

Antioxidant effect of garlic (*Allium sativum*) and black seeds (*Nigella sativa*) in healthy postmenopausal women

SAGE Open Medicine
I: 2050312113517501
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DOI: 10.1177/2050312113517501
smo.sagepub.com



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Abstract

Objectives: The objective of this study is to investigate the antioxidant effects of garlic extract and crude black seeds' consumption on blood oxidant/antioxidant levels in healthy postmenopausal women.

Methods: In total, 30 healthy postmenopausal women (mean age = 50.31 ± 4.23 years) participated. They ingested two garlic soft gels per day (each is equivalent to 1000 mg of fresh garlic bulb) and crude black seed grounded to powder in a dose of 3 g/day for 8 weeks. Oxidant (malondialdehyde) activity in plasma and antioxidants superoxide dismutase and glutathione peroxidase activities in erythrocytes were studied.

Results: Significant low levels of plasma malondialdehyde with increased erythrocyte glutathione peroxidase and superoxide dismutase activities.

Discussion: Menopause is associated with an increase in oxidative stress and a decrease in some antioxidant parameters. Consumption of garlic extracts and crude black seeds may have a beneficial effect on improved balance between blood oxidants and antioxidants in healthy postmenopausal women.

Keywords

Menopause, oxidative stress, garlic, antioxidants, superoxide dismutase, malondialdehyde, oxidation

Date received: 28 October 2013; accepted: 27 November 2013

Introduction

Menopause and its symptoms are major concerns for a large population of women. It is estimated that by the year 2025, 20% of the world's population will be above the age of 60 years. Therefore, women who spend two-thirds of their life in postmenopausal term will increase in number.¹ Menopause is a physiological process that occurs in healthy women usually between 50 and 55 years of age.²

Natural menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular, which is known to occur after 12 consecutive months of amenorrhea, for which there is no other obvious pathological or physiological cause.^{3,4} The final menstrual cycle marks not only the ending of the reproductive phase but also the beginning of a permanent phase of lowered estrogen secretion that leads to somatic and metabolic changes.³ Symptoms include hot flashes, night sweats, vaginal dryness, mood swings, declining libido, insomnia, lethargy/fatigue, irritability, anxiety, depression, heart palpitations and joint pain.⁵

Estrogen is a powerful antioxidant.⁶ Containing a phenol ring, estrogen can effectively locate hydroxyl radicals, giving rise to hydroxylated products.⁷ Besides, considerable

evidence has shown that estrogen decreases the production of reactive oxygen species (ROS) and oxidative stress both in vitro and in vivo.⁸⁻¹¹ Hence, the decrease in estrogen synthesis in postmenopausal women is thought to be responsible for enhancing oxidative stress.¹¹⁻¹³ Antioxidant enzymes protect aerobic cells against the oxidative injury caused by ROS that are generated during normal metabolism. Either an overproduction of ROS or a deficiency in enzymatic and

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nonenzymatic antioxidant systems may result in oxidative stress, which is considered responsible for a number of pathological conditions in humans and also for aging process.¹⁴ Oxidative stress has been associated with physiological changes occurring after menopause, as increased oxidative damage and changes in antioxidant enzymes have been observed in this phase.^{11,15–17}

Garlic (*Allium sativum*) belongs to the plant family Liliaceae, which is a genus of 500 species. It has been in use for many centuries and was recognized for its therapeutic and medicinal value as far back as the era of ancient Egypt.^{18,19} The antioxidant properties of garlic and different garlic preparations are well documented.^{20–30} It has distinct nutritional profile with special reference to its bioactive components and is used in different diet-based therapies to cure various lifestyle-related disorders.³¹

It is generally considered that health-related functions are mostly attributed to the fresh garlic content rich in γ -glutamylcysteine and many other sulfur-containing compounds in it, giving a characteristic flavor formed during storage and processing.^{23,24,27–29} However, additional constituents of garlic include a wide range of primary and secondary nonsulfur biomolecules, such as steroidal glycosides; essential oil;³² anthocyanins;³³ lectins²⁰ (the most abundant proteins in garlic);³⁴ prostaglandins; fructan; pectin; adenosine; vitamins B1, B2, B6, C and E; biotin; nicotinic acid; fatty acids; glycolipids; phospholipids and essential amino acids.²⁰ Their biological effects have been studied for over several decades, and the importance of biological and pharmacological activities, such as antifungal, antibacterial, anti-tumor, anti-inflammatory, antithrombotic and hypocholesterolemic properties of certain steroid saponins and saponins, such as pchlorogenin, has been recently demonstrated.³⁵ These series of biological benefits have been mainly attributed to the antioxidative activity.

