*J Caring Sci*, 2023, 12(3), 163-173
doi: 10.34172/jcs.2023.31875
https://jcs.tbzmed.ac.ir

Original Article

# The Efficacy of *Nigella sativa* L. and Curcumin Nanomicelle Alone or Together on Lipid Profile, Glycemic Control Indices, and Serum 17-B Estradiol in Postmenopausal Women

**Zeynab Sadeghzadeh[1], Alireza Ostadrahimi[2], Minoo Ranjbar[3], Azizeh Farshbaf-Khalili[4*]**

[1]Department of Biological Sciences, Tabriz Higher Education Institute of Rab-Rashid, Tabriz, Iran
[2]Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Department of Midwifery, Faculty of Nursing and Midwifery, Tabriz Medical Science, Islamic Azad University, Tabriz, Iran
[4]Physical Medicine and Rehabilitation Research Centre, Aging Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran

**Article Info**
**Article History:**
**Received**: November 21, 2022
**Accepted:** May 6, 2023
**e-Published:** August 6, 2023

**Keywords:**
Menopause, Curcumin, *Nigella sativa*, Lipids, Glucose metabolism disorders, Estrogens

***Corresponding Author:**
Azizeh Farshbaf-Khalili,
Email: farshbafa@tbzmed.ac.ir

**Abstract**
**Introduction:** Menopause is a condition for metabolic disorders. This study aimed to evaluate the effect of *Nigella sativa* (NS), curcumin nanomicelle (CN), lipid profile, glycemic status and 17-β estradiol (ES) levels in postmenopausal women.
**Methods:** Triple-blind randomized clinical trial was conducted on 120 postmenopausal women. Participants were randomly assigned to four groups: 1) NS capsule 1000 mg and CN placebo, 2) 80 mg CN capsule and NS placebo, 3) both NS and CN capsules and 4) NS and CN placebo. Participants received a single dose daily for 6 months. The serum lipid profile, glycemic control biomarkers, and ES were measured pre-and post-intervention using biochemical methods.
**Results**: Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, fasting blood sugar (FBS), fasting insulin (FI), insulin resistance (IR), and ES showed significant improvement in NS group. CN significantly reduced TC, FI, and IR, and significantly increased ES. The combination of NS-CN significantly decreased TC, LDL, FI, and IR, and increased HDL and ES. The comparison of the studied with the placebo groups showed that these changes were significant in glycemic indices and NS significantly increased estrogen.
**Conclusion**: NS, CN and NS-CN improved lipid profiles, blood sugar, and hormone levels. However, this improvement was significant in glycemic indices and estrogen levels compared to the placebo group. No superiority of combined NS-CN over NS or CN was found in this trial.

## Introduction

Menopause is an important physiological event in a woman's life that represents the end of a woman's reproductive life by stopping menstruation for at least a year resulting from the loss of ovarian function.[1] Natural menopause generally happens between 45 and 55 years worldwide, and some women experience normal menopause before the age of 40 years.[2]

After menopause, insulin resistance gradually develops and blood insulin levels increase. A significant decrease in serum estrogen and an increase in serum testosterone concentration during menopause is accompanied by an increased incidence of metabolic disorders including cardiovascular diseases and diabetes.[3,4] Estrogen therapy has been proven to have useful effects on carbohydrate and lipid metabolism but they have serious side effects.[5] Some compounds that act through the effect on estrogen receptors like phytoestrogens in some foods can reduce the risk of cardiovascular diseases and diabetes without side effects.[6] In recent years, there has been more interest in alternative therapies, especially those derived from plants.[7] Herbs have long been used as the basis of traditional medicine in human history and also serve as a source of modern medicine.[8]

Seeds and oils of *Nigella sativa* L. (NS) are the original medicinal source from old times. NS is one of the most common herbal plants used globally.[9] NS has effective compounds against many diseases like cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrheal, obesity, diabetes, paralysis, hemiplegia, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea.[10] It is also used to abscesses, nasal ulcers, orchitis, eczema, and swelling of the joints.[11] Its pharmacological potential results from its rich and various chemical compounds such as protein (contains eight essential amino acids), carbohydrate, crude fiber, unsaturated fatty acids

