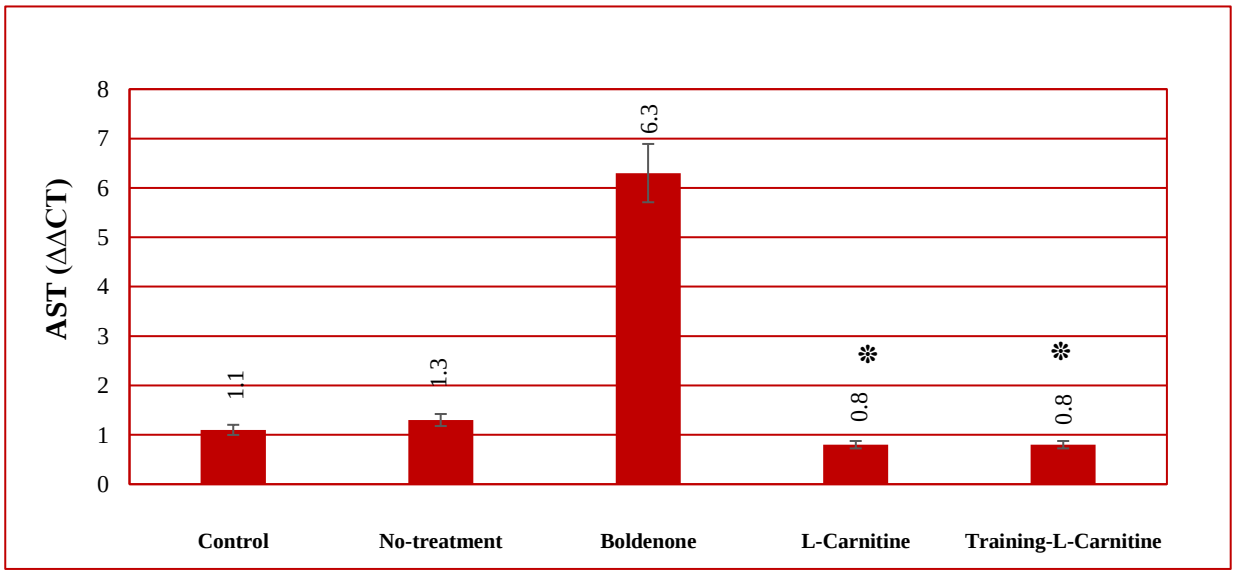


Chart 3. changes in the expression of AST gene in male wistar rats in different groups
*Significant difference compared to Boldenone group (P<0.05)

Discussion

The results of this study showed that the alterations of expression of liver enzymes (AST, ALT, ALP) genes in liver tissue was significantly increased in boldenone group compared to other groups. The results of the present study about the increase in liver enzymes following Boldenone consumption is consistant with the findings of Dornelles et al. (2017), El-Moghazy et al. (2012), Lamiaa et al. (2015) and Mayada et al. (2015) (6,7,23,24). Likewise, the results of Dornelles et al. (2017) showed that boldenone alters ALT activity at doses of 5 and 2.5 mg / kg per day, possibly indicating hepatotoxic effects (23). Also, the results El-Moghazy et al. (2012) showed an increase in ALT and AST enzymes after boldenone injection (5 mg / kg body weight) compared to the control group. Intramuscular injections of boldenone caused mild to severe histopathologic lesions in the liver tissue (6). Molecular mechanisms of adverse effects of

androgenic anabolic steroids on liver enzymes have not been well studied. It is believed that an increase in the activity of hepatic enzymes (AST, ALT, ALP) may be due to the release of these enzymes from the hepatocellular systole of hepatocytes into the bloodstream due to damage to hepatocytes (25). Overall, the findings suggest that boldenone abuse may be associated with permanent damage to the liver structure and function that may lead to advanced liver disease, so people who want to use such steroids to increase their endurance and strength should be careful.

Also, the results of this study showed that the changes of liver enzymes gene expression (AST, ALT, ALP) in liver tissue in L-carnitine supplement group were significantly decreased compared to boldenone group. Very few studies are available on the effect of carnitine on liver enzymes. Keskin et al. (2015) investigated the effects of L-carnitine on liver enzymes in rats on the cholesterol-rich diets. Carnitine groups received 75 mg / l standard carnitine. The results showed that L-carnitine significantly reduced the levels of AST, ALT, ALP (12). These results suggest that L-carnitine may prevent liver damage in rats. The results of the present study also support the idea that L-carnitine supplementation reduces liver damage and enzyme leakage from hepatocytes (12,26,27). L-carnitine has been reported to reduce fat accumulation in the liver. L-carnitine also reduces obesity caused by high-fat diets and excess carnitine inhibits increase in triglycerides and total lipids (28). The main function of L-carnitine is to facilitate lipid oxidation by transporting long-chain fatty acids to mitochondria where beta-oxidation is performed. Therefore, most dietary lipids can be used as an energy source in the body using carnitine (28). One of the beneficial mechanisms of L-carnitine on hepatotoxicity is the ability to stabilize the cell membrane fluidity by regulating the amounts of spinogumiline. L-carnitine has protective properties against mitochondrial drug-induced damage. So, L-carnitine can be safely used in therapeutic doses even with long-term administration without significant side effects. In addition, it has been shown that L-carnitine has antioxidant properties with protective effects against free radical damage (29). Thus, the decrease in hepatic enzyme activity with L-carnitine may be related to the effects of L-carnitine on fat reduction and its antioxidant properties.