The name “Black seed” is one of many colloquial names of the herb *Nigella sativa*. The seeds are known in Arabic as *Rabba Sawda* (black com) or *Rabbet EI Baraka* “Com of blessing.”³⁶ The black seed, *N. sativa* L. (NS), a member of the family of Ranunculaceae, contains more than 30% of fixed oil and 0.4%–0.45% wt/wt of volatile oil. The volatile oil contains 18.4%–24% thymoquinone (TQ) and 46% many monoterpenes such as p-cymene and α -pinene.³⁷ The fixed oil of NS oil consists of 50% linoleic acid, 25% oleic acid, 12% palmitic acid, 2.84% stearic acid, 0.34% linolenic acid and 0.35% myristic acid.³⁸ Recently, clinical and animal studies have shown that extract of the black seeds have many therapeutic effects such as immune-modulative,³⁷ antibacterial,³⁹ hypotensive,⁴⁰ hepatoprotective⁴¹ and anti-diabetic effects.⁴² NS oil has been reported to possess antioxidant activity.⁴³ Ohkawa et al.⁴⁴ also reported that NS oil and its derivative TQ inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation.^{45,46}

The different compounds in the oil were found to act in synergistic manner (i.e. more than the mere summation of

the actions of the individual compounds). This stresses the importance of using the whole oil (or the whole crude extract) of the seeds in different pharmacological and clinical studies.

The aims of this study are principally to investigate the antioxidant effects of garlic and crude black seeds' consumption on blood oxidant/antioxidant status in postmenopausal women.

Subjects and methods

In total, 30 postmenopausal women (mean age = 50.31 \pm 4.23 years) participated in this study. All women were at least 2 years naturally menopausal. All participants were normotensive, normocholesterolemic, nondiabetic, non-smokers and receiving no medication (including vitamin supplements). All of them denied having hot flashes for at least a year. All participants gave informed consent in accordance with the requirement of the Medical Research Ethics Committee of the University of Suez Canal, Egypt.

Garlic soft gels were purchased from Puritan's Pride, Inc, Oakdale, NY, USA. Each of the soft gel contained *A. sativum* (from 500:1 extract) equivalent to 1000 mg of fresh garlic bulb. Mature NS seeds were supplied by the Medicinal and Aromatic Research Department, Horticulture Research Station, Agriculture Research Center, Alexandria, Egypt. *Nigella* seeds were grounded to powder using an electric grinder (model MX-915 C; National, Japan) for 2 min and placed in a glass container prior to use.

The subjects consumed garlic at a dose of two garlic soft gels per day (equivalent to 2000 mg of fresh garlic bulb)⁴⁷ and crude black seed grounded to powder in a dose of 3 g/day for a period of 8 weeks.⁴⁸

Before and after this period, fasting blood samples were obtained, and oxidant malondialdehyde (MDA) was measured in the plasma fraction, based on the reaction with thiobarbituric acid to yield a colored product. The optical density of the reaction product was measured at 532 nm using 1,1,3,3-tetramethoxypropane as a standard.⁴⁹ Antioxidant (superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)) activities were studied in erythrocyte hemolysate as indices of oxidant balance. Erythrocytes were washed with isotonic solution of sodium chloride and centrifuged at 3000 r/min for 10 min where erythrocyte pellets were separated and resuspended in cold distilled water to obtain the erythrocyte lysate.^{50,51} The resulting erythrocyte lysate was used for measurement of SOD and determination of GSH-Px.⁵²

The GSH-Px activity was given as IU/mL and SOD activity as U/mL. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the nitroblue tetrazolium salt reduction rate. The MDA concentration was determined by using thiobarbituric acid reaction. MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid to form a colored complex that has maximum absorbance at 532 nm.

Table 1. MDA levels in plasma from postmenopausal women after consumption of garlic and black seeds for 8 weeks (mean \pm SD; n = 30).

Groups	MDA (nmol/mL)
Before garlic and black seeds	2.59 \pm 0.36
After garlic and black seeds	1.35 \pm 0.030**

SD: standard deviation; MDA: malondialdehyde.

**p < 0.001.

Table 2. Antioxidant parameters in erythrocytes from postmenopausal women after consumption of garlic and black seeds for 8 weeks (mean \pm SD; n = 30).

Groups	SOD (μ /mL)	GSH-Px (IU/mL)
Before garlic and black seeds	1.55 \pm 0.15	8.38 \pm 0.32
After garlic and black seeds	2.18 \pm 0.16*	9.41 \pm 0.58*

SD: standard deviation; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase.

*p < 0.05.

Statistical analysis was performed using GraphPad Prism version 0.5. Paired t-test was used. Data were given as mean \pm standard deviation (SD) with 0.05 as point of minimal statistical significance.

Results

As shown in Table 1, the plasma MDA showed significant decrease ($p < 0.001$) after 8 weeks of garlic and black seeds' consumption. MDA levels are good markers of lipid peroxidation during aging.

