

## The Effect of Gallic Acid Supplement and Resistance Exercise on the Bio-markers of Liver in Intoxicated Male Rats of Anabolic Steroid

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### Abstract

**Background and Objectives:** Liver is one of the vital organs of the human body. Antioxidants have been shown to play important roles in reducing liver injuries. The aim of this study was to examine the effects of gallic acid supplementation and resistance exercise on liver damage biomarkers in male rats intoxicated by steroid anabolic.

**Materials and Methods:** Forty-two male Wistar rats were randomly divided into six equal groups: control, sham, steroid anabolic (5 mg/kg), steroid anabolic (5 mg/kg) + gallic acid supplementation (50 mg/kg), steroid anabolic (5 mg/kg) + resistance exercise, and steroid anabolic (5 mg/kg) + gallic acid supplementation (50 mg/kg) + resistance exercise. Except for control and sham groups, all groups received (injection) steroid anabolic at a dose of 5 mg / kg body weight once a week. The resistance exercise protocol was comprised of three weekly exercise sessions by 5 rep/3 set of climbing ladder for eight weeks. Data were analyzed via ANOVA and Tukey's post hoc test at a significance level of  $P < 0.05$ .

**Results:** The hepatic enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) and bilirubin (total and direct), "In the gallic acid supplementation group ( $P < 0.01$ ,  $p < 0.01$ ,  $p < 0.01$ ,  $p < 0.02$ , and  $p < 0.05$ , respectively), resistance training group ( $p < 0.02$ ,  $p < 0.01$ ,  $p < 0.01$ ,  $p < 0.01$ , and  $p < 0.05$ , respectively), and the gallic acid supplementation group with resistance exercise ( $P < 0.02$ ,  $p < 0.01$ ,  $p < 0.01$ ,  $p < 0.01$ , and  $p < 0.01$ , respectively)" were significantly reduced compared to the anabolic steroid group.

**Conclusions:** Gallic acid supplementation and resistance exercise significantly reduce liver damage biomarkers. However, the simultaneous use of resistance exercise and gallic acid supplementation has no increasing effects on these biomarkers.

**Keywords:** Anabolic Agents, gallic acid, Resistance Training.

## INTRODUCTION

An increase has been recently observed in the tendency to use drug plants for the treatment of different diseases. Certain natural and non-natural compounds have antioxidant properties that play critical roles against the destructive factors of liver (1-4). Gallic acid (3, 4, 5 Trihydroxy benzoic acid) is a trihydroxybenzoic acid found in plants such as barberry pomegranate, nut fruits (5) berries and tea leaves (6). Gallic acid is considered as a highly natural antioxidant which reduces free radical activity (7). Moreover, gallic acid and its derivatives affect cancer cell duplication (8, 9), and different studies have shown the liver protection ability of this acid. For instance, Tung et al. reported that the herbal extract of gallic acid was able to reduce the liver poison derived from CCL4. They further observed that the reduction in the poison due to the prevention of lipid peroxidation, increased the activity of antioxidant enzyme and reduced ALT activity and AST (10).

Liver is the most metabolic organ and detoxification responsible of different material that have an important role in many processes of such as glucose homeostasis, making necessary protein of plasma, making lipoprotein and lipid, making and secretion of bile acids and storage of vitamins (1). Several endogenous and exogenous factors such as poisons (11), drugs (11), hormones (12) and some pathologic ingredients (13) can endanger the liver hepatocyte life. One of the damaging factors is anabolic steroid (14-16) which derives compounds from testosterone (15) to increase power and resistance in canine, equine and humans (athletes) through increasing the production of muscular proteins and constituent basic materials associated with sexual hormones (15,16). Boldenone (17-b-Bol) is an anabolic steroid with high anabolic and moderate androgenic properties. Tousson et al. showed that Boldenone damaged liver (14-16). Specifically, they reported that nine weeks of Boldenone usage induced damage in the liver, kidney and testicles of rats (14). Similarly, Matinhomae et al. showed that 12-week Boldenone consumption damaged the liver of rats, an effect which was more clear at higher doses (0.5 mg in kg), (15). On the other hand, some studies have shown that resistance exercises might improve liver enzymes (17-21). Zelber et al. studied the

effect of resistant exercise on non-alcoholic fatty liver. The results showed that three months of resistance exercise reduced fat and liver enzymes (17). Also, Shamsoddini et al. found that resistance exercises such aerobic exercises increased liver fat and reduced its enzymes (19). Similarly, Bashiri et al. showed that eight weeks of resistance exercise and supplementary creatine use had no significant effect on liver enzyme (2). In Damor et al. study, no changes were observed in liver enzymes following two months of high resistance exercise (22). Likewise, Petterson et al. observed that high resistance exercise was able to increase liver enzymes in hte blood (23). The aforementioned studies indicate contradictory results with regards to the effect of resistant exercise on liver enzyme. Research has shown that gallic acid is probably capable of reducing liver enzyme secretion. Therefore, the present research investigated the effect of eight weeks of resistance exercise coupled with gallic acid consumption on the liver enzymes of male rates intoxicated with anabolic steroid.

