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Combination effect of Voluntary Exercise and Garlic (Allium sativum) on oxidative stress biomarkers and lipid profile in healthy rats

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Running title: Voluntary exercise and garlic alone and in combination protect diabetic heart

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Conflict of interests

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Combination effect of Voluntary Exercise and Garlic (Allium sativum) on oxidative stress biomarkers and lipid profile in healthy rats

Abstract

Background: We evaluated the combination effect of voluntary exercise and garlic on serum oxidative stress biomarkers and lipid profile in healthy rats.

Methods: The rats were randomly assigned to four groups (n=7): Control, Garlic, Exercise, and Garlic+Exercise. Rats were fed with raw fresh garlic homogenate by oral gavage (250 mg/kg) or were subjected to voluntary exercise using stainless steel running wheels alone or together for 6 weeks. The samples were collected at the end of the experiment.

Results: After 6 weeks, total cholesterol, low-density lipoprotein (LDL), triglycerides (TG) and high-density lipoprotein (HDL) levels improved in both garlic and exercise group, compared with the control group. We also found that serum glutathione peroxidase (Gpx), Superoxide dismutase (SOD), Catalase (CAT), and Total antioxidant (TAC) levels enhanced significantly following the above-mentioned interventions. Furthermore, simultaneous treatment of rats with garlic and voluntary exercise had an additive effect on these parameters. However, malondialdehyde (MDA) level was not significantly different from control group during our protocol.

Conclusion: The findings revealed that simultaneous treatment of rats with garlic and voluntary exercise improved antioxidant defense system and lipid profile in an additive manner in healthy rats.

Keywords: Garlic; Voluntary exercise; Oxidative stress; Lipid profile

Introduction

Oxidative stress plays a key role in the pathogenesis of many disorders such as cancer, cardiovascular diseases, atherosclerosis, hypertension, diabetes, neurodegenerative diseases and rheumatoid arthritis[1] . It has been developed from an imbalance between the production and detoxification of oxygen/nitrogen free radicals which is induced by a large number of environmental factors[2]. It is obvious that increased oxidative damage in cells is associated with aging[3]. The advantages of garlic in the conditions mentioned above is generally related to its antioxidant effects[4]. Garlic (Allium sativum) is a common harsh flavoring agent used all over the world as food and medicinal plants. The most compelling evidence that garlic has a higher concentration of sulfur constituents than any other Allium species. It has long been used in traditional medicine and obviously has been reported to have various beneficial effects, including anti-carcinogenic, antiatherosclerotic, antithrombotic, antimicrobial, antiinflammatory and antioxidant effects[5]. It was reported that garlic exhibited significant radical scavenging activity and thus increased antioxidant capacity[4]. While many studies have reported a hypolipidemic effect of garlic[6], others have found no detectable lipid response[7-9]. Also, in healthy volunteers lipid parameters were not found to be significantly different between control and garlic treated subjects[4]. As mentioned above, there are reports suggesting that garlic can also decrease serum lipid parameters but the importance of this effect is debatable and its effectiveness on serum lipid levels remains questionable[10]. Exercise is a potent producer of ROS (reactive oxygen specious) and previous studies have indicated that free radicals cause compensatory response including increased antioxidant enzyme activity in animal models[11]. Various beneficial health effects, including reduced oxidative stress, improved lipid profile has been established in response to exercise training[12-14]. It has been reported that exercise training increases antioxidant defense system and decrease lipid peroxidation markers, which can reverse oxidative stress in animal models[15,16].

Thereby, regular exercise at moderate intensity is recommended for improving physiological and functional capacity in healthy subjects. Voluntary exercise is regarded as mild or moderate exercise and is effective in promoting healthy lifestyles. Despite the promising evidence of the effectiveness of garlic and voluntary exercise in reducing oxidative stress and improving lipid profile, little is known about the combination effect of these interventions in healthy animals. Thus the present study was designed to investigate the effect of garlic in combination with voluntary exercise on oxidative stress markers and lipid profile in healthy rats.

Materials and methods

Animals

All animal studies were performed in accordance with the "Principles of Laboratory Animal Care" and were approved by the Tabriz University of Medical Sciences Animal Care Committee. Twenty-eight male Wistar rats (200 - 250 g) were maintained under controlled environmental conditions (24 ± 2°C and 12 h light-dark cycle) and allowed free access to standard rat chow and tap water.

