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Effect of short-term garlic supplementation on DNA damage after exhaustive exercise in non-athlete men

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ABSTRACT

This study was performed to determine Effect of short-term garlic supplementation on DNA damage after exhaustive exercise in non-athlete men. Twenty male non-athletes (aged 21.05±1.35 years, weigth 67.15±7.30 kg and heigth 179.2±6.92 cm and BMI 22.02±2.95 kg/m ^{2}) in a randomized and double-blind design were allocated in two equal supplement and placebo groups (700mg/day garlic or dextrose for 14 days). After supplementation, all participants were participated in bruce test. The blood samples were taken in three phases (before and after the supplementation and after the exercise). The normal data (Mean ± SD) were analyzed by repeated measure ANOVA, Tukey and independent t-test (P≤0.05). The results showed that a 14-day garlic supplementation hadn't significant effect on 8-oxodG (P›0.05). Moreover, exercise-induced decrease of 8-oxodG in the supplement group were significantly more in comparison with those in the placebo group (P<0.05). Result of the study indicates that 14-day garlic supplementation can effected DNA damage. However, according to few studies conducted in this area, more research is needed.

Key words: Short-term garlic supplementation, DNA damage, exhaustive exercise

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INTRODUCTION

Oxidative stress is a condition in which the delicate balance existing between prooxidant (free radicals) production and their subsequent amelioration via the antioxidant defense system becomes skewed in favor of free radical expression(1). Both the radicals themselves as well as the non-radical species created via interaction with free radicals are collectively referred to as reactive oxygen/nitrogen species (RONS)(2) which arise as natural byproducts of normal cellular energy production or are generated in large amounts by exhaustive exercise or by chemical agents in the environment (3). It has been shown that excess ROS generation may lead to oxidative damage to DNA, lipids and proteins (4). DNA subjected to attack by RONS results in the formation of a variety of base and sugar

modification products. The presence of these modified products is used to indicate oxidative stress, as they are not present during normal nucleotide metabolism. Typically, the product 8-hydroxy- 2-deoxyguanosine (8-OHdG) has been measured as an index of exercise induced oxidation of DNA (5). An increase in reactive oxygen species (ROS) generation and DNA damage has been demonstrated following exercise (4). Aside from two investigations noting a significant increase in 8- OHdG, the majority of studies have reported no change following a variety of exercise protocols. Null findings may be partially due to the fact that moderate duration and/or intensity aerobic exercise may not be sufficient to elicit an increase in 8-OHdG, possibly due to the rapid repair of DNA following oxidation, as an increase in the activity of certain DNA repair enzymes has been observed following acute aerobic exercise. Aside from the measurement of 8-OHdG, assessment of DNA damage has also been performed using the single cell gel electrophoresis assay (Comet assay) which detects DNA damage with high sensitivity. In these investigations, increases have been noted in DNA damage post exercise (5).

