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Effect of Pistacia Atlantica Extract on Glutathione Peroxidase Tissue Levels and Total Oxidative Capacity of Liver and Plasma Lipid Profile of Rats

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Abstract

***Background*:** Exercise causes increased oxygen consumption, leaving cells exposed to oxidative stress. Antioxidants may have a protective effect by inhibiting lipid peroxidation. Thus, this study aims to examine the effect of *Pistacia atlantica* extract on glutathione peroxidase levels and total oxidative capacity of liver and plasma lipid profile of rats.

***Materials and Methods*:** In this experimental study, 28 female rats' weight 155.8±2.7 grams were randomly and equally divided into 4 groups of exercise-saline, control-saline, exercise-mastic, and control-mastic. The exercise groups exercised for 8 weeks (5 days per week, 60 minutes daily, 25 meters per minute, on a zero degree slope). The rats received equal volumes of mastic and saline orally for 4 weeks. Blood and tissue samples were taken 72 hours after the last exercise session. Data were analyzed using one-way variance analysis (ANOVA).

***Results*:** Consumption of *Pistacia atlantica* extract together with endurance exercising for 8 weeks did not significantly affect glutathione peroxidase concentration, total oxidative capacity, LDL, triglyceride, or cholesterol, but significantly reduced HDL (p=0.002).

***Conclusion*:** Results showed that antioxidant and lipid profile levels were not affected by consumption of supplements and endurance exercising. However, further studies are required to assess the long term effects of this herbal extract.

Introduction

Free radicals are atoms or molecules with a single electron that makes them highly reactive. Formation of free radicals in living organisms damages macro-molecules such as DNA, proteins, and lipids. To defend against free radicals, body has an antioxidant defense mechanism that includes enzymatic antioxidants like super-oxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), and non-enzymatic antioxidants such as vitamins A, C, and E, flavonoids, uric acid, bilirubin, ferritin, and thiols like glutathione (GSH), ubiquinone (coenzyme Q_{10}), and micronutrients such as, iron, copper, zinc, selenium, manganese [1]. The imbalance between the production of free radicals and antioxidant defense system causes oxidative stress that is involved in pathogenesis of various diseases [2]. Oxygen consumption and metabolism of the whole body is elevated by exercise, and consequently, cells are exposed to oxidative stress and reactive oxygen species [3, 4].

Da Cunaha et al. have reported that by increasing anti-oxidative activity of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in rat's cerebral cortex, exercise can have a protective role against oxidative imbalance and brain damage induced by homocysteine [5]. It is hypothesized that long-term aerobic exercise can significantly improve liver function and lipid profile [6, 7]. To date, many investigations have been conducted, in search of natural antioxidants from plant sources. Bing et al. point out that consumption of the antioxidant Kingobiloba extract increases endurance capacity, delays fatigue, improves athletic ability, and promotes the recovery process after strenuous exercise in rats. This improvement was the result of increased superoxide dismutase activity and lowering of liver malondialdehyde (MDA), causing reduced liver tissue lipid peroxidation damage [8]. Akil et al. declared selenium consumption increases brain tissue GSH concentration in rats following swimming exercise [9].

Pistacia atlantica or Mastic is a pistachio species of the Anacardiaceae family [10]. Different concentrations of phenolic compounds in the mastic skin are perfectly able to slow down the oxidation process [11].

In a study, consumption of edible mastic oil and powder increased HDL and decreased blood serum LDL in male and female rabbits [12]. It has also been suggested that dried mixed-nuts may have desirable effects beyond lipid reduction. Sari et al. in their prescription for the participants in a Mediterranean diet substituted saturated fat with pistachio (comprising about 20% of daily caloric intake) for a period of 4 weeks. The pistachio diet led to lower glucose, lipids, interleukin 6, total oxidant status, and malon dialdehyde, and increased superoxide dismutase [13]. As mentioned, most previous studies focused on consumption of antioxidant supplements and exercise separately, without producing comparable results.

Therefore, as no research has been carried out so far into the simultaneous effects of *Pistacia atlantica* extract (mastic) consumption and exercise on some liver tissue antioxidant indices and plasma lipid profile, this study intends to examine the effect of endurance exercise on glutathione peroxidase tissue level and total oxidative capacity of liver and plasma lipid profile of rats, with and without Pistacia plant extract.

