Original Article

Effect of *Nigella sativa* Oil on Early Menopausal Symptoms and Serum Levels of Oxidative Markers in Menopausal Women: A Randomized, Triple-Blind Clinical Trial

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Abstract

Background: The declining levels of estrogen during menopause are linked with numerous somatic and psychological complications. **Objectives:** This study aimed to examine the effect of *Nigella sativa* (*N. sativa)* oil on early menopausal symptoms and serum levels of some oxidative markers in postmenopausal women. **Methods:** This randomized placebo trial was conducted on 72 menopausal women aged 45–60 years. Participants were randomly allocated to placebo and intervention groups with an equal allocation ratio (1:1). Patients in the intervention group received one *N. sativa* oil capsule (1000 mg), whereas the placebo group received a placebo capsule at night for 8 weeks. Data were collected through the demographics questionnaire, the Greene's Climacteric Scale, and a form for recording the number of daily hot flashes. Furthermore, the serum levels of total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured before and 8 weeks after the intervention. Data were analyzed using the independent-samples t, Chi-square, Mann-Whitney U, and Friedman tests as well as the repeated-measures analysis of variance. **Results:** The participants were matched in baseline values. The mean baseline score of the Greene's scale was 22.5 ± 9.5 in the intervention group and 20.0 ± 8.0 in the placebo group (P = 0.397). Mean scores had significantly reduced in both groups at the end of weeks 4 and 8. However, the intervention group experienced a more remarkable decrease in Greene's score (adjusted MD_{Log10} = −0.16 (−0.29 to −0.05); P = 0.019). There were no significant differences between the two groups in the subscales of Greene's scale (P > 0.05). No significant difference was observed between the groups in serum levels of TAC (P = 0.250) and MDA (P = 0.444). **Conclusion:** *N. sativa* reduced the total score of menopausal symptoms and hot flashes in menopausal women; however, it had no significant effect on the serum levels of oxidative stress markers.

Keywords: *Hot flash, Menopause, Nigella sativa, Oxidative markers, Symptom*

Introduction

Approximately 74%–80% of women suffer from menopausal complications.[1] Menopause is generally characterized by follicular depletion and a marked decrease in estradiol and inhibin-B secretion, resulting in reduced blood estrogen levels and elevated levels of follicle-stimulating hormone to over 40 mIU/ml.[2] Menopausal transition or perimenopause

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is a period beginning with emerging irregular menstrual cycles until the last menstrual period. Menopause is a point in time 12 months after a woman's last menstrual period. Postmenopause refers to the period following the last menses.[3]

During menopause, women experience several early and late complications due to estrogen depletion that interfere with occupational and social activities and quality of life (QOL) and also undermine feelings being healthy and useful.[4] They also suffer from hot flashes, night sweats, vaginal dryness, dyspareunia, mood swings, joint pain and stiffness, palpitations, sleep disturbances, restlessness, forgetfulness, and urinary symptoms and also are at increased risk of osteoporosis.[5] Hot flashes are the most common, earliest, and most problematic symptom of menopause.[6] Since these symptoms generally affect women's QOL, measures to alleviate menopausal symptoms are crucial.[7]

Evidence suggests that estrogens have antioxidant properties,[8] which are related to their phenolic groups.[9] In addition to their antioxidant effects on low-density lipoprotein, estrogen may also reduce the risk of coronary heart disease by affecting serum lipids. Estrogen may also be beneficial in scavenging free radicals.[10] The reduced antioxidant activity of estrogen during menopause coincides with increases in reactive oxygen species and some pathological conditions such as osteoporosis, cardiovascular diseases, and some vasomotor symptoms.[11] Antioxidants contribute to the prevention of complications such as coronary and neurological diseases, cancers, and oxidative stress disorders.[12]

Several pharmaceutical and nonpharmaceutical interventions have been used for menopausal complications.[13] Hormone therapy with estrogen and progesterone is the most common pharmaceutical intervention for hot flashes.[14] However, hormone therapy causes some side effects,[15] including adverse effects on lipids and lipase activity, increased risk of stroke, breast and endometrial cancers, thromboembolic disorders, liver disorders, Alzheimer's disease, etc.[7] Therefore, seeking alternative treatments is of utmost importance.[16] Many women who seek natural or safer ways to treat menopausal symptoms often turn to complementary and alternative therapies.[17]