(including oleic acid and linoleic acid), saturated fatty acids (such as stearic acid, palmitic acid, and myristic acid), minerals, alkaloids, saponin, and others.[12,13] These chemical compounds have many pharmacological activities such as antibacterial, antiviral, anti-inflammatory, and wound healing effects and also be used for acne vulgaris, skin cancer, pigmentation, and many other anti-aging properties.[14] The most important medicinal properties of NS are mainly due to thymoquinone (TQ) which is the major active chemical mixture of the NS oil.[15,16] TQ has been defined to have anti-diabetic, anti-obesity, hypotensive, and hypolipidemic compounds.[17] NS has also been shown to have beneficial effects on some chronic diseases. For example, in patients with type 2 diabetes using NS compounds has decreased the levels of fasting blood glucose (FBS), 2-hour post-load plasma glucose (2hPG), glycated hemoglobin or hemoglobin A1c (HbA1c), insulin resistance, leptin[18] as well as an improved lipid profile.[19] Also, TQ of NS has a vital role as an antioxidant and effectively acts as a protective agent against chemically-induced hepatic damage and improved liver enzyme levels in non-alcoholic fatty liver.[20] NS can exert estrogenic effects on female fertility directly and indirectly by binding to estrogen receptors. NS has estrogen-like effects such as histological and cytological changes in vaginal cells and increasing blood estradiol levels.[21] The estrogenic effects of NS can be due to the unsaturated fatty acids in its content.[22]

Curcumin nanomicelles is an effective component of the medicinal plant and an active polyphenol from the dried yellow rhizome of turmeric (*Curcuma longa*). Curcuma longa, which is prepared from the powdered roots of the plant.[23,24] Curcumin has antioxidant, anti-inflammatory, and anti-atherosclerotic benefits. It has pharmacological effects in psoriasis, diabetes, multiple sclerosis, Alzheimer, HIV, septic shock, cardiovascular and lung problems, arthritis, and inflammatory bowel diseases.[25] Studies show that curcumin is involved in lowering hyperglycemia, blood glucose, and insulin resistance.[26] It has an HbA1c lowering effect on type-2 diabetes mellitus (DM) and partially decreases serum low-density lipoprotein (LDL) cholesterol and body mass index (BMI).[27] It increases insulin sensitivity and improves insulin resistance in type 2 diabetes.[28] Curcumin is rapidly eliminated from the body due to its insolubility in water and hydrophobicity. Nano-formulation solves the absorption problem.[29]

The core-shell structure of micelles does not allow water to penetrate inside the core. This important feature of micelles provides a suitable environment for the encapsulated drug compared to the free drug.[30] Some advantages offered by micelles as drug carriers, include easy production, suitable costs, easy transport of cargo across biological barriers, better solubility in aqueous media including unstirred water layer of the intestine, controlled release index, and protection against degradation.[31]

Nearly a third of a woman's life is spent in the postmenopausal period and the reduction of estrogen production and finally its termination causes different symptoms in menopausal women; so the above compounds can be effective in reducing the changes caused by the termination of estrogen.[32-34] Due to the determined properties of curcumin and NS on body metabolism, their popularity, the cost-effectiveness of these herbal compounds, and lack of studies about their effectiveness on postmenopausal metabolic changes in combination together, this study was designed to evaluate the efficacy of curcumin nanomicelle (CN) and NS oil soft capsules, separately and together, on lipid profile, glycemic control indexes, and serum 17-β estradiol (ES) levels in postmenopausal women.

## Materials and Methods

This study was a triple-blind randomized controlled clinical trial (researchers, participants, and statistical analyst did not aware of the type of received intervention) with a factorial design. After obtaining permission from the Regional Ethics Committee (IR.TBZMED.REC.1397.131), and registering it in the Iranian Registry of Clinical Trial (identifier: IRCT20131009014957N4), the researcher referred to healthcare centers to select participants. Inclusion criteria were women with normal menopause, sufficient literacy to fill out the questionnaires or together with a literate person in the family, less than 12 months after menopause, between 50 and 65 years old. Exclusion criteria were smoking, alcohol use, regular use of herbal medicines, gallstones, gallbladder obstruction, any detected malignancy, history of surgery and recent trauma (during the last six months), acute and uncontrolled chronic inflammatory diseases (including diabetes, rheumatoid arthritis, history of allergies to turmeric or other herbs [by asking the study participant], taking curcumin supplements or estrogen therapy during three months before the study and use of special diets. The size of the sample was calculated using G*Power version 3.1.2 (the mean difference between two independent groups formula) based on triglyceride and HDL-C. It was estimated according to Heshmati's study[35] 17 people by taking into account M1)SD1( = 38.2)5.3( and M2(SD2) = 45.4(7.1), $\alpha = 0.05$, $\beta = 0.1$, and power = 95% and according to Neerati and colleagues' study,[36] 25 individuals by taking into account M1(SD1) = 122.9(23.76) and M2(SD2) = 106(23.32), $\alpha = 0.05$, $\beta = 0.2$, and power = 95%. Finally, the sample size was calculated 30 women in each group and a total of 120 considering a 20% dropout rate.