## MATERIALS AND METHODS

Forty-two male Wistar rats were prepared from the animal houses of Sari Azad University and categorized into six equal groups: healthy control group, experimental group, Boldenone group, gallic acid supplement group, exercise group, and exercise and gallic acid supplement group. Except for the healthy control and experimental groups, all groups were intramuscularly administered with steroid anabolic (Boldenone brand equipoise made in Meditech Company, Germany) with a dosage of 0.5 mg/kg once a week.

Rats were weighed weekly and gallic acid with brand sigma Aldrichcst (St. Louis. Mo.USA) consumed orally at a concentration of 50 mg/kg. The animals were kept in a standard cage with 12-hour light, favorable ventilation and free access to water and food. This study was carried out in line with the Animal Breeding Guide (24).

The eight-week resistance program included ladder climbing (one meter height and 85-degree slope) with three weekly sessions, and resistance was created by putting the weight on animal tail. Each session consisted of three sets with five repetition that there were one-

minute rest between each repetitions and two minutes rest between each sets. In the first week, the weight of the rats was increased by 50%, with 10% weekly increase depending on the body weight of the mouse. A final 120% increase occurred in the body weight of the rats at the end of the week.

After anesthetizing, an anatomy was performed on the surgery board of rodents through fixing animal, and blood samples were taken from the animals' heart. Subsequently, the blood samples were transferred to the lab. The levels of aspartate aminotransferase, alanin aminotransferase, alkaline aminotransferase, albumin and bilirubin were measured using PARS AZMUN Kit and enzymatic method with an accuracy of 0.1 unit per liter. The bilirubin was measured by Diazo method and serum albumin was measured using photometric method. In

this study, descriptive and inferential statistics were used for statistical analysis. Smirnov-cologomograph test was employed to determine the natural distribution of data. The means of all the obtained values were compared with one-way analysis of variance. The differences obtained from the Tukey sample were used to compare group pairs, and the significance level was considered  $p < 0.05$ .

## RESULTS

Table 1 shows the significant increase in ALP, ALT and AST serum enzymes in the Boldenone group compared to the control group. However, no significant increase was observed in the gallic acid group with a dosage of 50 mg/kg and in the resistant training activity of the ALP, ALT, and AST enzymes. Further consumption gallic acid with resistance training (group 6) could inhibited the increase in the enzymes.

Table 1- comparison of serum ALP, ALT and AST enzymes based on U/lit in different groups

Number	Groups	AST (IU/lit)	ALT (IU/lit)	ALP (IU/lit)
1	Control	146±17.08	55.33±18.29	225.33±74.71
2	Experimental	143.4±22.72	53.8±16.05	221.4±59.05
3	Boldenone	219.5±22.3*	95.16±12.2*	360.66±36.3*
4	Boldenone+resistance exercise	173.74±24.71**	70.22±25.85**	249.85±55.61**
5	Boldenone+ gallic acid	169.71±30.65**	69.14±8.90**	265.71±56.30**
6	Boldenone+gallic acid+resistance exercise	165.58±22.13**	70.42±8.93**	269.83±27.60**
Difference between groups		F	4.168	3.395
		p	0.001	0.006
				3.256
				0.008

\*Each column represents a significant difference with the control group.

\*\*Each column represents a significant difference with the gallic acid group.

Table 2 shows a significant increase in the total bilirubin and direct bilirubin activity in the Boldenone group compared to the control and experimental groups. However, there was no significant increase in the gallic acid consumption group and resistance training

group; moreover, in the gallic acid and resistant training group, the total bilirubin and bilirubin activity were not significantly higher than the control and experimental groups. Also, there was no significant difference between the levels of albumin groups in the table.

