Electronic Physician (ISSN: 2008-5842) http://www.ephysician.ir
November 2017, Volume: 9, Issue: 11, Pages: 5670-5675, DOI: http://dx.doi.org/10.19082/5670

The effect of consumption of garlic tablet on proteins oxidation biomarkers in postmenopausal osteoporotic women: A randomized clinical trial

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Type of article: Original

Abstract

Background: Osteoporosis (OP) is one of the most prevalent metabolic bone diseases at higher ages, especially in postmenopausal women.

Objective: To determine the effect of consumption of garlic tablet on proteins oxidation biomarkers in postmenopausal osteoporotic women.

Methods: The present study was a double-blind randomized controlled clinical trial that included 42 postmenopausal women in Yazd during 2014-2015. Osteoporotic women were randomly assigned into two groups: the garlic group (GG) and the placebo group (PG). Participants in GG took two garlic tablets daily for 1 month and the participants in PG took placebo tablets in the same manner. After 30 days, the plasma level of carbonyl groups (PCO), total antioxidant capacity (TAC), and advanced oxidation protein products (AOPPs) were assessed by spectrophotometric assays. Also, Malondialdehyde (MDA) content was measured according to the procedure of Thiobarbituric Acid (TBA). Data were analyzed by SPSS version 18, using paired-samples t-test, independent-samples t-test, Wilcoxon, and Mann-Whitney U test.

Results: This study showed that garlic tablets had decreased PCO plasma levels (47.37±5.98 vs. 19.62±3.40 nM, $p \leq 0.001$, before and after the study, respectively), AOPPs (738.95±151.86 vs. 585.12±209.99 µM, $p \leq 0.008$, before and after the study, respectively), and increased TAC (11.34±10.80 vs. 47.93±17.80, $p \leq 0.001$, before and after the study, respectively). The parameters in placebo groups showed no significant differences before and after the study, respectively. The levels of MDA before taking the drug in comparison to before Garlic group was also reduced (1.30±1.04 vs. 0.92±0.81 µM, $p=0.01$, before and after the study, respectively).

Conclusion: The role of oxidative stress in the pathophysiology of many diseases such as osteoporosis has been demonstrated. The present study showed that garlic consumption can reduce the oxidative stress.

Trial registration: The protocol of trial was registered at the Iranian clinical trial register (www.irct.ir) with ID: IRCT138811183273N1.

Funding: This study funded by Shahid Sadoughi University of Medical Sciences (Yazd, Iran).

Keywords: Oxidative stress, Osteoporosis, Garlic tablets

1. Introduction

Osteoporosis (OP) is a silent dangerous disease associated with lifestyle. OP causes fractures which can be mortal or disabling, and increases health care expenses (1). In 1991, the World Health Organization (WHO) announced OP as

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Received: February 07, 2017, Accepted: August 30, 2017, Published: November 2017
iThenticate screening: August 31, 2017, English editing: October 07, 2017, Quality control: October 15, 2017

the fourth health-threatening factor after cancer, myocardial infarction and cerebrovascular disease (2). So, developing OP prevention strategies became one of the main goals of physicians, particularly nutritionists (3). It is estimated that about 61 million people will suffer from OP by the year 2020 (4). Without promotion in preventive strategies, its costs could rise to over 200 billion dollars by 2040 (5). Annually, about 1.5 million fractures occur in osteoporotic patients in the United States (6). OP begins in the fourth decade of life and is more common in women. One out of three females, one out of 12 males, half of the women older than 45 years, and 90% of 75+ year-old women develop OP (7). The main cause of the higher prevalence of this disorder in the female population is menopause and reduction in sexual steroid levels which lead to bone turnover and reduced bone density (8). Other underlying factors associated with OP are low mineral density like CA, protein and vitamin D deficiency, smoking, thyroid diseases, low level of sexual hormone, rheumatoid arthritis (RA), diabetes mellitus (DM), Cushing disease, Body index less than 10% than when at a younger age, body mass index (BMI) lower than 19, alcoholism, malnutrition and certain drugs (such as corticosteroids, furosemide, heparin and phenytoin (9). One of the biggest factors associated with OP is oxidative stress. Under normal conditions, the levels of free radicals such as reactive oxygen species (ROS) are maintained at low levels by and antioxidant systems (enzymes and chemical compounds) in biological systems. If an imbalance between free radicals and antioxidants, referred to as oxidative stress, occurs in the body (10), sex hormones (estrogens and androgens) can influence the growth and structure of the skeleton. So estrogen depletion at menopause in elderly women can cause the development of osteoporosis (11). There is some evidence showing the correlation between oxidative stress and loss of bone density (12). Some studies revealed that estrogen deprivation in menopause increases oxidative stress and bone loss (13). Sendur et al. confirmed the role of oxidative stress over bone density in OP with a study conducted on 45 women in the postmenopausal period (14). On the other hand, estrogen and other chemical therapies have their own complications and can increase the risk of some malignancies, so, extensive efforts have been made to find an effective treatment with the lowest side-effects. Herbal medicine proposed garlic as a treatment of bone loss process due to low estrogen level (15). Garlic is an antioxidant plant and its role in many diseases (like hyperlipidemia [HLP], DM, hypertension [HTN], malignancies) was proved in previous studies (16). Vazquez-Prieto et al. revealed its impact on reducing oxidative stress and vascular cell adhesion molecule-1imperssion in rats (17). A study confirmed that garlic extract antioxidant capacity can effectively decrease oxidative stress (18). As oxidative stress has an important role in OP and garlic has phytoestrogens and anti-oxidative effects, this study investigated the efficacy of garlic tablet in oxidative stress indexes in menopausal women with osteoporosis.