Experiments Protocol

Twenty-eight male Wistar rats were randomly assigned into four groups (n=7): Control, Garlic, Exercise, and Garlic+Exercise. In the control group, the animals were kept 6 weeks in the regular cages and were fed with vehicle only (tap water). Homogenized garlic (250 mg/kg) was fed orally 6 days a week for a period of 6 weeks. For preparation homogenized garlic, garlic (Allium sativum) bulbs were purchased from a local market. Cloves were peeled, sliced, ground into a paste and then suspended in distilled water. (For example, the homogenized garlic was prepared by adding 250 mg of garlic with 2 ml of distilled water (for 4 rats, weight: 250 g) and crushed in a mixing machine). The garlic homogenate was prepared freshly each day. Voluntary exercise performed in exercise groups. Animals were housed in the running wheel equipped cages (1.00 m circumference, Tajhiz Gostar), and were allowed free access to the wheel 24 h per day for 6 weeks. Running distance was monitored daily[17]. The animals eliminated from the study if the running distance was less than 2000 m/day. For evaluation the combination effect of garlic and voluntary exercise (group 4), the rats were housed in the cages equipped with running wheel and once a day garlic homogenate was gavaged according to our protocol at morning. Our sample size was determined based on our similar previous studies[17,18]. After the experiments, the rats were anesthetized with pentobarbital sodium (Sigma-Aldrich; St. Louis, Missouri, United States) (35 mg/kg, i.p.)[18]. Blood samples were directly obtained from the heart of anesthetized rats and then centrifuged at 3500 rpm for 10 min at 4°C immediately. The resulting serum stored in tubes at −70°C for measuring biochemical parameters. Then the animals were sacrificed under deep anesthesia.

Lipid profile measurement

After collecting blood samples by cardiac puncture and isolating serum by centrifuge, Triglycerides levels were evaluated by enzymatic kits (ZiestChem Diagnostic kits, Iran) using glycerol as standard. Moreover, high- density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were measured by enzymatic methods using diagnostic kits, (ZiestChem, Iran) choosing cholesterol as standard. Total cholesterol level measured by an auto blood analyzer (Bayer Corp. USA). The related kit was obtained from Pars Azmoon CO, Iran.

Measurement of antioxidant enzymes activities

Whole blood samples were used for determination of GPX and SOD and CAT.

Superoxide dismutase (SOD) activity was determined using a commercial kit (RANSOD, Randox co., Antrim, United Kingdom) according to Delmas-Beauvieux, et al. SOD activity was assessed at 505 nm by a spectrophotometer (Pharmacia Biotech; Cambridge, England). According to this method, xanthine and xanthine oxidase were used to produce superoxide radicals that react with 2-(4-iodophenyl)-3 (4-nitrophenols)-5-phenyl tetrazolium chloride (ITN) to create a red formazan dye. Concentrations of substrates were 0.05 mmol/L for xanthine and 0.025 mmol/L for ITN. SOD activity was measured by the degree of inhibition of this reaction. After calculating the percent of inhibition by using related formula, SOD activity value was calculated by comparing with the standard curve and was expressed as U/g hemoglobin (Hb) in blood and U/mg protein in tissue[19,20].

Glutathione peroxidase (GPX) activity was determined using the commercial kit (RANSEL, Randox co., Antrim, United Kingdom) according to the method of Paglia and Valentine. GPX catalyzes the oxidation of glutathione (at a concentration of 4 mmol/L) by cumene hydroperoxide. In the presence of glutathione reductase (at a concentration ≥ 0.5 units/L) and 0.28 mmol/L of NADPH, oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm (37°C) was measured using a spectrophotometer (Pharmacia Biotech; Cambridge, England), and then GPx concentration was calculated by the related formula[19,20].

Catalase activity was measured using the Aebi method .According to this method, measurement was performed based on the dissociation rate of H2O2 in 240 nm at 20°C. Myocardial homogenate aliquots and whole blood lysates were centrifuged. The adequate amount of supernatant was added to a reaction mixture that contained 0.002% Triton X-100, 0.1 mM EDTA, 0.5 M potassium phosphate buffer, and 15 mM H2O2 in 1 mL final volume at pH 7.0. Activity was calculated as the decomposition rate within the initial 15 s and expressed as K/gHb in blood and K/mg protein in the heart[19].

TAC was evaluated by a Randox (Crumlin, County Antrim, United Kingdom) total antioxidant status kit in which 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfanate) (ABTS) is incubated with a peroxidase and H2O2 resulting in the radical cation ABTS + production. This has a stable blue-green color, which is evaluated at 600 nm[20].

MDA Assessment

Malondialdehyde (MDA) as the end-product of lipid peroxidation was measured in the blood samples and tissue extracts according to the Esterbauer and Cheeseman method. MDA reacts with thiobarbituric acid (Sigma-Aldrich; St. Louis, Missouri, United States) and produces a pink pigment that has a maximum absorption at 532 nm[19,20].