As a herbal medicine, *Nigella sativa* (*N. sativa*) has been traditionally used to treat various conditions. *N. sativa* has been reported to promote lactation and ameliorate menstrual complications.[18,19] It contains compounds with phytoestrogenic and antioxidant properties that may help control menopausal symptoms.[20] It also contains thymoquinone (TQ)[21] which has anti-inflammatory, anti-aging, and hepatoprotective effects.[22] It is also rich in calcium, iron, and potassium, and its beneficial effects in gynecological disorders have been reported.[19] Although there is a long list of examples demonstrating the usefulness of *N.* sativa in medicine, few studies have reported its effects on the reproductive system, focusing on male reproduction. However, many studies have demonstrated the safety of this herb in both animals and humans.[23,24]

The results of an animal study showed that *N. sativa* exerts estrogenic effects confirmed through uterotrophic assay, vaginal cell examination, and measurement of blood estrogen levels. Furthermore, a study showed that low-dose *N. sativa*, methanol extract, and linoleic acid had prominent estrogen-like effects.[24] An open-label, cross-over study also examined the effect of l2-week using of *N. sativa* capsule in perimenopausal women and reported that *N. sativa* significantly reduced the incidence and severity of menopausal symptoms and improved some components of QOL.[24] Given the role of inflammatory and oxidative processes in the etiology of menopausal symptoms and the side effects of current medications,[25,26] and considering the anti-inflammatory and antioxidant effects of *N. sativa,*[23,27-31] the question arises whether *N. sativa* can alleviate early menopausal symptoms and improve the serum levels of oxidative markers in menopausal women.

Objectives

The present study aimed to examine the effects of *N. sativa* on the early menopausal symptoms and serum levels of oxidative markers in menopausal women.

Methods

Design and participants

A triple-blind, randomized, placebo trial was conducted on menopausal women attending the comprehensive health centers of Tabriz, Iran, from March 2019 to January 2020. The inclusion criteria were women with normal menopause, literacy enough to respond the questionnaires or being accompanied by a literate person in the family, married, <5 years of menopause, age between 40 and 60 years, hot flashes at least twice a week, score 15–42 on the Greene's scale, and having normal blood pressure (100/60–135/85 mmHg).[32] Noninclusion criteria were tobacco and alcohol abuse, use of herbal remedies, experiencing severe stressors (e.g., job loss and death of first-degree relatives in the past 6 months), psychiatric or systemic disorders (e.g., arthritis, cardiovascular, gastrointestinal, hepatic, hematologic, and endocrine disorders, as self-reported by the women), use of antidepressants and sedatives (e.g., serotonin,

norepinephrine, antihistamines, barbiturates, narcotics, diazepam, amphetamines, and cocaine), anticoagulants (e.g., heparin, warfarin, and enoxaparin), and medication affecting hot flashes (e.g., clonidine, methyldopa, gabapentin, selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, and soy isoflavones), use of medications affecting sexual responses (e.g., antihypertensive drugs and thiazide diuretics), allergy to turmeric, other medications, foods, and food dyes. The exclusion criteria were bleeding, exacerbation of hot flashes, stomach pain, and any unpleasant side effects.

We calculated the sample size using G-power and the formula for examining the difference between two means. In a previous study, the mean ± standard deviation (SD) of the Greene's score was 33.8 ± 6.4 in the treatment group and 31.1 ± 6.4 in the control group. Considering $\mu_1 = 31.1$, $\mu_2 = 33.8$, $\delta_1 = 6.3$, $\delta_2 = 6.4$,[33] and with a $\alpha = 0.05$ and a power of 95%, the sample size was estimated at 23 per group. Moreover, in another study, the mean total antioxidant capacity (TAC) in postmenopausal and nonmenopausal women was 11.4 ± 4.4 and 10.3 ± 1.2 mMTrolox, respectively. Then, considering $\mu_1 = 11.4$, $\mu_2 = 10.3$, $\delta_1 = 4.4$, $\delta_2 = 1.2$,[34] and with a α=0.05 and a power of 95%, the sample size was estimated at 30 per group. However, considering a possible dropout of 20%, we selected 36 participants in each group.