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later corrections or comparable ethical standards. The researcher referred to health care centers (with different socio-economic statuses) and extracted a list of postmenopausal women aged 50-65 along with their telephone numbers and addresses. Then, according

to proportional sampling (based on the number of postmenopausal women covered by each health center), the required sample of menopausal women was selected. The selected postmenopausal women were contacted by phone, the goals and methods of the study were briefly explained, and if they were eligible and willing to participate in the study, they were asked to visit the health center on a specific day. An informed consent form was initially signed by all eligible participants.

The participants were randomly divided into four groups (three intervention groups and one control group) using block randomization with a 1:1:1:1 allocation ratio generated by random allocation software (RAS version 1.0.0). The assignment sequence was hidden from the researcher and participants by using sequentially numbered packages from 1 to 120. Each capsule and its placebo were prepared in the same appearance by the pharmaceutical company in identical dark and closed containers. A person who had no role in sampling, data collection, and analysis, prepared the packages. For each participant, three closed opaque containers containing 60 capsules of NS, CN, or their placebos were prepared and the participants received medications daily for 6 months. So, intervention groups received NS + CN, NS + CN placebo, or CN + NS placebo, and the control group received both placebos of NS and CN. Receiving the medications every two months was accompanied by the delivery of a checklist for the daily consumption of the previous medications and their containers. After the end of the consumption period, 5 ml blood samples were taken from the participants for tests (FBS, FI, ES, triglyceride [TG], total cholesterol [TC], LDL cholesterol, and HDL cholesterol) and centrifuged for ten minutes at 3500 rpm. The serum was stored at -70 °C until assayed. Due to ethical considerations, all participants received the routine treatments and required supplements. They were given dietary and physical activity recommendations and a training pamphlet in this regard according to the national guidelines.[37]

CN soft gelatin capsules were provided by Exir Nano Sina (Mashhad, Iran), which according to the drug catalog, each capsule was standardized based on 80 mg of nano curcumin. The placebo capsules of CN were prepared from microcrystalline cellulose (MCC) with the same color, smell, shape, and size by the above company. NS oil soft gelatin capsules were prepared from Barij Essence Pharmaceutical Co. (Kashan, Iran). Each capsule of NS contained at least 6.5 mg of TQ and 495-605 mg of linoleic acid based on the company brochure. The placebo capsules of the NS were MCC, which were provided with similar size, shape, color, and smell by the mentioned company.

A checklist of inclusion and exclusion criteria, a socio-demographic questionnaire, a short form of the International Physical Activity Questionnaire (IPAQ), a midwifery and medical profile questionnaire, and laboratory tests recording questionnaire were used to obtain the necessary information in our study. IPAQ was used to state the physical activity.[38] This questionnaire was transformed to metabolic equivalent-hour/week. This questionnaire was used in Iran by Moghaddam et al and Cronbach's alpha coefficient (0.7) showed that this instrument has good internal consistency.[39] To analyze the serum levels of FBS, TG, TC, and HDL, an Alcyon model autoanalyzer using Pars Azmoon commercial diagnostic kits; and for insulin and estrogen, a mono binding kit and Stat Fax enzyme-linked immunosorbent assay (ELISA( reader were used. The checklist of side effects was designed by the research team. Adverse effects were noted by the participants in the relevant checklist. Serum LDL concentrations were determined using the Friedewald-Fredrickson formula[40] as mentioned below.

LDL = Total Chol - (Triglyceride / 5) - HDL

The following formula was used to calculate of homeostatic model assessment for insulin resistance (HOMA-IR): HOMA-IR = fasting glucose (mg/dL) *fasting insulin (mIU/L) /450.[41]

After completing the information, the data was analyzed through SPSS version 13 (SPSS Inc., Chicago, Ill., USA). Data normality was checked out by the Kolmogorov-Smirnov test. For comparing baseline and demographic characteristics one-way analysis of variance (ANOVA), chi-square, and Fisher's exact test were applied. Analysis of covariance (ANCOVA) adjusted for confounders (baseline values and physical activity) was used for between-group analyses after intervention for normal variables and Mann-Whitney was used for non-normal variables. Paired samples *t* test was used for intra-group comparison if the data distribution was normal and the Wilcoxon test was used if it was non-normal. The significance level was $P < 0.05$.