Currently, it is clear that both acute aerobic and anaerobic exercise has the potential to result in increased free radical production, which may or may not result in acute oxidative stress. In order for oxidative stress to occur, the RONS produced during exercise must exceed the antioxidant defense system present, thereby resulting in oxidative damage to specific biomolecules (6). Different exercise protocols may induce varying levels of RONS production, as oxidative damage has been shown to be both intensity and duration dependent. During low-intensity and duration protocols, antioxidant defenses appear sufficient to meet the RONS production, but as intensity and/or duration of exercise increases, these defenses are no longer adequate, potentially resulting in oxidative damage to surrounding tissues (7). Recently, different studies indicated that natural antioxidants contained in vegetables and fruits (flavonoids, carotenoids) may be useful in preventing deleterious consequences of oxidative stress (8). garlic known as Allium sativum belonging to Liliaceae family, is a herbaceous plant with small bulbs (9) and effective medicinal substances such as allein, allicin, allinase enzyme, inulin, vitamins A, B and C. Garlic is a traditional plant used not only as a spice, but also for various biological qualities like anticancer, anti-arthrosclerosis, antithrombotic, antimicrobial, anti-inflammatory and antioxidant(10). Garlic's current principal medicinal uses are to prevent and treat cardiovascular disease by lowering blood pressure and cholesterol, as an antimicrobial, and as a preventive agent for cancer. The active constituents are several complex sulfur-containing compounds that are rapidly absorbed, transformed and metabolized. Pooled data from numerous randomized trials suggest that garlic lowers total cholesterol concentrations by approximately 10% and favorably alters HDL/LDL ratios. Randomized trials also support garlic's effectiveness as a mild antihypertensive which lowers blood pressure by 5-7%. Garlic also inhibits platelet aggregation and enhances fibrinolytic activity, reducing clots on damaged endothelium. In vitro data suggest antiviral and antibacterial effects, but these have not been evaluated in controlled trials in humans. Epidemiologic data, in vitro studies and animal data suggest that garlic may help prevent some solid tumors, but no randomized trials have evaluated its effectiveness as a therapeutic agent in oncology (10). Since, in Iran, the effects of garlic supplementation and exercise, has not been studied simultaneously, and also there are few studies in abroad, therefore, the present study aims to investigate the impact of short-term garlic supplementation on DNA damage of non-athlete men, to answer the some of the ambiguities and contractions.

MATERIALS AND METHODS

Subjects

20 non-athlete male students of Islamic Azad University of Ahar(aged 21.05±1.35 years, weigth 67.15±7.30 kg and heigth 179.2±6.92 cm and BMI 22.02±2.95 kg/m^2), to participate in this research, as the samples were selected voluntarily and with their consent. Subjects were selected according to the following criteria: nonsmoking and apparently healthy. Furthermore, all selected subjects were not taking any antioxidant supplements (such as vitamin A, C, or E) 2 month before and during the study; they were asked to follow a rigorously standardized basal diet, avoid of garlic products. Volunteer subjects randomly replaced in two groups of receiver of garlic supplements (700 mg daily for two meals a day for fourteen days) and placebo (dextrose capsule). For control of subjects Nutrition, dietary questionnaire of 24-hour retention was used.

Experimental protocol

First day of study, height, weight and percentage body fat was measured in all subjects. Initial blood sample, at baseline, before starting supplementation, were taken from the Antecubital vein from all the participants. Second samples were taken after completion of 14-day period of supplementation and before Bruce test. After the Bruce test, third samples were taken from subjects. At each stage of depletion, phlebotomy (5 mL for measurement of plasma levels of DNA damage (8-hydroxy- 2-deoxyguanosine)) was performed at rest and before starting work. All

measurements were done in same temperature, humidity, ventilation and lighting. In addition, subjects, 48 hours before the test, don't any heavy physical activity, and their meal before the test was similar.

Measurement of plasma 8-OHG. Determination of plasma 8-OHG levels was performed using the method of Park et al. For the measurement of plasma 8-OHG, 1 ml plasma was spiked with 1,000 cpm [14C]-OHG. Plasma protein was precipitated by the addition of an equal volume of acetonitrile, and precipitated protein was separated by centrifugation at 3,000*g* for 15 min at 4°C. Supernatant was transferred to a new tube and mixed with eight volumes of water. The resulting sample was applied to the preconditionedC18/OH solid-phase extraction column. The solid-phase extraction column was washed with 5 ml of 50 mmol/l KH2PO4 buffer (pH 7.5), and then retained.

Compounds were eluted with 3 ml of 15% methanol in the same buffer. The elute was loaded into the immunoaffinity column prepared with monoclonal antibody for 8-OHG. Purified 8-OHG was dissolved in 50 _l water and injected into an HPLC device equipped with a Beckman Ultrasphere ODS column (5 _m, 4.6 mm _ 25 cm) and an electrochemical detector. The height of the 8-OHG peak and the total radioactivity of the elute were measured. The height of the peak was used to determine the total amount of 8-OHG injected, which was the sum of the plasma 8-OHG and [14C]-OHG added. The amount of 8-OHG was determined by subtracting the amount of [14C]-OHG injected from the total amount of 8-OHG injected. The amount of [14C]-OHG injected was determined from the calibration curve of the peak height of [14C]-OHG. The radioactivity of the elute was used to determine the amount of loss of 8-OHG during the purification procedure using the immunoaffinity column.