$$n = \frac{\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 \left(\delta_1^2 + \delta_2^2\right)}{\left(\mu_1 - \mu_2\right)^2}$$

First, four health-care centers in Tabriz city, Iran, were randomly selected from the list of all health-care centers in the city. Postmenopausal women who consecutively referred to the four selected health-care centers were then screened for eligibility and 18 eligible women inclining to participate in the study were selected from each health-care center. Then, using a random allocation software, the women were allocated to either a placebo or an intervention group. A block randomization method with blocks of four and six and a 1:1 ratio was used allocate the subjects in the placebo and intervention groups to receive placebo or the *N. sativa* oil capsules. The allocation sequence was concealed from the researcher and participants using sequentially numbered, opaque black and identical envelopes containing oil or placebo capsules. The envelopes were prepared by a third party who was not involved in the clinical trial. The envelopes were coded from one to 72 according to the randomly generated allocation sequence. The first subject received envelope numbered 1, the second participant received envelop numbered 2, and sampling continued to the last participant according to this pattern.

Serum TAC and malondialdehyde (MDA) levels were, respectively, measured using the Naxifer kit (NS-15012, NS-15013) and the Naxifer kit (NS-15022, NS-15023) produced by Navand Salamat Urmia Co. In the first round, 5 ml of blood was drawn in the laboratory before the start of intervention and after about 12 h of fasting. To harvest the serum, the samples were centrifuged at 3000 rpm for 10 min and then stored at -70°C until the test was performed. The second phase of blood sampling was performed 8 weeks after the start of the intervention under the same conditions as in the first phase.

Intervention

The intervention group received an oil capsule (1000 mg) containing at least 5.6 mg TQ and 495−605 mg standard linoleic acid every night after dinner for up to 4 weeks. To ensure proper intake of the prescribed medications, each participant was provided with two 30-capsule packs. After they used up the first pack and returned its empty pack, they received the second pack for another 4 weeks. The placebo group received placebo capsules containing carboxymethyl cellulose. The *N. sativa* oil and placebo capsules were produced by Barij Essence Co., Kashan, Iran. All capsules were identical in weight, shape, color, and odor. The author followed up the participants once a week to ensure medication adherence. During the trial period, patients were asked to record the frequency of their daily hot flashes. To monitor the occurrence of side effects, participants were requested to complete a side effect checklist. Data were collected in two phases (4 and 8 weeks after the intervention). If a participant experienced severe and intolerable menopausal symptoms during the study, the intervention was discontinued and she was referred to a gynecologist.

Data collection instruments

Data were collected using a demographic questionnaire, the Greene's Climacteric Scale (GCS), and a form for recording the number of daily hot flashes. The demographic data included age, the time elapsed since menopause, age at menopause, education level, employment status, education level and employment status of spouse, adequacy of monthly family income for living expenses, number of family members, weight, height, and smoking habits of the participants and their spouses. The GCS was developed by Greene in 1975.[35,36] This scale contains 21 items on menopausal symptoms. All items are rated by the participant on a 4-point scale from "0: No symptom" to "3: Severe." Items 1–11 measure psychological symptoms including depression and anxiety (items 1–6 for depression

and 7–11 for anxiety), items 12–18 measure somatic menopausal symptoms (dizziness, pressure in the head or body, insentience in parts of the body, headache, joint or muscle pain, numbness in the hands or feet, and difficulty breathing), items 19 and 20 measure vasomotor symptoms, and item 21 measures sexual dysfunction. The total score range between 0 and 63. The Persian translation of the GCS was validated by Askari *et al*. through content validity method and its test-retest and Cronbach's alpha coefficients were reported 0.74 and 0.87, respectively.[37]

The hot flash form was used to document the frequency of hot flashes. Participants were requested to record the frequency of hot flashes during one week on a checklist one week before, and 4 and 8 weeks after the intervention. We defined hot flashes at the beginning of this form and explained verbally to the participants.

