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Research Paper:

Assessment of Propolis Supplementation on Serum Concentrations of Liver Enzymes in Endurance Athletes with Four Weeks Aerobic Training

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ABSTRACT

Background: Aerobic and intense exercises with an increase in free radicals cause damages at the cellular level, heart disease, cancer, and the development of aging processes, which one of its symptoms is increased serum concentrations of liver enzymes.

Objective: The purpose of this study was to investigate the concurrent effect of four weeks of aerobic training and propolis supplementation on the activity of liver enzymes, including ALT, AST, and SOD in endurance athletes.

Methods: Thirty-two male athletes (age: 21±1.4 years) in track and field were randomly divided into three groups: exercise group, exercise with placebo group, and exercise with supplement group. Propolis supplementation was taken as two tablets (500 mg) twice a day and aerobic exercise was performed for 4 weeks and in 24 sessions with an intensity of 60 to 65% of heart rate. The statistical method was done using one-way ANOVA and Tukey post hoc test by SPSS v. 18 software.

Findings: The results showed that there was a statistically significant difference between groups in serum levels of SOD, AST, and ALT ($P<0.05$). There was not a statistically significant difference between the exercise group and placebo+exercise group in serum levels of SOD, AST, and ALT ($P>0.05$).

Conclusion: The results showed that aerobic exercise alone can increase SOD levels and propolis supplementation with aerobic exercise can reduce AST and ALT serum levels and lead to improved liver cell function.

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1. Introduction

Reactive Oxygen Species (ROS) or free radicals are naturally produced in the human body during various reactions. Free radicals are highly reactive due to the presence of unpaired electrons in their molecular orbitals [1]. Uncontrolled production of ROS inside the cell causes oxidative stress and disturbs the balance of oxidants and antioxidants. ROS can induce oxidation due to the interaction of biological molecules, such as nucleic acids, proteins, and lipids, resulting in the alteration of genetic information and proteins as their expression products [2].

More than 60 diseases in the human body are known to be caused by free radicals, such as Alzheimer's disease, allergies, heart failure, and kidney and liver disorders [3]. During endurance exercise, where oxygen consumption is increased 10 to 20 times higher than the rest free radicals are produced.

In this condition, about 2 to 5% of the body's respiratory oxygen is converted to superoxide. Strenuous or prolonged exercise increases oxidative stress in both sexes [4, 5]. and the production of ROS and the activity of antioxidant enzymes can increase depending on the intensity and volume of the exercise [6]. Physical fatigue after work, especially sports activities can affect many components and functions of the immune system [7].

One of the injuries caused by exercise fatigue is acute liver damage that increases the levels of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) enzymes. Regarding the effect of long-term exercise, it has been found that 4 to 12 weeks of continuous and intermittent exercise significantly increases the levels of the ALT, AST, and Alkaline Phosphatase (ALP) enzymes [8]. Kaynar et al. (2016) examined the effect of short-term intense training on liver enzymes and serum lipids in kickboxing athletes and found that AST and ALT activity increased this exercise [9].

In recent years, much attention has been paid to the effectiveness of supplements, especially herbal and natural supplements in dealing with the damage caused by the production of free radicals. Synthetic antioxidants used in the food industry, such as butylated hydroxytoluene and butylated hydroxyanisole are associated with several side effects and the carcinogenicity of these two compounds has been proven [10].

Propolis is one of the most powerful natural antioxidants among the bee products with high concentrations of flavonoids and phenolic, which its activity according to the Oxygen Radical Absorbance Capacity (ORAC) is four times higher than vitamin E [11]. Propolis can prevent further damage of liver cells due to its antioxidant properties [12, 13] and leading to a decrease in the levels of liver enzymes.

In previous studies, the effects of propolis on serum levels of AST, ALT, and SOD activity have been studied separately. Because during endurance exercise, oxygen consumption increases 10 to 20 times compared to the rest and 2% to 5% of respiratory oxygen in the body is converted to free radicals, the present study was done to investigate the simultaneous effect of aerobic exercise and propolis supplementation on serum levels of these liver enzymes in athletes.

2. Materials and Methods

In this quasi-experimental, field, and laboratory study 32 eligible male athletes in Qazvin board of athletes, Iran who had about five years of experience and exercised at least four sessions per weeks were purposefully selected by the G*Power software and randomly assigned to three groups, including the aerobic exercise and propolis supplement, aerobic exercise and placebo, aerobic exercise after minor changes in their weight.