2. Material and Methods

This double-blinded study was conducted as a clinical trial on 42 menopausal women referred to the Milad Center for bone mass densitometry in Yazd during 2014-2015. An informed written consent was received from each patient. They could freely leave the study at any time during the study. All patients were continuing their previous medical treatments and no interruptions were made. Research Ethics Committee of SSUMS approved research proposal of the study. The protocol of trial was registered at the Iranian clinical trial register (www.irct.ir) with ID equal IRCT138811183273N1. Inclusion criteria included: proved OP (femoral or lumbar bone mass index lower than 2.5 g/cm^2), and being aged between 45 and 65 years. Exclusion criteria included patients with heart diseases, HTN, HLP, gastric ulcer, drug use (aspirin, captopril and anticoagulants) a history of allergic reaction for garlic, and dermatitis. Informed written consent was obtained from all participants. Patients were randomly divided into case and control groups using random block method. In the case group, women received 2 garlic tablets daily with meals for one month and the control group received the same shaped tablet as placebo. Garlic tablets (Garlet) contained 1200 µg Alcin equal to 2 g fresh garlic and the placebo was obtained from Amin Industry, Esfahan, Iran. Before and after intervention, 5 cc of venous blood sample were obtained in non-fasting condition. Samples were frozen in -70° C. Plasma protein carbonyl (PCO), advanced oxidation protein products (AOPP), and total antioxidant capacity (TAC) (with 2, 2-diphenyl-1-picrylhydrazyl [DPPH] oxidation) were measured with the following methods: Walwadkar (19), Kataaha et al. (20), Girbal et al. (21), Kitajima (22) and Janaszewska et al. (23). Ikatsu (MDA) measurement was performed by thiobarbituric acid (TBA) (24). Data were coded and entered into SPSS version 18 (SPSS Inc., Chicago, Illinois, USA). Kolmogorov-Smirnov test was used to determine the cases normality. Inferential statistical analyses performed in this study were paired-samples t-test, independent-samples t-test, Wilcoxon, and Mann-Whitney U test. P-values less than 0.05 were considered significant. An informed written consent was received from each patient. They could freely leave the study. All patients were continuing their previous medical treatments and no interruptions were made. The Research Ethics Committee of SSUMS approved research proposal of the study.

3. Results

Forty-two women participating in this study were divided into two groups (Figure 1). Demographic characteristics of patients are summarized in Table 1. As table 1 showed at the beginning of the study, there was no significant difference between the averages of the baseline characteristics in two groups. Serum protein peroxidation measurements are shown in Table 2. Mean PCO level was significantly lower in the garlic group before and after intervention ($p<0.001$), and there was also a significant difference between the two groups ($p<0.001$). TAC mean level was lower before than after Garlet tablet administration ($p<0.001$). Also, the mean TAC level was significantly different between the two groups ($p<0.001$). AOPP level was higher before intervention ($p=0.02$) and this difference was significant between the groups ($p=0.008$). Moreover, MDA was reduced significantly after garlic treatment ($p=0.01$). However, no difference was observed in the control group ($p=0.39$).