In erythrocytes, GSH-Px was significantly higher ($p < 0.05$) after 8 weeks of consumption of the combination of garlic and black seeds as shown in Table 2. Furthermore, SOD activities were also significantly higher ($p < 0.05$) after consumption of the combination of garlic and black seeds for the same period as shown in Table 2.

Discussion

There were more than 477 million menopausal women in the world in 1998, and the number expected to reach 1.1 billion by 2025.⁵³ Menopause is a process of normal aging, during which the level of estradiol secreted by the ovaries gradually declines.⁵⁴

Aging is a complex biological phenomenon which involves progressive decline in different physiological functions of various tissues.⁵⁵ Oxidative stress, an unavoidable consequence in the metabolism of oxygen by aerobic cells, is a major factor not only in the normal aging process but also in many age-related degenerative processes.^{56,57}

Oxidative stress is defined as an imbalance between higher cellular levels of ROS and the cellular antioxidant

defense. Generation of ROS is ubiquitous since ROS are generated during aerobic metabolism, that is, mitochondrial oxidations and other monoamine oxidations. In order to scavenge ROS, different defense systems exist, such as enzymatic (SOD, GSH-Px and catalase), nonenzymatic (glutathione and uric acid) and dietary (vitamins A, E and C; β -carotene; quinones and flavons) antioxidants. If ROS are not effectively eliminated, they can cause the oxidative cell injury, that is, peroxidation of cell membrane phospholipids, proteins (receptors and enzymes) and DNA.⁵⁸

Thiobarbituric acid reactive substances primarily reflect MDA, a breakdown product of lipid peroxides, and are commonly used as a measure of oxidative stress.⁵⁹ MDA can affect membrane proteins by cross-linkage, rendering them useless as receptors or enzymes.⁶⁰

Natural remedies have been investigated for centuries for a wide variety of ailments⁶¹ and are generally perceived as safe by the public as, unlike pharmacological preparations, they are "natural." Among them, garlic has received special attention for its beneficial effects,^{22,61–63} and black seeds (NS), a herbaceous product, have been used for thousands of years for medical purposes. NS oil is an effective free radical scavenger showing antioxidant activities and protecting against the damage caused by free radicals.^{22,58}

In this study, plasma MDA levels showed significant reduction in the plasma of normal postmenopausal women after 8 weeks of garlic and black seeds' consumption. This effect may be due to increasing activities of the antioxidant enzymes, as our data showed significant increase in the activities of SOD and GSH-Px in erythrocytes. SOD is an antioxidant enzyme and rapidly converts O₂ to less dangerous H₂O₂ which is further degraded by endogenous antioxidant enzymes GSH-Px and catalase to water.⁵⁷

Our results are in good agreement with some previous reports, which have already revealed a decrease in SOD activities in aged rats^{57,64,65} and demonstrated that GSH-Px activity dwindles in aged rats.^{57,65} Durak et al. found that dietary supplement of garlic extract activated the antioxidant system and decreased peroxidation in aortic tissue in their experimental model.⁶⁶ Pedraza-Chaverri et al.⁶⁷ found that the protective effect of garlic powder prevented the decrease in SOD and GSH-Px activities and the rise of lipoperoxidation in renal cortex of rats with gentamicin nephrotoxicity.⁶⁷ In other previous studies, it was established that garlic extract ingestion caused increased activities of some antioxidant enzymes. For example, Chen et al.⁶⁸ reported increased SOD and decreased GSH-Px activities in hepatic tissues of rats fed a garlic oil-rich diet.⁶⁸

In gentamicin-induced toxicity, treatment with NS oil produced a dose-dependent amelioration of the biochemical and histological indices of nephrotoxicity, which coincided with the increase in the scavenger defense system including the total antioxidant status in renal cortex.^{69,70} The published findings provide clear evidence that the oil or NS seed

constituents, in particular TQ, possess reproducible effects through enhancing the oxidant scavenger system.⁷⁰

Our data contradict with those of Durak et al.,⁶¹ as their results showed that ingestion of garlic extract does not show any increase in the activities of antioxidant enzymes SOD and GSH-Px.⁶⁶ However, there is agreement that garlic prevents oxidation and lowers MDA levels in all tissues and cells.^{66,71,72} The oxidation preventive potential of garlic is possibly related to its high content of antioxidant sulfur compounds.⁷³

Conclusion

Aging is associated with an increase in oxidative stress and a decrease in some antioxidant parameters. Our results indicate that in postmenopausal healthy women, the combined ingestion of two garlic soft gels per day (each soft gel equivalent to 1000 mg of fresh garlic bulb) and crude black seed grounded to powder in a dose of 3 g/day for 8 weeks improved the balance between blood oxidants and antioxidants. It is quite possible that reduced peroxidation processes due to this combination may play a part in some of their beneficial effects as a nonpharmacological, food complementary substances in healthy postmenopausal women. Further studies with different doses and intervals will be needed.

Declaration of conflicting interests

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

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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