Materials and Methods

For the purposes of this experimental study, 28, 6-8 week-old female rats weight 150-200 grams were procured from the laboratory research and animal reproduction center at Amol Pasteur Institute and kept in polyethylene cages at 22-24ºC temperature conditions and 12:12 hour light-dark cycle with no food or water restrictions. They were then randomly divided into 4 equal groups of exercise-saline, control-saline, exercise-mastic, and control-mastic.

The mastic preparation procedure was as follows [14]: Ten grams of mastic fruit powder in 150 ml water was simmered for 45 minutes, and then cooled down to room temperature, and twice filtered through Wattman Number-4 paper. For every 200 grams weight of rats, 1.5 ml (7.5 µl for every gram weight) of the filtered solution was fed to the rats in control-mastic and exercise-mastic groups, 5 days per week, for 4 weeks after the end of the second week, 3 hours after exercise. Simultaneously, equal volume of saline was fed to the rats in control-saline and exercise-saline groups in the same manner.

The exercise program for the two exercise groups included 8 weeks of endurance running exercises (5 days per week, at specified intensity and duration) on a specially designed treadmill. After a 5-day induction, the exercise program began as follows: First week: first session; rats exercised on the treadmill at speed of 20 m/min for 20 minutes. By the fifth session, speed was increased to 25 m/min and duration to 35 minutes. Second week: speed was increased to 25 m/min and duration to 60 minutes for each session. These conditions were maintained through to the end of the eighth week.

On each day after completion of the exercise course, 2 rats were randomly selected from each of the exercise and control groups. Seventy-two hours after the last exercise session, and after 4 hours of fasting, these rats were anesthetized by intra-peritoneal injection of ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg). Then, immediately, blood samples were taken from the right ventricle with an EDTA-coated syringe, and transferred to EDTA-coated test tubes.

Blood samples were then centrifuged at 1500 rpm for 10 minutes. Plasma and liver tissue were frozen in liquid nitrogen and kept at -80ºC for subsequent measurements. To avoid the nightly rhythm effect, sampling began at 8 o'clock in the morning and finished by 11.30 am. To examine glutathione peroxidase and total oxidative capacity of liver, first, the liver tissue was homogenized in a phosphate buffer (17 mMol and 7.4 pH) at the speed of 3000×g and temperature of 4ºC for 15 minutes using a mechanical homogenizer (Polytron, Germany).

The top liquid from homogenized centrifuged samples was used for tests. Glutathione peroxidase level and total oxidative capacity were analyzed using ELISA method in accordance with the guidelines of German manufactured kit, Cayman (Sensitivity: 0.09, Intra-Assay CV%: 4.5), and values of 8.7 mg/ml and 11.4 U/ml were obtained for Glutathione peroxidase and total oxidative capacity, respectively. These results were read using ELISA reader kit (Sunrise model, Takken Corporation, Vienna, Austria).

Cholesterol was measured using enzymatic colorimeter method in accordance with kit manufacturer's guidelines (Greiner Bahlingen Company, Germany, with 4 mg/dl sensitivity). HDL was measured using the same method and with Randox Company kit (sensitivity: 3 mg/dl) of Antrim, U.K. Triglyceride was measured by the German, Greiner Bahlingen kit with 1.5 mg/dl sensitivity. LDL was measured using the values of HDL-c, TG, and TC. Tests results were analyzed by ELISA- reader kit (Ststfax, U.S.).

Normal distribution of data was assured by Kolmograv-Smirnov test, and significant differences between groups were determined using one-way variance analysis (ANOVA). Tukey test was used for assessment of changes between groups. Data were processed by SPSS-16 software, with significance level of $p \leq 0.05$.

Results

After 8 weeks of endurance exercise and consumption of mastic, the following results were obtained:

Glutathione peroxidase concentration reduced in the exercise-mastic group compared with the three other groups. But this reduction was not significant. The difference between exercise-mastic and exercise-saline groups in terms of changes in glutathione peroxidase was not significant, either. In the exercise-mastic group, compared with the three other groups, only an insignificant reduction in total oxidative capacity concentration was observed. Also, the changes in total oxidative capacity in exercise-mastic and exercise-saline groups were insignificant.

HDL concentration in exercise-mastic group, compared with other groups reduced significantly (p=0.002), but changes in HDL in exercise-mastic and exercise-saline groups was not significant.

