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Garlic tablet supplementation reduces lipopolysaccharide-induced TNF-alpha production by peripheral blood mononuclear cells

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Abstract

This study was designed to investigate whether garlic tablets possess anti-inflammatory and lipid-lowering effects in healthy adults. Twelve healthy adults participated in a randomized, cross-over design with a three-week treatment and a two-week washout period. Participants received either garlic powder tablets twice daily or two placebo tablets/day for three weeks. Plasma and peripheral blood mononuclear cells (PBMC) were isolated from fasting blood samples at baseline and after each three-week treatment with garlic or placebo. PBMC were cultured, stimulated with lipopolysaccharide (LPS), and changes in cell culture supernatants tumor necrosis factor-α (TNF-α) levels were determined. In addition, changes in plasma high-sensitivity C-reactive protein (hs-CRP), as well as plasma levels of lipids were determined. After three weeks of supplementation, LPS-stimulated TNF-α release in cell culture supernatant was lower after garlic than placebo (P <0.05) whereas no significant changes were observed in unstimulated TNF-α release or plasma TNF-α. There were no significant differences in plasma hs-CRP, cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol levels between garlic and placebo. In healthy individuals, garlic supplementation did not change plasma levels of TNF-α and hs-CRP while it caused lower TNF-α release into cell culture supernatant after stimulation by LPS.

Keywords

C-reactive protein, garlic, lipids, peripheral blood mononuclear cells (PBMC), tumor necrosis factor-α (TNF-α)

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Introduction

Atherosclerosis is the dominant cause of cardiovascular disease and its progress is affected by many modifiable risk factors such as increased low-density lipoprotein cholesterol (LDL-C) and decreased level of high-density lipoprotein cholesterol (HDL-C). In addition, atherosclerosis can be characterized as an inflammatory disease and low-grade chronic inflammation, as indicated by levels of the inflammatory marker C-reactive protein (CRP), influences risk of atherosclerotic complications.[1] Activated monocytes/macrophages are abundant in atherosclerotic lesions and release substantial amounts

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of cytokines. Peripheral blood mononuclear cells (PBMCs) could be involved in this process through migration to atherosclerotic lesions. Activated monocytes/macrophages could release inflammatory mediators such as tumor necrosis factor-α (TNF-α).[2] Some reports suggest that inflammatory cytokines levels, such as TNF-α, may be stronger predictors for the incident of cardiovascular events than CRP level.[3] TNF-α, which is produced largely by macrophages in response to stimuli such as lipopolysaccharide (LPS), could be involved in the atherosclerosis pathological pathway.

Garlic has been used as both food and medicine for thousands of years. It contains high levels of organosulfur compounds and flavonoids, as well as a variety of compounds that function synergistically to provide various health benefits.[4] Various studies have shown the positive impact of garlic effects on lipid levels; however, some contradictory results are also reported.[5] Besides, extensive laboratory studies have demonstrated that garlic has a wide range of biological activities including antioxidant activity, lipid-lowering, and anti-inflammatory activity by suppressing inflammatory mediators.[6–8] Data from human studies are, however, limited and inconsistent. There are many types of garlic products available on the market including fresh garlic, garlic powder (dried garlic), aged garlic extract, and garlic oils. These products have varying quantities of constituents. Dried garlic powder tablets are relatively similar to fresh garlic with respect to chemical components. Dried garlic powder contains diallyl disulfide and alliin, the inactive precursor of the biologically highly potent and strongly smelling allicin.[9,10] Garlic powder extract, alliin, diallyl sulfide, and other garlic-derived compounds have been shown to possess inhibitory potency on several molecules involved in inflammatory responses.[10,11] We propose that supplementation with dried garlic tablet will result in lower plasma systemic anti-inflammatory effects associated with modulated cytokine production by PBMCs in healthy adults. Furthermore, we assessed the effects on plasma lipids.

Methods

Study participants

The study was conducted in a crossover design with two three-week intervention periods. There was a two-week break between periods. Twelve apparently healthy, free-living adults participated (age range, 25–55 years). Volunteers were recruited from the Nutrition Clinic, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The participants included in the study had no diseases demanding continuous administration of any drugs or nutritional supplements. All participants were from urban regions. All participants gave written and informed consent and the study was approved by the National Nutrition and Food Technology Research Institute Ethics committee.