Statistical analysis

Results were statistically analyzed using one-way analysis of variance (ANOVA) and a subsequent Tukey test. The significant rank was set at $p < 0.05$. Data are expressed as means ± S.E.M.

Results

Serum lipid profile

Table 1 summarizes the effect of garlic and voluntary exercise on lipid profile. As shown in Table 1, garlic and voluntary exercise significantly reduced serum LDL, cholesterol and triglyceride levels ($P<0.05$) in comparison with the Control group. Also, the combination treatment of these interventions decreased LDL ($P<0.05$), cholesterol ($P<0.001$) and triglyceride ($P<0.01$) levels compared with the Control group. Furthermore, HDL content increased in garlic ($P<0.05$) and exercise ($P<0.01$) groups alone or together ($P<0.001$) as compared with Control animals. Moreover, the HDL level was significantly higher in Garlic+Exercise group than garlic ($P<0.01$) and exercise ($P<0.05$) groups alone. In parallel, the amount of cholesterol more decreased after 6 weeks of combination treatment of garlic and voluntary exercise ($P<0.05$) compared with garlic and exercise alone.

Antioxidant enzymes and lipid peroxidation

Table 2 depicts that garlic consumption increased SOD ($P<0.01$), Gpx, CAT and TAC ($P<0.05$) levels and also, voluntary exercise exhibited higher levels of SOD, Gpx, CAT ($P<0.01$), and TAC ($P<0.05$) than those in Control group. Furthermore, their combination treatment increased these parameters ($P<0.001$) in comparison to Control animals. After 6 weeks intervention data analysis showed simultaneous treatment with garlic and voluntary exercise significantly more raised SOD, TAC ($P<0.05$), and Gpx ($P<0.001$), compared to the exercise group. Similarly, SOD, TAC ($P<0.05$), CAT ($P<0.01$) and Gpx ($P<0.001$) levels were higher in Garlic+Exercise group than those in garlic group. ANOVA analysis showed that there was no significant difference in MDA level among the groups.

Discussion

The results of the present study indicated that garlic consumption with or without exercise improved oxidative stress and lipid profile in healthy rats as manifested by a significant elevation in serum SOD, Gpx, CAT, TAC levels and an improvement in LDL, HDL, TG, cholesterol contents. Moreover, our data demonstrated the favorable and additive effects of garlic in combination with voluntary exercise in improving oxidative stress and serum lipid profiles of healthy rats.

Elevated serum lipid levels including LDL, cholesterol, and triglyceride are associated with an increased risk of cardiovascular disorders, particularly with coronary events and atherosclerosis[21]. These simple markers are widely accepted as a risk factor for cardiovascular system disease, leading cause of death in developed countries[21].

Several studies declared that garlic preparations have been linked to cardiovascular benefits[22,23]. Both antiatherogenic and antiatherosclerotic effect of garlic in part is due to lowers the contents of free cholesterol and cholesteryl esters in arterial walls[5]. Other possible contributors to protection against cardiovascular disease are largely due to its high content of organosulfur compounds and antioxidant activity[24]. It was indicated that garlic has sulfur-containing compounds named γ-glutamyl cysteine, which can be hydrolyzed and oxidized to form alliin. In turn, alliin converts to allicin by alliinase, when it is cut or crushed. These sulfur compounds are responsible for the antioxidant activity of garlic[18].

Previously, to have the promising antioxidant potential of garlic, Koseoglue et al also demonstrated that garlic, used as a dietary supplementation, increased the antioxidant capacity of the body in healthy volunteers. Although in this study total cholesterol, LDL cholesterol, HDL cholesterol, and TG were not found to be significantly different in garlic consumers[25]. There are some studies that declared lipid-lowering effects of different preparation of garlic, suggesting garlic to be effective in reducing total serum cholesterol and low-density lipoprotein with elevated HDL levels in individuals[26-28]. Although there are many reports demonstrating the beneficial effects of garlic on serum lipid parameters, some studies declared inconsistent results in the above-mentioned parameters[4,10]. Current study results showed that garlic consumption improved lipid profile in healthy rats that were manifested by a decrease in cholesterol, LDL and increase in HDL levels. The controversial results in some studies may be due in part to the use of different garlic preparations, duration of treatment and baseline cholesterol levels[29]. The mechanisms involved in garlic-induced lipid-lowering effect are diverse. It has been suggested that garlic attenuates the hepatic activities of lipogenic and cholesterogenic enzymes[30], increases the excretion of cholesterol[31] and inhibits cholesterol synthesis[30]. In this study, we demonstrated that the combination of garlic with voluntary exercise resulted in a higher increase of antioxidant levels and a more improved lipid profile contents than either of them alone in the serum of healthy rats.