Each subject was randomly given the number 1 to 32 and then, the numbers 1 to 12 were assigned to the exercise or placebo group, the numbers 13-22 to the exercise group, and the numbers 23-32 to the exercise+supplement group. The inclusion criteria were male gender, age, obtaining a physical health card from a general practitioner, no history of consuming any types of energizers or propolis supplement (at least one month before the test), and providing informed consent. Exclusion criteria were underlying diseases, such as diabetes, cardiovascular disease, musculoskeletal injury, lack of regular attendance at training sessions, and dissatisfaction with the training program. Follow-up of those absent and encouragement to participate in compensatory sessions in case of being absent was done by making a phone call to the subjects.

The data collection tool was the Superoxide Dismutase (SOD) ELISA kit manufactured by the Nond Salamat Company with a sensitivity of 0.2 units/l [14] and Pars Azmoon kits for the measurement of AST with a sensitivity of 2 units/l and ALT with a sensitivity of 4 units/l [15]. Propolis supplement was prepared as tablets (500 mg) [16, 17] made by Soren Tak Toos Company. Also, for measuring weight, the Seca scale made in Germany with an accuracy of 0.1 kg, for measuring height, a Seca caliper made in Germany with an accuracy of 0.1 cm, and a Suunto heart rate monitor made in Germany were used.

Blood samples of the participants were taken once in the first stage (pre-test) in the morning after at least 11 hours of fasting at a rate of 5 cc in a sitting position and then for the second time (post-test) after four weeks of supplementation and training and 48 hours after the last training session and the last supplementation.

The groups one week before the protocol for answering the questionnaires (food frequency, sleep, and appetite), obtaining the consent, baseline diagnosis, and providing necessary explanations on how to conduct the research, including the time required to complete the research, number of days of supplementation, number of blood sampling, the exact date of each of these steps, participated in the briefing session. They were also reassured that taking pills and taking blood would be a positive and safe process.

The meetings, testing, and blood sampling in all stages of the research were conducted in the morning. The schedule of taking the pills was two pills a day after lunch and dinner meals, which were given to the subjects from the first day of use to the last day, including 30 days. This program was reserved for observing the exact time intervals until the end of the action plan.

Exercise program

The subject's exercises were performed for four weeks and four days a week. The exercises were performed two days a week, twice in the morning and in the afternoon, and for two days only in the morning. Afternoon exercises were endurance training (Fartlek training for 10000, 5000, 2000, 1000 m). Morning training included sprint training for 800, 400, 200, and 100 m. The duration of each exercise session was repeated for 2 to 2.5 hours.

At each stage of the exercise (60% to 65% of maximal reserve heart rate), its intensity was monitored by a pacemaker. In order to observe the principle of overload, the training volume (with increasing distance and repetition of runs) was increased every week.

Statistical methods

Differences between contextual variables (age, weight, and Body Mass Index (BMI)) were assessed at the beginning of the study by ANOVA. To test the hypothesis of normality of research variables, the Kolmogorov–Smirnov test was used. In order to analyze the data due to the normality of the variables, a one-way analysis of covariance was used to adjust the effect of the pre-test to compare the mean scores of the groups (placebo+exercise, exercise, and supplement+exercise) in the post-test. The means were compared using Tukey's post hoc test and the mean differences were significant at the level of 0.05.

3. Results

The results of ANOVA analysis showed that there was no significant difference between the variables (age, height, weight, and BMI) of the subjects in different groups at the beginning of the study. The Kolmogorov–Smirnov test also showed that the model errors before and after training had a normal distribution in all variables (P>0/05).

The Mean±SDof pre-test and post-test levels of ALT and AST liver enzymes and SOD levels are shown in Table 1. The results of the Tukey post hoc test showed that the level of hepatic AST enzyme was significantly lower in the exercise+supplement group compared to the exercise (P=0.000) and the exercise+placebo (P=0.001) groups. There was no significant difference between the exercise group and the exercise and placebo group (P=0.188). Also, the results showed that the level of ALT was significantly lower in the exercise+supplement group compared to the exercise (P=0.008) and exercise+placebo (P=0.000) groups. There was no significant difference between the exercise group and the exercise+placebo group (P=0.644).

The levels of SOD were significantly higher in the exercise and supplement group compared to the exercise (P=0.041) and the exercise+placebo (P=0.004) groups. There was no significant difference in SOD levels between the exercise group and the exercise+placebo group (P=0.875).