Statistical analysis

Standard descriptive statistics were used to report means and standard deviation for baseline characteristics. A repeated measure ANOVA and Tukey post-hoc test and independent t-test used to analyze the data ($P \leq 0.05$). All data was analyzed by using SPSS for windows software version 16.0(SPSS Inc, Chicago, IL)

RESULTS

Table 1 shows Personal Characteristics of garlic and placebo group. T-test results showed no significant differences between the variables of height, weight , age , BMI and percentage body fat of two groups' subjects that indicated to be homogeneous both groups in these variables. Also in the baseline measurement of 8-hydroxy- 2-deoxyguanosine showed no significant difference between placebo and garlic subjects (P=0.541) (table 2).

Table 1: Personal Characteristics of garlic and placebo groups

Variable	Group	N	Mean	SD
Age(years)	Garlic	10	21.20	1.22
	Placebo	10	20.90	1.52
Height(cm)	Garlic	10	178.1	8.45
	Placebo	10	180.30	5.20
Weight(kg)	Garlic	10	72.30	9.65
	Placebo	10	69	9.13
BMI(kg/m^2)	Garlic	10	22.69	2.39
	Placebo	10	21.08	3.27
Percentage body fat (%)	Garlic	10	16.25	3.29
	Placebo	10	15.31	2.91

Table2: plasma 8-hydroxy- 2-deoxyguanosine in baseline of garlic and placebo groups

Variable	Group	N	Mean	SD
8-OHdG (Ng/ml)	Garlic	10	0.45	0.11
	Placebo	10	0.41	0.15

In Table 3, the mean and standard deviation values plasma indices of DNA damage is shown after supplementation and the exercise protocol(Bruce test) in garlic and placebo groups.

Table3: plasma 8-hydroxy- 2-deoxyguanosine after supplementation and the exercise protocol of garlic and placebo groups

Variable	Group	N	Mean	SD
8-OHdG (Ng/ml) after supplementation	Garlic	10	0.30	0.13
	Placebo★★	10	0.43	0.11
8-OHdG (Ng/ml) after exercise protocol	Garlic★	10	0.32	0.04
	Placebo	10	0.62	0.05