Following the baseline assessment, participants were randomly allocated to receive garlic powder tablets (Garlet, commercially manufactured tablets, 1200 mg allicin/tab): six participants, twice daily; or six participants, two placebo tablets/day, for three weeks. At the end of this period, participants discontinued treatment for two weeks (washout phase) before crossing to the alternate treatment for a final three-week period. Participants were instructed to maintain their usual dietary and exercise habits during the study.

Height, weight, and blood pressure were measured before and after garlic or placebo treatment. Blood pressure was taken by a trained research nurse using a single calibrated mercury sphygmomanometer with appropriate sized cuffs. Measurements were taken with the patient in a seated position with their arm supported at heart level and after 5 min rest. Two serial measurements at intervals of 5 min were recorded and the mean of the two blood pressure measurements was used in the analysis.

Blood sampling and cell culture

Before and after each treatment, peripheral venous blood samples were taken into heparinized bottles after an overnight fast. The blood samples were centrifuged and the resulting plasma was stored at − 80°C. After plasma isolation, each 5 mL of remaining blood was diluted with 10 mL of pyrogen-free phosphate buffered saline (PBS) and PBMCs were isolated by density-gradient centrifugation over Lympholyte-H (density 1.077 g/cm^3, Cedarlane, Burlington, Canada). Isolated PBMC were then washed twice with PBS and cell viability and count were determined using the trypan blue. Then the cells were cultured in 12-well plates (2×10^6 cells/well) in Roswell Park Memorial Institute

Table 1. The effect of garlic on body weight, blood pressure, and plasma parameters.

	Treatment time	Baseline	After three weeks	Change from baseline*
Body weight (kg)	Garlic	69.7 ± 3.9	69.9 ± 3.8	0.1 ± 0.1
	Placebo	70.0 ± 3.8	70.0 ± 3.7	0.0 ± 0.1
Diastolic BP (mmHg)	Garlic	75.0 ± 4.7	74.0 ± 4.0	0.9 ± 3.1
	Placebo	73.6 ± 4.2	73.1 ± 3.8	0.4 ± 1.4
Systolic BP (mmHg)	Garlic	119.0 ± 5.5	115.4 ± 5.7	–3.6 ± 3.6
	Placebo	109.1 ± 4.3	111.6 ± 5.0	2.5 ± 1.4
Plasma AST (mg/dL)	Garlic	19.7 ± 1.9	18.8 ± 2.3	1.0 ± 1.8
	Placebo	18.5 ± 2.0	20.0 ± 1.8	–0.6 ±1.4
Plasma ALT (mg/dL)	Garlic	19.1 ± 2.8	16.2 ± 2.8	2.7 ± 1.63
	Placebo	19.0 ± 3.9	20.3 ± 3.6	0.2 ± 8.6
Fasting plasma glucose (mg/dL)	Garlic	91.4 ± 4.8	89.4 ± 5.5	2.2 ± 3.3
	Placebo	101.5 ± 4.3	88.0 ± 2.2	–0.6 ± 2.5

Data represent mean ± SEM.
*Change from baseline = baseline values – three-week values.
BP, blood pressure.

(RPMI 1640) medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin/streptomycin at 37°C in a humidified incubator with a 5% CO_2/95% air atmosphere for 24 h. Afterward, cells were incubated with or without LPS (Sigma-Aldrich, Heidelberg, Germany) to achieve a final concentration of 1 μg/mL LPS during the culture.[6] After 24 h of incubation, culture plates were centrifuged and the supernatant was removed and frozen at −80°C for the analysis of TNF-α production.

Laboratory measurements

Plasma glucose and lipids were measured using commercially available kits (Pars Azmoon, Iran). Plasma hs-CRP concentrations were measured in plasma with an immunoturbidimetric assay (Pars Azmoon, Iran). Plasma and cell supernatant concentrations of soluble TNF-α were measured using ELISA kit (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions.

The Kolmogorov–Smirnov test was used to determine the normal distribution of continuous variables using SPSS statistical package (version 11, SPSS Inc, Chicago, IL, USA). Values are expressed as means and standard error. Mean changes from baseline were calculated and compared among treatments. A paired *t*-test or Wilcoxon test was used to evaluate differences in each outcome from baseline to the end of the period and among treatments. All *P* values are two-tailed and considered to be statistically significant if $P < 0.05$.