In addition, the results of the present study showed that the changes of liver enzymes gene expression (AST, ALT, ALP) in liver tissue were significantly decreased in aerobic training and L-carnitine supplementation compared to boldenone group. It is important to increase the understanding of the side effects of anabolic androgenic steroids in order to find the appropriate treatment and care for athletes and people who abuse anabolic androgenic steroids. The effect of exercise on liver enzymes (AST, ALT, ALP) has been investigated in several studies. The results of the present study indicate that the reduction of liver enzymes (AST, ALT, ALP) following exercise training is consistent with the findings of previous studies (30-33). Studies have shown that aerobic exercise significantly reduces visceral fat and improves insulin resistance. Aerobic exercise may also reduce liver fat (34). In addition, regular aerobic exercise reduces triglyceride, cholesterol, and LDL levels as well as increases HDL levels. These positive metabolic changes resulting from aerobic exercise may eventually lead to an improvement in liver condition, which can be characterized by a decrease in serum levels of hepatic enzymes. Hepatic enzymes decreased, possibly due to a decrease in liver cell damage following steroid use. Also, regular aerobic exercise increases the body's antioxidant capacity, thereby reducing the liver cells damage. However, lipid profile and antioxidant status were not evaluated in the present study. However, the results of some research contradict the results of the present study. The results of Uadia et al. (2016) showed that exercise and flexibility (2 hours per day for 6 weeks) had no significant effect on plasma levels of ALT, ALP, and bilirubin levels in both men and women (35). Ghadampour et al., also investigated the effect of anabolic steroid named Stanozolol along with eight weeks of endurance training on structural changes in liver tissue in male rats. According to the findings, endurance training was not able to prevent hepatic damage caused by Stanozolol and this damage was observed despite injection and in low dose (36). The inconsistency in the previous studies can also be related to factors such as type, intensity, duration, length of course of exercise. The inconsistency of results may be due to the use of different methods. Even in some

cases, the different conditions of the subjects such as age, sex, and fitness may affect the heterogeneous results.

Conclusions

In summary, L-carnitine supplementation along with regular aerobic exercise appears to reduce liver damage caused by androgenic anabolic steroids. According to the results of the present study, it is suggested that L-carnitine supplementation with regular aerobic exercise be used to prevent and treat the liver damage caused by steroidal supplements. However, definitive recommendation needs further studies at the cellular levels and on animal models.

Acknowledgments

This study with research ethics code 19753/3 has been approved by research ethics committee at Ferdowsi University. In this way, all those who have collaborated in this research are kindly thanked.

Conflict of Interests

The authors declare no conflicts of interest.

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مقاله پژوهشی

تاثیر تمرین استقامتی و مصرف ال-کارنیتین بر بیان ژن آنزیم های کبدی (AST,ALT,ALP) در رت های نر ویستار مسموم شده با بولدنون

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چکیده

زمینه و هدف: هدف از این پژوهش تاثیر یک دوره تمرین استقامتی و مصرف ال-کارنیتین بر بیان ژن آنزیم های کبدی (AST,ALT,ALP) در رت های نر ویستار مسموم شده با بولدنون بود.

مواد و روش ها: در این تحقیق تجربی، تعداد ۳۰ سر موش نر ویستار با سن ۱۲هفته و میانگین وزن ۷/۹۴±۱۹۵گرم به‌طور تصادفی در ۵ گروه تقسیم شدند: کنترل، بدون درمان، بولدنون(۵ میلی‌گرم به ازای هر کیلوگرم وزن)، ال-کارنیتین، ال-کارنیتین+تمرین هوازی با شش سر موش در هر گروه تقسیم شدند: برنامه تمرین استقامتی با شدت متوسط (۵۵-۵۰درصد اکسیژن مصرفی بیشینه) به مدت شش هفته و پنج جلسه در هفته اجرا شد. تزریق دارو یک بار در هفته، در یک روز مقرر، و در عضلات همسترینگ به صورت عمیق انجام شد. پس از بیهوشی، کالبد شکافی انجام و بافت کبد برداشته شد. میزان بیان آنزیم های کبدی به روش Real Time PCR اندازه گیری شد. داده‌ها به روش t همبسته، تحلیل واریانس یک‌طرفه و آزمون تعقیبی شفه در سطح معنی‌داری $P<0.05$ تجزیه و تحلیل شدند.

نتایج: نتایج نشان داد بین میانگین بیان ژن آنزیم های کبدی (AST,ALT,ALP) موش های نر ویستار در گروه های مختلف تفاوت وجود دارد (P=۰/۰۰۰). تغییرات بیان ژن آنزیم های کبدی (AST,ALT,ALP) در گروه های ال-کارنیتین و تمرین- ال-کارنیتین نسبت به گروه بولدنون به طور معنی داری کمتر بود(P=۰/۰۰۰).

نتیجه‌گیری: با توجه به یافته های تحقیق حاضر به نظر می رسد مکمل ال-کارنیتین همراه با تمرینات هوازی منظم سبب کاهش آسیب کبدی ناشی از استروئیدهای آنابولیک آندروژنی می شود.

کلمات کلیدی: تمرین هوازی، بولدنون، ال کارنیتین، آنزیم های کبدی، رت‌های ویستار

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