Ethical considerations

This study was conducted after approval by the Ethics Committee of Tabriz University of Medical Sciences (IR. TBZMED. REC.1397.1034), registration the study in the Iranian Registry of Clinical Trials (IRCT20110606006709N19) and obtaining permission from the Health Deputy of Tabriz University of Medical Sciences. This research complies with all relevant national regulations and institutional guidelines. We explained the research objectives and methods to all participants and they all signed a written informed consent before participating in the study.

Data analysis

We used the SPSS-16 software (IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA) to analyze the data. The Kolmogorov–Smirnov test was used to check the normality of the quantitative data. Data were described using the descriptive statistics including frequency, mean, SD, or median (Inter-quartile range). The Fisher's exact, the Chi-square, and the independent-samples *t*-tests were used to compare the two groups respecting their demographic characteristics. Repeated measures analysis was used to compare the mean total menopause symptoms score and hot flashes across the three measurement time points. Analysis of covariance was used to compare postinterventional serum MDA and TAC between groups and paired-*t*-test was used for within group analyses. In addition, the Friedman and Mann-Whitney U tests were used to between groups and within-group analyses of the sub-domains of menopause symptoms. Logarithm (Log) 10 conversion was performed to normalize the total scores of menopausal symptoms and hot flashes. The significance level was set at <0.05.

Results

This study was conducted on 72 menopausal women, 36 in each group. Five women in the intervention group and four in the placebo group discontinued the intervention due to side effects. All participants were followed up and included in the analysis using the intention-to-treat approach [Figure 1]. The mean age of participants was 53.91 ± 3.10 and 53.63 ± 3.38 years in the intervention and placebo groups, respectively. The mean age at menopause was 51.36 ± 3.07 in the intervention group and 50.30 ± 3.25 in the placebo group. No significant difference was found between the two groups respecting demographic characteristics ($P > 0.05$) [Table 1].

The mean baseline score of the Greene's scale was 22.5 ± 9.5 in the intervention group and 20.0 ± 8.0 in the placebo group ($P = 0.397$). Mean scores had significantly reduced in both groups at the end of weeks 4 and 8. However, the intervention group experienced a more remarkable decrease in Greene's score (adjusted $MD_{Log\ 10}$ = −0.16 (−0.29 to −0.05);

Table 1: Demographic characteristics of participants by study groups

Variables	Nigella sativa, *n* (%)	Placebo, *n* (%)	*P*
Age (years)[a]	53.91 ± 3.10	53.63 ± 3.38	0.718[b]
Menopause age (years)[a]	51.36 ± 3.07	50.30 ± 3.25	0.162[b]
Paritya	2.63 ± 4.220	2.78 ± 1.49	0.683[b]
Weight (kg)[a]	73.45 ± 11.51	72.31 ± 12.04	0.682[b]
BMI (kg/m^2)[a]	29.95 ± 4.40	29.54 ± 4.80	0.705[b]
Maital status			
Married	36 (10.0)	35 (97.2)	1.000[d]
Divorced	0	1 (2.8)	
Education			
Less than a diploma	23 (63.9)	27 (75.0)	0.541[c]
Diploma	6 (16.7)	5 (13.9)	
University	7 (19.4)	4 (11.1)	
Occupation			
Housekeeper	28 (77.8)	31 (86.1)	0.308[c]
Employed	6 (16.7)	2 (5.6)	
Retired	2 (5.6)	3 (8.3)	
Spouse education			
Less than a diploma	22 (61.1)	23 (63.9)	0.659[c]
Diploma	6 (16.7)	7 (19.4)	
University	8 (22.2)	6 (16.7)	
Family income sufficiency			
Less than adequate	12 (33.3)	16 (44.4)	0.469[d]
Adequate	24 (66.7)	20 (56.6)	
Smoker spouse			
Yes	10 (27.8)	7 (19.4)	0.580[d]
No	26 (72.2)	29 (80.6)	

[a]Mean ± SD, [b]Independent *t*-test, [c]Chi-square test, [d]Fisher's exact test. SD: Standard deviation, BMI: Body mass index