4. Discussion

The aim of this study was to evaluate the effect of aerobic exercise and propolis supplementation on serum levels of ALT, AST, and SOD in endurance runners. The results showed that four weeks (24 sessions) of combined high-volume aerobic and anaerobic exercise, in the exercise group and the exercise and placebo group increased the serum levels of ALT and AST enzymes. However, in the supplement+with exercise group, the serum lev-

Journal of
Inflammatory Diseases
(Previous Title: The Journal of Qazvin University of Medical Sciences)

Table 1. Descriptive data Mean±SD of the variables

Variables	Mean±SD		Percentage of changes	Mean±SD		Percentage of changes
	Placebo (n=12)			Exercise(n=10)		
	Pre-test	Post-test		Pre-test	Post-test	
aspartate aminotransferase (AST) (IU/Lit)	26.17±10.91	30.83±10.29	0.178	27.10±8.56	36.90±7.00	0.287
alanine aminotransferase (ALT) (IU/Lit)	20.17±9.97	24.83±8.77	0.231	22.30±7.62	25.60±6.55	0.98
Superoxide dismutase (SOD) (IU/ml.lit)	207.17±20.49	215.42±16.89	0.39	197.30±22.07	209.20±23.97	0.6

Variables	Mean±SD		Percentage of changes
	Pre-test	Post-test	
aspartate aminotransferase (AST) (IU/Lit)	28.30±12.59	26.50±12.96	-0.63
alanine aminotransferase (ALT) (IU/Lit)	20.20±7.27	19.40±9.71	-0.39
Superoxide dismutase (SOD) (IU/ml.lit)	201.80±22.44	227.70±12.54	0.26

Journal of
Inflammatory Diseases

els of ALT and AST decreased. A significant reduction in ALT and AST was found in the exercise+supplement group in comparison with the exercise group and the exercise+placebo group. This result may indicate a reduction in liver damage caused by exercise with concomitant use of propolis supplement.

According to the American Society for the Study of Liver Disease, ALT and AST are good markers of liver health and disease and elevated serum ALT is a characteristic of non-alcoholic fatty liver disease [18]. No research has been done on the effect of a period of endurance aerobic training and propolis supplementation on serum levels of ALT and AST.

Sharma et al., Kolankaya et al., and Türkez et al., used propolis in mice to counteract elevated ALT and AST levels in their study, but unlike the present study, aerobic exercise was not used [19-21]].

Zakerkish et al. also showed that 90 days of consumption of Iranian propolis reduced liver enzymes of ALT and AST in type 2 diabetic patients. Oršolić et al. in their study used propolis to regulate serum levels of hepatic lipids and reduce weight in mice [22, 23].

Other supplements have been used in some previous studies to reduce the levels of liver enzymes. For example, Basirat-Dehkordi et al. investigated the effect of aerobic exercise on AST and ALT enzymes, but their antioxidant supplement was embryonic gel [24]. Masoodsinaki et al. investigated the effect of omega-3 supplementation along with a course of selected aerobic exercise on liver enzymes (ALT and AST) [25]. The similarity of these studies with the present study is the positive effect of antioxidant supplements on liver enzymes.

Studies on propolis supplementation have shown that it contains high amounts of flavonoids. Its extract has different effects, including anti-cancer, anti-viral, anti-inflammatory, anti-bacterial, anti-fungal, and antioxidant properties [26, 27]. The strong antioxidant effect of propolis extract prepared from different regions is known to be due to high flavonoid levels in the extract [28].

Our studies on propolis for various therapeutic purposes began several years ago. In 2017, we conducted a study on the effect of propels polyphenols to improve learning and memory in mice and positive results were achieved [29]. In another study, the antioxidant and antimicrobial properties of propolis were shown to accelerate wound healing [30].

The results of the present study also showed that four weeks (24 sessions) of combined high-volume aerobic and anaerobic exercise in all study groups increased the serum levels of SOD. However, in the propolis+exercise group, this increase was significant compared to other groups. SOD is considered as the first line of defense against the harmful effects of oxygen free radicals in the cell and reducing its activity leads to increased cell damage. Increased SOD levels indicate that propolis supplementation along with exercise can have antioxidant effects by stimulating SOD production. Propolis is a combination of polyphenolic compounds that has antioxidant properties and positive effects on liver function.

According to the results, it is recommended that athletes who do high-volume and intense exercise, considering the approved roles of propolis in increasing the body's antioxidant capacity and reducing enzymes that indicate liver damage, consider taking this supplement. Considering the beneficial effects of propolis in the present study and in case of repetition of such effects by other studies on athletes and non-athletes, its use can be recommended to treat and reduce liver damage.

5. Conclusion

The results of this study showed that four weeks of endurance aerobic training increased AST and ALT levels in the exercise and exercise+placebo groups; however, exercise and consumption of propolis prevented this increase, which could be a sign of reduced liver damage. On the other hand, increased SOD levels in the exercise and propolis supplementation group reduced cell damage. By reaffirming the antioxidant effect of propolis and reducing liver enzyme levels in athletes who took this supplement, the research group recommends the use of this supplement to the sports community of the country for liver health until further research.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Qazvin University of Medical Sciences (Code: IR.QUMS.REC.1396.44).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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