LDL concentration increased insignificantly in exercise-mastic group, compared with three other groups. In addition, changes in LDL between exercise-mastic and exercise-saline groups were insignificant.

Triglyceride concentration in exercise-mastic group showed an insignificant reduction compared with three other groups. Changes in triglyceride in exercise-mastic and exercise-saline groups were insignificant. Cholesterol concentration in exercise-mastic group reduced insignificantly compared with three other groups. Changes in cholesterol in exercise-mastic and exercise-saline groups were not significant, either (Table 1).

Table 1. Descriptive indices of study main variables (mean±SD)

Variable	Control-saline group	Exercise-saline group	Control-mastic group	Exercise mastic group	*p*-Value
Glutathione peroxidase (nmol/ml)	0.10±0.001	0.11±0.0009	0.105±0.0007	0.105±0.002	0.898
Total oxidative capacity (U/ml)	0.037±0.006	0.04±0.006	0.036±0.003	0.035±0.003	0.242
HDL(mg/dl)	49.13±4.81	53.35±6.85	47.086±2.87	45.12±1.351	0.002*
LDL(mg/dl)	40.065±19.75	28.84±15.52	26.29±23.29	44.61±20.58	0.259
Triglyceride (mg/dl)	92.14±17.72	91.71±23.16	97.71±16.97	105.29±14.90	0.493
Cholesterol (mg/dl)	108.29±20.33	100.43±16.98	93.00±23.88	110.71+22.33	0.4

*$p \leq 0.05$ significance level

Discussion

Results of the study showed, after 8 weeks consumption of *Pistacia atlantica* extract in combination with endurance exercises, the desired effects were not observed in glutathione peroxidase concentration, liver total oxidative capacity, and LDL, triglyceride, or cholesterol concentrations. However, there was a significant reduction in plasma HDL.

Evidence shows a change in enzymatic antioxidants due to exercise leads to oxidative stress [5]. Studies in the past have provided evidence showing that adaptation of antioxidant activity with exercise is induced by a change in mRNA gene expression and regulation of protein enzyme levels. This adaptation is effectively influenced by physiological and environmental factors such as gender, age, and diet [16, 17]. It is hypothesized that consumption of mastic as an antioxidant, increases activity of anti-oxidative enzymes and reduces cholesterol levels in exercised people, and consequently, exercise-induced oxidative stress is effectively reduced [11, 12]. However, in this study, glutathione peroxidase level was lower in exercise-mastic group compared with other groups, but statistically, this was insignificant. Also, between exercise-mastic and exercise-saline groups, the difference in glutathione peroxidase changes was not significant. This meant that, consumption of mastic was not able to increase glutathione peroxidase activity, and could not help improve antioxidant defense system. Glutathione peroxidase reduction have also been shown in other studies [18, 19]. There is evidence that shows intense physical activity disrupts glutathione peroxidase balance through reducing its level in tissues, changing redox status of cells (cell regeneration), and interfering in its production and transfer [20]. Gul et al. described reduced glutathione peroxidase activity level in heart tissue following endurance exercise as exhaustive [21]. Unlike results of this study, consumption of ginkgo boliba [8] and selenium [9] caused an increase in glutathione peroxidase. Along the same lines, Leelarungrayub et al. study showed reduced malondialdehyde and nitrite oxide, and increased Q_{10} and glutathione peroxidase following 9 days of exercise and 300 mg of Q_{10} intake, 12 days after that, in young swimmers [22]. But, Tiidus et al. reported no significant change in glutathione peroxidase in men and women following 8 weeks of cycling exercise [19]. It has been reported that animals lacking antioxidants such as vitamin E and selenium in their food, have higher antioxidant and exercise compatibility compared with those having normal diet [23, 24]. Also, it has been suggested that antioxidant supplements should not be taken prior to exercise, as they impede adaption of muscle cells. In other words, consumption of antioxidants just before competition, when probably feeling exhausted, leads to production of Reactive Oxygen Species (ROS), and may be ineffective [25]. In this study, subjects were animals that freely received supplements during the 8 weeks of experiment.