Table 1. Baseline characteristics of groups under study

Characteristics	Garlic group (Mean±SD)	Placebo group (Mean±SD)	p-value
Age (year)	56.10±5.81	57.27±5.84	0.51
Age of menarche (year)	13.55±0.95	13.68±1.39	0.72
Age of menopause (year)	48.55±4.40	48.22±5.38	0.83
BMI (kg/m^2)	27.27±2.58	27.59±2.93	0.51
BMD in lumbar spine (g/cm^2)	3.28±0.60	3.28±0.83	0.98
BMD in neck femur (g/cm^2)	1.91±0.90	3.28±0.82	0.91

Table 2. Comparison of oxidation proteins and lipids products before and after intervention in garlic group and placebo group

Variables		Garlic group (Mean±SD)	Placebo group (Mean±SD)	p-value
PCO (nM)	Before	47.37±5.98	43.61±7.35	0.079
	After	19.62±3.40	42.48±11.58	0.001
	p-value	0.001	0.92	
TAC (% DPPH Reduction)	Before	11.34±10.80	11.69±7.77	0.07
	After	47.93±17.80	16.64±8.15	0.001
	p-value	0.001	0.92	
AOPP (μm)	Before	738.95±151.86	736.50±95.69	0.08
	After	585.12±209.99	666.03±208.22	0.02
	p-value	0.008	0.16	
MDA (μm)	Before	1.30±1.04	1.28±1.08	0.72
	After	0.92±0.81	1.54±1.50	0.01
	p-value	0.01	0.39	