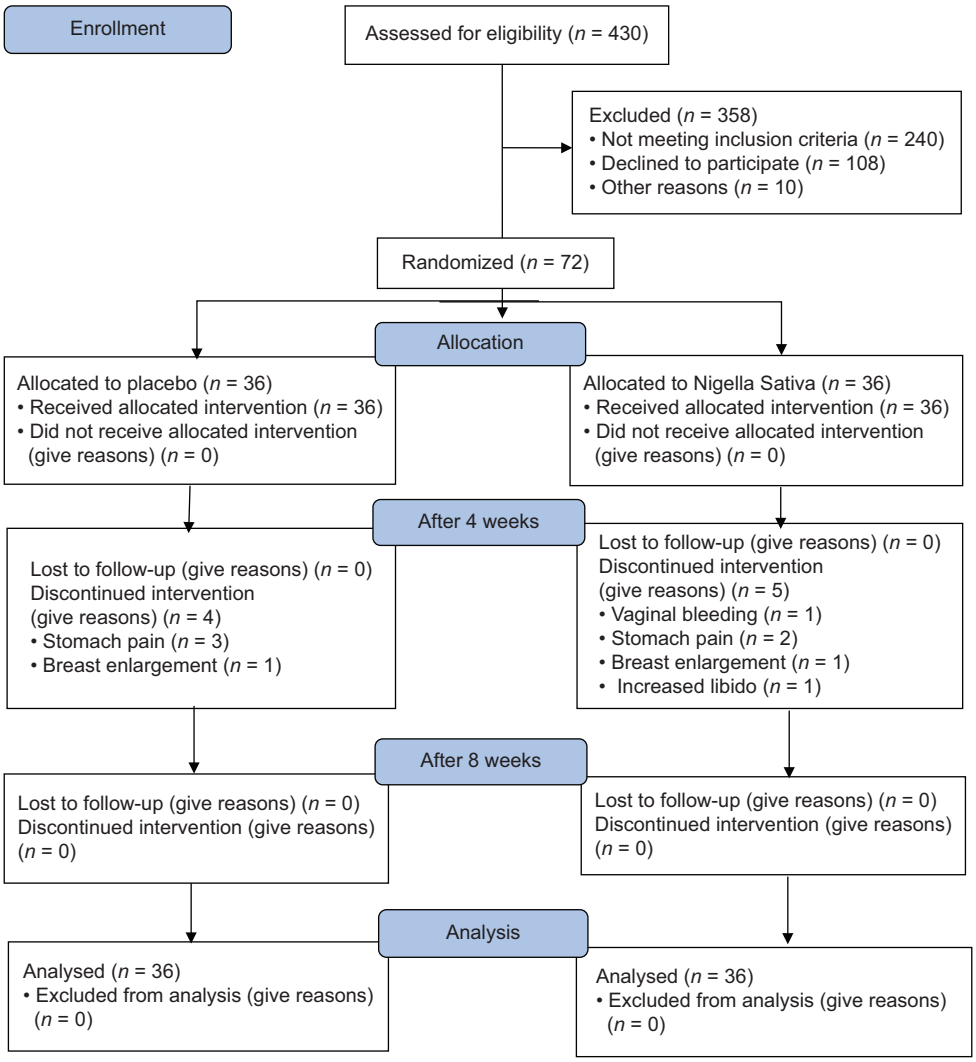

Figure 1: Flow diagram of the trial

$P = 0.019$). No significant difference was found between the two groups in anxiety ($P = 0.226$), depression ($P = 0.312$), physical ($P = 0.131$), psychological ($P = 0.220$), sexual ($P = 0.695$), and vasomotor symptoms ($P = 0.670$) [Table 2].

The mean frequency of hot flashes one week before the intervention was 23.0 ± 19.0 in the intervention group and 18.0 ± 13.0 in the placebo group ($P = 0.055$). The frequency of hot flashes was significantly reduced in both groups at the end of weeks 4 and 8. However, the intervention group experienced a more notable reduction in hot flashes (adjusted $MD_{Log\ 10=}$ −0.13 (−0.25 to −0.02); $P = 0.020$) [Table 3].

The mean serum levels of TAC and MDA before the intervention were 0.48 ± 0.07 and 0.51 ± 0.01 in the intervention group and 0.51 ± 0.08 and 0.05 ± 0.01 in the placebo group. There was no significant difference between the two groups in terms of MDA ($P = 0.986$) and TAC ($P = 0.060$) levels. Moreover, no significant difference was observed between the groups in serum levels of TCA ($P = 0.250$) and MDA ($P = 0.444$) 8 weeks after the intervention [Table 4].