Results

The study group included 12 asymptomatic healthy men and women. The mean age of participants was 38.00 ± 1.86 years (age range, 32–55 years). From 12 participants, six participants (50%) were men and six (50%) were women. In this study, men had a higher weight than women ($P = 0.02$). In all participants, weight was positively correlated with systolic blood pressure ($r = 0.55$, $P = 0.008$) and diastolic blood pressure ($r = 0.52$, $P = 0.01$).

Weight and blood pressure did not change significantly during garlic and placebo treatment (Table 1). Garlic had no significant effect on plasma AST and ALT or FBS. After three weeks of taking garlic tablets, there was a non-significant trend towards a lower total cholesterol ($P = 0.07$), while no similar trend was observed in the placebo treatment (Table 2). In addition, LDL-C tended to increase in the placebo treatment ($P = 0.08$), while it did not change in participants taking garlic. However, the mean change of cholesterol or LDL-C did not differ between garlic and placebo. There were no significant differences in plasma triglyceride and HDL-C in each of the two treatments.

Garlic was associated with the significantly lower production of TNF-α by LPS-stimulated PBMCs than with the placebo treatment ($P = 0.004$) (Table 3). No significant difference was observed between treatments in the plasma TNF-α ($P = 0.9$) or in the production of TNF-α by unstimulated PBMCs ($P = 0.3$). In addition, no significant changes were observed in plasma hs-CRP in both treatments.

Table 2. The effects of garlic on plasma lipids.

	Treatment time	Baseline	After three weeks	Change from baseline*
Cholesterol (mg/dL)	Garlic	181.5 ± 7.3	175.6 ± 5.7	7.1 ± 2.9
	Placebo	178.0 ± 7.9	177.9 ± 8.2	1.8 ± 5.6
HDL-C (mg/dL)	Garlic	50.5 ± 4.7	53.0 ± 4.1	–1.7 ± 1.8
	Placebo	55.5 ± 3.8	56.3 ± 4.5	1.0 ± 1.8
LDL-C (mg/dL)	Garlic	91.2 ± 3.9	94.4 ± 4.1	–1.3 ± 3.0
	Placebo	92.4 ± 3.0	99 ± 4.5	–5.5 ± 2.9
Triglycerides (mg/dL)	Garlic	130.9 ± 21.6	127.0 ± 22.1	11.3 ± 9. 9
	Placebo	120.6 ± 15.9	107.5 ± 18.9	10.3 ± 9.6

Data represent mean ± SEM.
*Change from baseline = baseline values – three-week values.
HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. The effects of garlic on inflammatory markers.

	Treatment time	Baseline	After three weeks	Change from baseline*
hs-CRP	Garlic	1.96 ± 0.69	2.19 ± 0.75	–0.50 ± 0.30
(mg/L)	Placebo	1.70 ± 0.45	1.80 ± 0.59	0.20 ± 0.30
Plasma TNF-α	Garlic	244.99 ± 66.08	243.61 ± 170.95	1.28 ± 171.90
(pg/mL)	Placebo	249.99 ± 80.67	273.93 ± 135.69	–24.58 ± 118.69
Unstimulated supernatant	Garlic	125.84 ± 37.39	110.66 ± 23.29	21.44 ± 39.41
TNF-α (pg/mL)	Placebo	124.00 ± 35.32	98.40 ± 22.36	29.45 ± 36.13
Stimulated supernatant	Garlic	474.08 ± 119.16	230.11 ± 53.32‡	–282.76 ± 146.25§
TNF-α (pg/mL)†	Placebo	246.33 ± 38.11	489.63 ± 117.17‡	243.29 ± 87.74

Data represent mean ± SEM.
*Change from baseline = baseline values – three-week values.
†Peripheral blood mononuclear cells stimulated with lipopolysaccharide.
‡P <0.05 compared to baseline.
§P <0.01 compared to placebo.

Most of the participants found it easy to take garlic tablets. Most of the participants were interested to see if the treatment was effective as they would be willing to continue taking the tablets after the trial was finished.