★ Significant difference between garlic and placebo groups

★★ Significant difference after supplementation and after exercise protocol

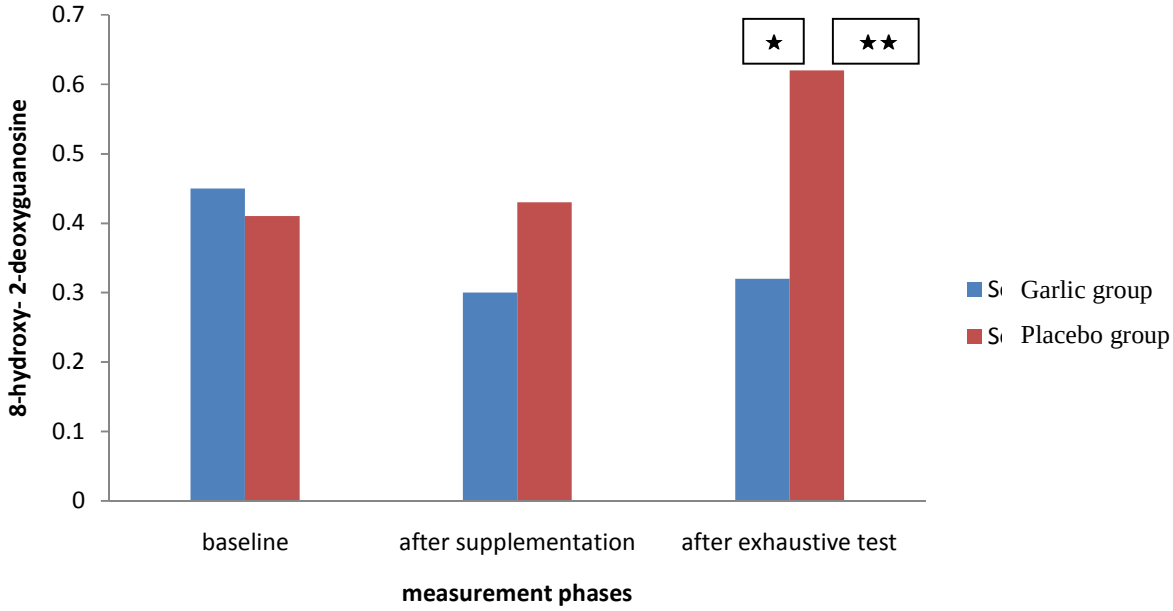

Diagram 1: plasma 8-hydroxy- 2-deoxyguanosine in baseline and after supplementation and the exercise protocol of garlic and placebo groups

★ Significant difference between garlic and placebo groups

★★ Significant difference after supplementation and after exercise protocol

The results of two way repeated measure ANOVA, showed significant difference between measurement phases (3 phases) and group(2 groups)($F=10.103$, $P\leq0.05$). Also, main effect of measurement phases ($F=4.146$, $P\leq0.05$) and group ($F= 30.351$, $P\leq0.05$) was significant. The results of repeated measure ANOVA for any groups, show don't significant difference in effect of measurement phases in garlic group ($F=2.215$, $P\geq0.05$), but, significant difference showed in placebo group ($F=1.16$, $P\leq0.05$). The results of Tukey post-hoc test for placebo group showed significant difference in before and after bruce test.

The results of independent t-test showed that garlic and placebo groups have only significant difference after exhaustive exercise (bruce test) ($F=-14.295$, $P=0.0001$).

DISCUSSION

The purpose of this study was the survey of effect of short-term garlic supplementation on DNA damage after exhaustive exercise in non-athlete men. The results of this study showed that exhaustive exercise (Bruce test), significantly increased 8-hydroxy- 2-deoxyguanosine. Inconsistent with findings of this study Sumida and colleagues (1997), studied Effects of a physical activity session (including exhaustive jogging on a treadmill) on the urinary excretion of 8-hydroxy- 2-deoxyguanosine and Due to lack of significant changes in the amount it offered so much damage to the DNA is not (11). The same group reported that exhaustive activity has not increased amount of 8-hydroxy- 2-deoxyguanosine in untrained individuals, but, beta-carotene supplementation led to reduce steady

amount of damage inflicted on the DNA. Duration, intensity and training conditions are among the factors that are effective in showed signs of DNA damage. In Short-term exercise or exercises with the meantime, there was no DNA damage, but after an exhaustive Bruce test, was observed signs of DNA damage (11).

Also, the present results indicate that short-term garlic supplementation significantly decreased 8-hydroxy- 2-deoxyguanosine after exhaustive activity in the garlic group compared to the placebo group. The research that are compatible with the present study, we could mentioned researches such Avci et al (2008),sener et al(2005), parasad et al(2009) and bhatia et al(2008)(12-15). Effective mechanism of garlic in 8-hydroxy- 2-deoxyguanosine in this way is that garlic through increased enzyme of superoxide and antioxidant enzyme reduce DNA damage (14). Also, the garlic with effect on total antioxidant capacity can decrease DNA damage (13). Possible mechanism proposed in relation to the effects of garlic on increasing total capacity of antioxidant is to that Garlic with increasing intracellular antioxidants such as bilirubin, uric acid, and serum albumin can enhance the total capacity of antioxidant (16). In conclusion, evidence from the present investigation suggests that exhaustive exercise increased oxidative DNA damage as measured by plasma 8-OHdG level and also short-term garlic supplementation decreased oxidative DNA damage caused by exhaustive exercise. It should be noticed that, in the present study, we have analyzed acute effects of exercise and short-term supplementation. Further studies are necessary to evaluate chronic effects of exercise protocols and long-term garlic supplementation on oxidative DNA damage.

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