More than half of the patients in the intervention group and 25% in the placebo groups were satisfied with the medication used [Table 5]. Medication adherence was 91% in the intervention group and 93% in the placebo group, based on the count of the remaining capsules in the returned packs. One case of vaginal bleeding was reported in the intervention group, 5 participants in the intervention and the placebo groups reported stomach pain, one participant in each group reported enlargement of the breasts, and one participant in the intervention group reported increased libido. Five women in the *N. sativa* group and four in the placebo group discontinued the assigned intervention because of the aforementioned side effects. Since the study was designed based on the intention-to-treat approach, all women were followed and analyzed.

Table 2: The score of menopause symptoms among study groups in different time

Menopause symptoms	Median (IQR)		*P*
	Nigella sativa	Placebo	
Anxiety (0-3)			
Baseline	5.5 (3.5)	5.0 (4.0)	0.811[a]
After 4 weeks	2.0 (4.0)	3.0 (3.0)	0.070[a]
After 8 weeks	2.0 (4.57)	3.50 (2.36)	0.226[§]
P[b]	<0.001	<0.001	
Depression (0-3)			
Baseline	5.0 (4. 5)	4.0 (4.0)	0.381[a]
After 4 weeks	2.5 (4.0)	3.0 (5.0)	0.174[a]
After 8 weeks	3.0 (4.57)	3.0 (3.0)	0.312[a,§]
P[b]	<0.001	0.017	
Psychological (0-3)			
Baseline	10.0 (4.0)	9.0 (6.0)	0.448[a]
After 4 weeks	4.0 (7.5)	6.0 (6.0)	0.080[a]
After 8 weeks	5.0 (9.0)	7.0 (6.0)	0.220[a]
P[b]	<0.001	0.007	
Physical (0-3)			
Baseline	7.0 (3.75)	6.0 (3.0)	0.296[a]
After 4 weeks	3.0 (3.75)	4.0 (3.0)	0.244[a]
After 8 weeks	3.0 (5.0)	4. 0 (3.0)	0.131[a]
P[b]	<0.001	<0.001	
Vasomotor (0-3)			
Baseline	4.0 (3.75)	4.0 (3.0)	0.398[a]
After 4 weeks	2.0 (2.75)	2.0 (2.0)	0.426[a]
After 8 weeks	2.0 (1.0)	2.0 (2.0)	0.670[a]
P[b]	<0.001	<0.001	
Sexual (0-3)			
Baseline	2.0 (2.0)	2.0 (2.0)	0.787[a]
After 4 weeks	2.0 (2.0)	2.0 (2.0)	0.843[a]
After 8 weeks	2.0 (2.0)	2.0 (3.0)	0.695[a,§]
P[b]	0.378	0.032	
Total score (0-63)			
Baseline/mean ± SD	22.5 ± 9.5	20.0 ± 8.0	0.397[c]
Logarithm10/mean ± SD	1.33 ± 0.12	1.31 ± 0.10	
After 4 weeks/mean ± SD	13.0 ± 9.25	16.0 ± 7.0	
Logarithm10/mean ± SD	1.05 ± 0.26	1.16 ± 0.24	
After 8 weeks/mean ± SD	12.5 ± 14.5	16.0 ± 9. 0	
Logarithm10/mean ± SD	1.02 ± 0.33	1.13 ± 0.26	0.019[d]
MD (95% CI)	−0.31 (−0.41-−0.22)	−0.18 (−0.26-−0.10)	
P[c]	<0.001	<0.001	

[a]Mann–Whitney *U*, [b]Friedman test, [c]Independent *t*-test, [d]Between group repeated measure ANOVA (adjusted mean difference $_{\text{Log 10}}$ (95% CI); *P*: −0.16 (−0.29-−0.05); *P* = 0.019), [c]Repeated measure ANOVA, within group. Significant difference was observed between groups in the total green during the intervention (interaction between group and time) using the sphericity assumed repeated measures ANOVA test. *P* = 0.037. Total score of green scale and all its subscales had an abnormal distribution. Green's total score was normalized using logarithm 10 and was shown using mean ± SD and parametric test was used for comparison within and between groups. MD (95% CI). CI: Confidence interval, SD: Standard deviation, IQR: Interquartile range, MD: Mean difference

Discussion

The present study showed that the postintervention mean scores of total menopausal symptoms and hot flashes decreased remarkably in the group received *N. sativa* compared to the placebo group. However, there was no significant difference between the two groups in the postintervention mean scores of anxiety and depression, as well as psychological, physical, vasomotor, and sexual symptoms.