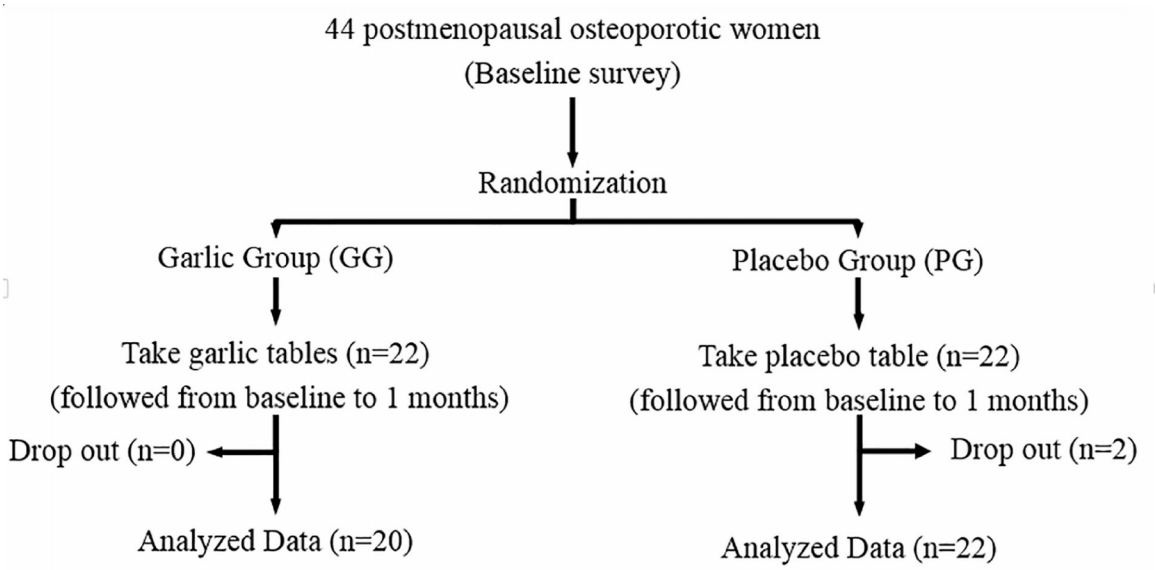

Figure 1. Flowchart of study design and sampling scheme

4. Discussion

Osteoporosis is a bone metabolic disorder in women, particularly in post-menopausal ones. Chemical drugs administration and estrogen therapy have their own side-effects. The traditional medicine and new advantages in herbal drugs reveal the role of medicinal herbs on oxidative stress reduction; onion, ginger, Nigella sativa, Berberis vulgaris extract. In this study, we showed the beneficial effect of garlic tablet on lipid and protein oxidation markers in post-menopausal osteoporosis. The beneficial effects of garlic against oxidative stress are numerous: Garlic decreases NADPH oxidase activity and VCAM-1 expression and therefore decreases inflammation.. Inflammation has a strong association with oxidative stress (17). The S-allyl cysteine (DAS) and s-allyl methyl cysteine (DADS) are the main components of garlic that have high antioxidant properties. DADS can protect osteoblasts from reactive oxygen species (ROS) formation. DADS can protect osteoblasts from reactive oxygen species (ROS) formation in smokers (22). Also, according to the past studies, these results could be due to allicin (inhibits down regulation of the c-Jun N-terminal Kinase Signaling Pathway and so, cell adhesion molecules expression), the protective role of S-allyl cysteine, in oxidized-LDL formation and inhibition of activation of nuclear factor kappa B activated by or hydrogen peroxide. However, some recent studies have been mentioned the anti-inflammatory effect of quercetin (another flavonoid in onion with antioxidant activity) (25-27). Quercetin can attenuate the TNF-alpha and NO production (28). Garlic extract has the anti-hypertrophic and anti-apoptotic role on the myocardial cell (29). As in some recent studies, our study confirmed that garlic tablet can prevent PCO incidence in menopausal women (30). Lai et al. showed that DADS of garlic can prevent problems associated with fatty liver by inhibiting oxidative stress in obese mice (32). Our findings revealed that garlic tablet reduced AOPPs levels in patients. Avci et al. proposed the efficacy of garlic in reducing AOPPs, osteoporosis, and oxidative stress in 66 rats exposed to mobile radio frequency waves (33). The animal study by Sun et al. on rats, showed that AOPPs can prevent bone stem cells proliferation and differentiation, and it might be important in osteoporosis process (34). Zeng et al. proposed that AOPPs accumulation in old mice, increases bone destruction most probably by activation of NADPH oxidase, and also by inhibition of bone reabsorption which has a great role in osteoporosis development (35). In this study, free radical-trapping capacity was statistically different between groups and there was a significant difference before and after garlic tablet administration. Muhammad et al. evaluated oxidative stress in rats after ovariectomy and observed that ovariectomy leads to osteoclasts and osteoblasts proliferation, and bone regulation antioxidant administration such as vitamin E can increase osteoblasts, so improve bone density indices (36). Leain et al., in their study showed that ROS has a negative correlation with estrogen and increases in rats and mice after ovariectomy which activates osteoclasts and suppresses osteoblasts (37). The study by Ehnert et al. assessed cell culture of osteoblasts derived from the femoral bone head of smokers and showed that garlic oil can prevent oxidative stress and promote their activity. They found that DADS was more effective compared to DAS in ROS reduction (38). Mukherjee et al. showed that garlic oil can reduce oxidative stress in lymphocytes and macrophages by estrogen levels compliance in rats and also antioxidant and interleukins increase can protect bone density. They suggested that garlic has a preventive role in bone mass reduction (39). Our study confirmed that MDA (results from the oxidative breakdown of polyunsaturated fatty acids) was reduced significantly after garlic administration. Free radicals' formation occurs in physiologic and pathologic conditions in mammal tissue (40), which react with polyunsaturated fatty acids and produce lipid peroxidation. Fatty oxidation reaction requires the attack to free bases of polyunsaturated fatty acids. Lipid damage occurs after double bond allelic base formation. Weak double bonds can combine with oxygen and produce lipid peroxide base, while lipid peroxide decomposes to aldehyde (like MDA). This component can attach to cellular lipid, protein or Deoxyribonucleic acid (DNA) and lead to structural change (41). Avci et al. showed that garlic can reduce MDA in plasma, red blood cells, and this herb also decreases oxidation reaction and increases antioxidant enzymes (33). Garlic neutralizes the effect of estrogen reduction with calcium absorption. Some reports showed anti-osteoporotic effects of garlic by the preventive role of biomarkers of estrogen deficiency and bone mass decrease and also serum alkaline phosphatase levels (7, 8).

5. Conclusions

Regarding the findings our study and previous ones, it can be concluded that garlic-derived medicine or raw garlic can be used in osteoporosis to perform calcium absorption. It also might have a great impact on carbonyl groups, AOPPs, and ROS. Garlic influences osteoporosis by oxidative stress reduction. In our study, increased oxidative stress index such as free radicals was observed in the garlic-treated group but it was reduced in the carbonyl group and AOPPs significantly after garlic administration. MDA also decreased significantly after tablet usage.

Acknowledgments:

The authors would like to express their thanks to the Board of Directors of Shahid Sadoughi University of Medical Sciences.

Trial registration: The protocol of trial was registered at the Iranian clinical trial register (www.irct.ir) with ID: IRCT138811183273N1.

Funding:
This study funded by Shahid Sadoughi University of Medical Sciences (Yazd, Iran).

Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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