Table2- Comparison of bilirubin total, bilirubin and albumin activity

Number	Groups	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Albumin (g/dL)
1	Control	0.53±0.04	0.13±0.05	3.99±0.20
2	Experimental	0.58±0.08	0.14±0.05	4.00±0.14
3	Boldenone	0.75±0.1*	0.22±0.07*	4.05±0.30
4	Boldenone+resistance exercise	0.60±0.07**	0.15±0.04**	4.06±0.05
5	Boldenone+Gallic acid	0.57±0.05**	0.16±0.07**	3.98±0.12
6	Boldenone+Gallic acid+resistance exercise	0.61±0.08**	0.14±0.05**	3.95±0.09
Difference between groups		F	4.747	2.32
		P	0.001	0.03
				0.440
				0.87

\*Each column represents significant difference from the control group.

\*\*Each column represents a significant difference from the Gallic acid.

**DISCUSSION**

Boldenone is an anabolic steroid which increases muscle mass and positive nitrogen balance and reduces protein degradation, and water, nitrogen, sodium, potassium and calcium intake (15). Similar to all other anabolic-androgenic steroids, Boldenone is a banned substance (14). Research has shown that Boldenone damages liver (14, 15), the largest gland in the body involved in many metabolic functions such as proteinization and detoxification (25). The change in the integrity of the liver cell membrane results in the introduction of naturally occurring enzymes within the cytosol located in the blood, which is a good indicator of liver status. Increased activity of liver enzymes in serum is the main indicator of liver damage (1). The return of the liver enzyme activity to its normal state is one of the main indicators of liver therapy (25).

The results of this study showed a significant increase in the liver enzymes in the Boldenone group (Dosage 0.5 mg/kg) compared to the control and the experimental groups, which is indicative of liver damage. Furthermore, a significant change was observed in the liver enzymes in the gallic acid consumption and Boldenone group compared to the boldenone group alone. Such decrease is indicative of the improved hepatic status following the use of gallic acid. Various studies have attributed the cause of hepatic protection to antioxidant properties (7,8). Tung et al. investigated the effects of *Acacia cofusa* herb extract and active ingredient (gallic acid) on the liver affected by tetrachloride. They reported that gallic acid intake protected liver, modified the liver enzymes, and inhibited liver prooxidant (10). Mahboob et al. observed that gallic acid intake protected the liver against the damage induced by paracetamol in mice (26). Gallic acid has also been shown to modify liver biomarkers (26). Bouasla et al. showed that the three-week consumption of gallic acid reduced the liver biomarkers. It is to be noted that levels of liver biomarkers increased in the groups that took sodium fluoride. However, the use of gallic acid protected the liver (27). Also, Ozman et al. showed that a weekly consumption of gallic acid resulted in liver protection in rats exposed to Ischemic reperfusion (28). The results of the above-mentioned studies are consistent with the results of the present study in that gallic acid

reduced the liver damage biomarkers. Furthermore, gallic acid eliminated free radicals (26, 27). The present study showed a significant change in the amount of liver enzymes in the resistance group with Boldenone consumption compared to Boldenone administration alone. Karampour et al. showed that three weekly sessions of resistance training for two months resulted in a significant decrease in liver enzymes (ALT, AST), which is consistent with the results of the present study (21). Waldron also investigated the changes in ALT, AST, ALP Urea, bilirubin and creatinine in the weightlifters participating in the Olympics. These athletes simultaneously underwent intense resistant training for five weeks and used creatine. The results of the present research showed that in the first weeks, ALT, ALP, and AST increased while in the final weeks of the first level, they decreased. Also, the bilirubin level was reduced the initial level. However, Alnumin did not significantly change during the training period (18). Similarly, Zellber et al. studied the effects of resistance training on non-alcoholic liver. They reported that resistance training for three months reduced the amount of fatty liver and the amount of ALT and AST, which is consistent with the results of the current study (17). Radak et al. showed in an overview article that exercise coupled with the increased need for ATP augmented aerobic and anaerobic metabolism, increasing the reactive oxygen species (29). The inhibitory effect of regular exercise is at least partly due to the compatibility with oxidative stress. The process of compatibility associated with exercise isn't just about generates ROS levels, but also increases the antioxidants and the activity of the enzymes, regenerating the oxidative-degradation. Perhaps the reduction in the liver enzymes is attributed to these compatibility, indicating hepatic tissue repair.

**CONCLUSION**

The enzymes of liver (AST, ALT, ALP) decreased in Group 6 (boldenone+exercise+gallic acid) compared with Group 3 (Boldenone). However, no significant difference was observed between Group 6 and Group 4 (boldenone+ exercise), Group 6 and Group 5 (boldenone+gallic acid), and Group 4 and Group 5. These results show

that gallic acid supplementation with resistance training than gallic acid or resistance training by itself, does not has a greater impact on the modification of liver damage. It is important that this issue be accurately investigated in hepatic histology studies.

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#### CONFLICT OF INTEREST

We have no conflict of interest to declare.

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