The exact mechanism and physiology of hot flashes are not clear. However, the estrogen level is the probable cause because it regulates body temperature by acting on the hypothalamus. During menopause, a decrease in estrogen levels leads to a decrease in serotonin levels and an increase in norepinephrine levels, which in turn leads to hypothalamic dysfunction and an increase in body temperature.[6] *N. sativa* has phytoestrogenic properties[20] and therefore may inhibit this process. Consistent with our findings, a review study reported the effectiveness of *N. sativa* inalleviating the menopausal symptoms.[38] An animal study also reported that *N. sativa* can improve climacteric symptoms due to its pseudoestrogenic properties.[19] A study also reported that the combination of *N. sativa* and citalopram could reduce hot flashes but had no effect on sexual function in menopausal women.[28] In another study, Carmignani *et al*. compared the effects of dietary phytoestrogen supplementation of soy compared to estrogen and placebo on menopausal symptoms in Brazilian women aged 40 − 60 years. The results showed that psychological, physical, and urogenital symptoms improved in all groups; but no significant improvement in urogenital symptoms was observed in the placebo group.[39] A study also compared the effect of a mixture of *N. sativa*, Melissa officinalis, and fennel fruit with citalopram and found that this combination had no significant effect on improving menopausal symptoms compared to citalopram.[40] Valadan *et al*. also reported that *N. sativa* failed to treat sexual dysfunction in menopausal women.[41] In general, *N. sativa oil* appears to have beneficial effects on hot flashes and menopausal symptoms. However, the insignificant change in the sexual dimension in the present study might be attributed to the fact that sexual function was assessed only with a question about "decreased libido." It is therefore recommended that future studies use more specific instruments to measure sexual function. The improvement in menopausal symptoms in the placebo group might also be attributable to the indoctrination effect of taking the placebo on the study participants. The postintervention analysis of MDA revealed no significant difference in its levels over time in any of

Table 3: Comparison of the mean frequency of hot flashes per week among study groups in different time points

Hot flashes	Mean ± SD		aMD (95% CI)	*P*
	Nigella sativa	Placebo		
Baseline	23.0 ± 19.0	18.0 ± 13.00		
Logarithm10	1.37 ± 0.21	1.25 ± 0.25	-	0.055[a]
After 4 weeks	12.5 ± 9.75	14.0 ± 8.0		
Logarithm10	1.09 ± 0.31	1.13 ± 0.18		
After 8 weeks	10.0 ± 12.75	11.0 ± 8.0		>0.001
Logarithm10	1.0 ± 0.32	1.02 ± 0.32	−0.13 (−0.25-−0.02)	0.020[b]
MD (95% CI)	−0.37 (−0.48-−0.26)	−0.22 (−0.03-−0.08)		
P[c]	<0.001	<0.001		

[a]Independent *t*-test; MD (95% CI), *P* values were analyzed based on Log 10 hot flashes due to abnormal distribution of hot-flashes. Distribution of data was normal after conversion. Significant difference between groups in the hot flashes during the intervention (interaction between time and group) using the sphericity assumed repeated measures ANOVA test. $P = 0.023$, [b]*P*-value by repeated measure ANOVA; between group, [c]Repeated measure ANOVA, within group. MD (95% CI): MD: Mean difference, CI: Confidence interval, aMD: Adjusted mean difference, SD: Standard deviation

Table 4: Comparison of the mean serum level of malondialdehyde and total antioxidant capacity among study groups before and after intervention