Discussion

The main finding of the study is that ex vivo LPS-stimulated TNF-α production in PBMC is attenuated by short-term supplementation with garlic powder tablets, whereas no detectable effects on plasma TNF-α or hs-CRP are observed. CRP is a circulating pentraxin that plays a major role in the human innate immune response and is secreted by the liver in response to a variety of inflammatory cytokines, including TNF-α.[12] Our findings regarding plasma levels of hs-CRP or TNF-α agree with results of Van Doorn et al. in which garlic powder had no significant effect on plasma TNF-α or hs-CRP.[13] Mozaffari-Khosravi et al. assigned postmenopausal osteoporotic women to garlic (two garlic tablets/day for one month) or placebo and found no significant difference in serum TNF-α between the two groups, although TNF-α levels were significantly reduced in garlic group when compared to baseline.[14]

Secretion of cytokines by PBMC may be related to the pathogenesis of chronic diseases. Increasing clinical observations reveal that persistent low-grade inflammation is associated with the pathogenesis of chronic diseases such as atherosclerosis, diabetes, and aging-related neurological diseases and low levels of circulating Gram-negative bacterial endotoxin LPS appear to be one of the key culprits in provoking a non-resolving low-grade inflammation.[15]

Our finding regarding lower LPS-stimulated TNF-α production is consistent with those from in vitro studies in which different garlic extracts or its active metabolites reported having anti-inflammatory properties in LPS-activated

monocyte/macrophage cells. Sulfur-containing compounds of garlic have reduced the levels of LPS-induced TNF-α in mouse macrophage cells (RAW264.7).[8] Hodge et al.[6] investigated the effects of unfractionated garlic extract on ex vivo cytokine production. Whole blood and PBMCs from healthy donors were stimulated with LPS in the presence of various concentrations of garlic extract and the effects were evaluated on cytokine production in vitro using a flow cytometric method. Inflammatory cytokine production, including TNF-α, were reduced significantly in the presence of garlic extract. Keiss et al.[7] showed that extracts of dried garlic powder and diallyl disulfide reduce LPS-induced production of TNF-α and IL-1β in human whole blood. Garlic compounds may attenuate inflammatory mediator production by interfering with the clustering of LPS with Toll-like receptor 4 and this could result in blocking the activation of nuclear factor-κB (NF-κB) signaling pathway which is a master switch for the regulation of inflammatory genes, including TNF-α.[8] However, our finding disagrees with the result of a previous study in which 12 weeks of treatment with garlic powder (2.1 g/d) in overweight and smoking participants had no detectable effects on whole blood LPS-stimulated TNF-α release.[13] This may partly be related to the experimental approach used. We used a higher concentration of LPS (1 μg/mL) to activate the PBMC, whereas, in that study, whole blood incubated with 10 ng/mL LPS.

The present study suggests that in healthy adults, short-term treatment with dry garlic powder could not improve plasma lipids, although it tended to reduce total cholesterol. Clinical trials using different types of garlic preparations in hypercholesterolemic patients or healthy participants have demonstrated different results and it was assumed that these discrepancies may have resulted due to the differences in the composition of garlic preparations and the response they may induce. Our results are consistent with previous observations in hypercholesterolemic or healthy participants that found no significant differences in lipid levels between those treated with garlic and placebo.[5] Baseline plasma values appear to be an important factor in demonstrating the lipid-lowering capacity of the treatment under investigation. In a German placebo-controlled study, those participants with baseline higher levels of cholesterol (250–300 mg/dL) experienced a greater decrease in cholesterol levels with ingestion of 800 mg/d of dried garlic powder.[4]

Several limitations of the current study should be considered when interpreting the results. The two main limitations are small sample size and short duration of the study. In addition, we assessed a limited number of parameters related to lipid metabolism or inflammation.

In conclusion, data obtained in the present study using dried garlic powder tablets suggest that supplementation could mitigate TNF-α production by PBMC upon LPS-stimulation. The decreased TNF-α production may be important in individuals who are at a higher risk of low-grade inflammation. However, further studies are needed to address the anti-inflammatory roles of different garlic products. Furthermore, studies with longer duration and different dosage of supplementation are needed to confirm and increase the clinical application of the present results.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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