Regular training is one of the most effective ways to improve quality of life. Most importantly, exercise can help reduce the risk of several illnesses[12]. Previously, it was demonstrated that regular exercise simultaneous with garlic consumption have not significant effect on triglyceride, cholesterol, low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) levels. Even though they showed the HDL-C level increased in the trained group, but this difference was not significant[32]. However, many studies have reported that exercise training improves lipid profiles including LDL, HDL, TG, cholesterol contents, although HDL levels are more sensitive to aerobic exercise than both LDL and TG[33-35]. Also, in previous studies, it has reported that exercise can alleviate oxidative stress by improving the endogenous antioxidative system and exhibiting greater resistance to oxidative stress[15,16]. Therefore, exercise training can reduce oxidative stress and successively, protect against several diseases that are associated with an imbalance in oxidant/antioxidant status[36].

Indeed, it has been reported that moderate-intensity physical activity has more health benefits. It is considered that exhaustive and vigorous intensity activity causes inflammation and enhances oxidative stress associated with ROS[37]. Thus, in the present study, we performed a voluntary exercise facility for the animals, which is regarded as a moderate intensity of physical activity. Although the main mechanism of exercise-induced lipid alterations is not fully understood, it has been reported that exercise probably decreases blood lipid levels by increasing blood lipid consumption[38] and also increased lipoprotein lipase activity[39]. In addition, mitochondrial biogenesis, improved efficiency of cardiac mitochondrial oxidative phosphorylation, reduced mitochondria permeability transition, elevated antioxidant capacity, and induction of myocardial heat shock proteins are the other mechanisms involved in cellular adaptation induced by regular exercise[12,40]. The data of our study revealed that regular administration of garlic in combination with voluntary exercise may induce beneficial effects in individuals providing a healthy life style. One limitation of our study is that we did not investigate further molecular mechanisms for the protective effect of these interventions. So, future research must focus on the mechanisms involved in the protective effect of garlic and voluntary exercise together.

Conclusion

The present study results confirmed that combination of garlic with voluntary exercise treatment may cause more beneficial effects in the antioxidant defense system and lipid profile than the use of garlic or voluntary exercise alone. These finding highlighted the importance of combined effect of these interventions.

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Conflict of interests

The authors declare no conflict of interests.

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Table 1. Lipid profiles in healthy rats following garlic and voluntary exercise and their combination after 6 weeks (means ± SEM). Low-density lipoprotein (LDL), High-density lipoprotein (HDL), Triglycerides (TG), cholesterol.

Groups / parameters	Control	Garlic	Exercise	Garlic+Exercise
LDL (mg/dl)	47±3.2	39±0.81*	39.3±0.7*	38.33±1.02*
HDL(mg/dl)	28.83±1.07	33.6±0.6*	34.8±0.8**	40.1±1.6***#$$
TG (mg/dl)	32.16±2.3	23.8±1.9*	23.8±1.7*	20.66±1.7**
Cholesterol (mg/dl)	82±2.8	70.5±2.1*	68.1±3.5**	57.1±1.6***#$

* p< 0.05, ** p< 0.01, *** p< 0.001 versus Control group. $ p < 0.05, $$, p < 0.001 versus Garlic group. # p < 0.05 versus Exercise group.

Table 2. The oxidative stress biomarkers in the serum of different groups following garlic and voluntary exercise and their combination after 6 weeks (means ± SEM). MDA (malondialdehyde), glutathione peroxidase (GPX), Superoxide dismutase (SOD), Catalase (CAT), Total antioxidant (TAC).

Groups / parameters	Control	Garlic	Exercise	Garlic+Exercise
MDA (nmol/gr protein)	3.2±0.6	3.1±1.2	3.2±0.7	2.8±0.83
Gpx (U/ gr protein)	9.77±0.8	20.49±2.7*	24.45±2.2**	41.04±2.5***###$$$
SOD (U/ gr protein)	1.01±53.7	1.71±2**	1.69±1.02**	2.24±58.7***#$
CAT (nmol/min/mg protein)	6.5±1.5	18.2±2.7*	23.4±2.3**	31.4±3.06***$$
TAC (mmol/lit)	0.75±0.01	1.06±0.08*	1.04±0.07*	1.33±0.03***#$

* $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$ versus Control group. $ $p < 0.05$, $$ $p < 0.01$, $$$ $p < 0.001$ versus Garlic group. # $p < 0.05$, ### $p < 0.001$ versus Exercise group.