Increased oxygen intake and tissue damage during exercise, may stimulate the activity of inflammatory cells like neutrophils, which leads to ROS production by NADPH oxidase (Nicotinamide Adenine Dinucleotide Phosphate-oxidase). Also, increasing concentration of catecholamines and temperature during exercise causes oxidative stress. Hence, increasing ROS requires more antioxidants activity [4].

In this study, liver total oxidative capacity reduced in exercise-mastic group, with no significant difference between groups. Also, liver total oxidative capacity increased in exercise group. This increase was also observed in other similar studies [25]. In Aguilo et al. study, increased levels of thiobarbituric acid (total oxidative damage indicator) was observed in aerobic exercise group after a tiring practice session [26]. A possible reason for this result could be short exercise period or non-consumption of supplements during exercise. The general public is of the opinion that regular physical exercise and supplement intake leads to adaptations that elevate total oxidative capacity for defending against oxidative stress. Sacheck et al. in their study observed a significant reduction in oxidative stress indicators after exercise in experimental groups, and a significant increase in total oxidative capacity compared with control group [27]. The reason for contradicting results of this study and Sacheck's may be non-consumption of plant extract.

Other results of this study revealed significant reduction in HDL concentration after 8 weeks of exercise and mastic intake in exercise-mastic group compared with others. However, no significant change was seen in LDL concentration, triglyceride, or cholesterol. This may be the very first study that showed an unexpected reduction in HDL level in exercise-mastic group. Along these lines, Young Choi et al. in an investigation into the effect of coffee on lipid profile and antioxidants in fit rats observed a reduction in HDL level and an increase in total oxidative capacity [28]. In another study by George et al. on the effects of progressive resistance exercise on lipids

and lipoproteins in adults, a reduction in HDL and cholesterol was reported [29].

In confirmation of previous studies [12, 13], and the desirable effects of pistachio on lipid profile, Edwards et al. [30] and Kocyigi et al. [31] reported that 3 weeks of pistachio diet significantly reduced cholesterol, TC/HDL, and LDL/HDL and significantly increased HDL in patients and volunteers with normal fat. Sheridan et al. reported, 4 weeks of consumption of pistachio caused a significant reduction in TC/HDL and LDL/HDL, and significantly increased HDL in patients with moderate hypercholesterolemia. But, cholesterol, LDL, and triglyceride levels did not change [32]. In Edward's study, pistachio replaced recommended daily fat intake (20% daily calorie). Therefore, positive effects of pistachio on lipid profile may have been due to reduced daily calorie from fat intake [30]. Previously, pistachio was regarded as a rich diet of MUFA and effective in reducing CHD risk in diabetic patients [33]. High fiber content of pistachio may explain its desirable effects on lipid parameters. Most studies have recommended pistachio consumption of 15-20 percent of daily calorie intake. In a recent study by Aksoy, pistachio consumption level equal to 20% of daily calorie intake in laboratory rats showed positive effects on lipid profile and LDL oxidation. But, when consumption was doubled (equal to 40%), these effects were reversed [35].

Given that pistachio is a fatty nutrient, could increased daily calorie (by 20%) and the subsequent weight gain be detrimental? Results showed that addition of varying amounts of pistachio to the daily calorie did not tangibly increase subject's weight. As rats are genetically similar and had the same diet, therefore, consumption of pistachio as 20% of daily calorie appears rational. Even though, cholesterol reducing mechanisms of pistachio are unknown, but the following may have an impact: 1- improved endothelial function, 2- A compound of pistachio called Arginine (nitric oxide precursor) may enhance EDV, 3- Pistachio contains fiber, vitamins, micronutrients, and fitsterol, and with a significant reduction in total oxidant status, it may play an important role in improving the pathogenesis and progression of atherosclerosis [33, 34]. Oxidation and antioxidant components may be influenced by different factors, but total oxidant status is more practical to measure than other variables. Therefore, reduction in TOS after a pistachio diet could provide extra data about positive effects of pistachio on lipid reduction.

Generally, this study showed that consumption of *Pistacia atlantica* plant extract together with endurance exercises did not have significant effects on liver tissue antioxidant indicators, or on plasma lipid profile (LDL, triglyceride, and cholesterol) of exercised rats, but caused a reduction in HDL. Therefore, not only the results of other studies about positive effects of pistachio diet with exercise on lipid profile was not confirmed, but also, the idea that pistachio intake with exercise has effects beyond lipid reduction must be more prospectively investigated.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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