Variable	Mean ± SD		aMD (95% CI)	*P*[a]
	Nigella sativa (*n*=36)	Placebo (*n*=36)		
MDA (nmol/ml)				
Baseline	0.05 ± 0.01	0.05 ± 0.01	-	0.986
After 8 weeks	0.05 ± 0.01	0.05 ± 0.01	0.00 (−0.00-0.01)	0.444
MD (95% CI)	−0.00 (−0.01-0.00)	−0.00 (−0.01-0.00)		
P[b]	0.617	0.084		
TAC (mmolfe^{2+}/l)				
Baseline	0.48 ± 0.07	0.51 ± 0.08	-	0.060
After 8 weeks	0.53 ± 0.11	0.57 ± 0.10	0.03 (−0.07-0.02)	0.250
MD (95% CI)	0.05 (−0.08-0.01)	0.05 (−0.09-0.02)		
P[b]	0.016	0.005		

[a]Analysis of covariance adjusted for baseline; aMD (95% CI), [b]Paired *t*-test. TAC: Total antioxidant capacity, MDA: Malondialdehyde, aMD: Adjusted mean difference, CI: Confidence interval, SD: Standard deviation

Table 5: Satisfaction with medication among study groups

Satisfaction	Nigella sativa, *n* (%)	Placebo, *n* (%)	*P*[a]
Completely satisfied	19 (52.8)	9 (25.0)	0.003
Satisfied	11 (30.6)	10 (27.8)	
Neither dissatisfied nor satisfied	4 (11.1)	9 (25.0)	
Dissatisfied	1 (2.8)	7 (19.4)	
Completely dissatisfied	1 (2.8)	1 (2.8)	

[a]Mann–Whitney *U* test

the study groups. This finding suggests that *N. sativa oil* could not significantly affect the serum levels of oxidative stress markers. In contrast to our finding, a study of 30 postmenopausal women reported that a daily dose of 3 g of *N. sativa* powder significantly reduced MDA.[42] This inconsistency is probably due to the higher dose of medication in the former study. However, in a study in rats fed *N. sativa* oil, it was reported that 8 weeks intake of *N. sativa oil* significantly reduced MDA levels in liver tissue. However, the decrease was dose-dependent, as higher doses caused a greater decrease in MDA levels.[43] Hadi *et al*. also investigated the effect of *N. sativa* oil on inflammatory and oxidative stress responses in patients with rheumatoid arthritis. The results showed that a daily dose of 500 mg of *N. sativa* over an 8-week period increased interleukin-10 levels and decreased MDA.[44] Their results also contradict our findings. The contradictory findings might be attributable to the sample size, because 68.40% of the participants in the latter study were premenopausal, whereas all participants in our study were menopausal. The effect of premenopausal endogenous estrogen might explain the difference in the results.

In addition, postintervention analysis of TAC revealed no significant difference in its levels over time in any of the study groups. Although Al-Azzawi *et al*. reported that *N. sativa* oil significantly reduces oxidative and inflammatory markers,[45] a review of 50 clinical trials concluded that *N. sativa* oil has a negligible effect on serum levels of TAC and MDA.[46] Consistent with our findings, a clinical trial has also reported that a daily dose of 1000 mg of *N. sativa* oil had a negligible effect

on TAC and MDA.[47] Their results are consistent with the findings of this study.

One of the strengths of this study was its adherence to all clinical trials principles, including random assignment and allocation concealment to prevent selection bias. The use of standard scales and a triple-blind trial were other strengths of this study. Participants were also quite satisfied with the *N. sativa* oil capsules.

A limitation of this study was the lack of control over the effects of psychological factors such as indoctrination due to medication in both groups. Given the contradictory results of various studies, it is suggested that similar studies be conducted in larger groups of patients over a longer period. This study was conducted in women aged 45–60 years, and the results cannot be generalized to other age groups. Using vaginal lubricants is an important factor that can affect the results of Greene's climacteric scale but we did not consider it in this study. Another limitation is that we did not measure serum concentrations of estrogen; we therefore recommend that this be measured in future studies.

Conclusion

Based on the study results, we can conclude that *N. sativa* oil can significantly reduce the total score of Greene's menopausal symptoms and the frequency of hot flashes. However, it had no significant effect on oxidative stress markers. Therefore, physicians and midwives can prescribe *N. sativa* oil to reduce menopausal symptoms and the frequency of hot flashes in menopausal women.

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Conflicts of interest

There are no conflicts of interest.

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