## Results

At the beginning of this study, 445 women aged 50 to 65 years old were randomly selected and after contacting them, the inclusion criteria were evaluated. One hundred twenty of them met the inclusion criteria for admission to the study and were referred to the health care centers. They were assigned into four groups. Each group included 30 women. Five women were left out of the study. Of those, two belonged to the combined CN and NG groups. Two of them belonged to the NS group and one from the placebo group who was reluctant in the follow-up. Finally, 115 women continued the study until the end (Figure 1).

Except for total physical activity, no statistically significant difference was observed between the four groups in terms of general characteristics ($P > 0.05$). The average age of women participating in the study was 58.4 (3.7) years and the average duration from menopause was 3.8 (1.7) years, 29.1% of participants were illiterate and 87.5% of them were housewives (Table 1).

**Table 1.** Baseline characteristics of the study participants

| Variable | Curcumin (n = 30) | *Nigella sativa* (n = 30) | Curcumin & *Nigella sativa* (n = 30) | Placebo (n = 30) | *P* |
|---|---|---|---|---|---|
| Age (years) | 58.0 (3.4) | 57.2 (4.3) | 57.4 (3.8) | 58.4 (3.4) | 0.58[a] |
| Parity | 3.4 (1.6) | 3.5 (1.5) | 4.2 (2.1) | 4.1 (1.4) | 0.25[a] |
| Age at menopause (years) | 48.7 (3.6) | 47.8 (4.6) | 48.6 (3.8) | 48.3 (3.6) | 0.81[a] |
| Live with husband | 22 (73%) | 22 (73%) | 25 (83%) | 23 (77%) | 0.77[b] |
| Education | | | | | 0.19[c] |
| Illiterate | 6 (20%) | 5 (17%) | 14 (47%) | 10 (33%) | |
| Primary school | 11 (37%) | 10 (33%) | 11 (37%) | 12 (40%) | |
| Higher education | 13 (43%) | 15 (50%) | 5 (16%) | 8 (27%) | |
| Unemployed | 22 (73%) | 28 (93%) | 28 (93%) | 27 (90%) | 0.10[b] |
| Insufficient household income | 3 (10%) | 5 (17%) | 6 (21%) | 4 (13%) | 0.96[c] |
| Exposure to direct sunlight at least 60 min/wk | 9 (30%) | 6 (20%) | 14 (47%) | 15 (50%) | 0.05[b] |
| Total physical activity (MET-min/wk) | 792.00(2493.00) | 346.50(4026.00) | 749.25(4666.50) | 359.25(4426.50) | 0.01[d] |
| History of lactation (Yes) | 23 (85%) | 25 (86%) | 27 (93%) | 28 (96%) | 0.45[b] |
| Duration of lactation (months) | 62.8 (38.0) | 62.3 (31.9) | 75.6 (46.9) | 69.1 (42.0) | 0.59[a] |
| History of oral contraceptive use | 16 (53.3%) | 15 (50%) | 13 (43.3%) | 8 (26.7%) | 0.06[b] |
| History of injectable contraceptive use (Yes) | 3 (11.5%) | 1 (4%) | 1 (3%) | 2 (7%) | 0.58[a] |
| Weight (kg) | 64.9 (8.9) | 67.6 (10.8) | 65.6 (8.2) | 70.1 (10.1) | 0.16[a] |
| Height (cm) | 153 (0.06) | 155 (0.06) | 153 (0.7) | 156 (0.5) | 0.19[a] |
| BMI (kg/m$^2$) | 27.7 (3.8) | 27.9 (4.03) | 28.07 (3.7) | 28.8 (3.8) | 0.75[a] |
| Waist circumference (cm) | 91.2 (9.6) | 92 (8.5) | 91.2 (7.7) | 95.5 (10) | 0.21[a] |
| Hip circumference (cm) | 104.7 (6.3) | 105.0 (8.3) | 104.3 (6.3) | 105.9 (8.1) | 0.86[a] |
| Waist to hip ratio | 0.8 (0.07) | 0.8 (0.06) | 0.8 (0.06) | 0.9 (0.7) | 0.27[a] |
| Arm circumference (cm) | 31.3 (2.5) | 30.9 (2.6) | 30.8 (2.5) | 31.6 (2.7) | 0.65[a] |

Note: The values presented as N (%) or mean (SD), BMI: Body mass index.
* Significant level < 0.05; [a] One-way ANOVA; [b] Chi-squared test; [c] Chi-squared for trend; [d] Kruskal-Wallis.

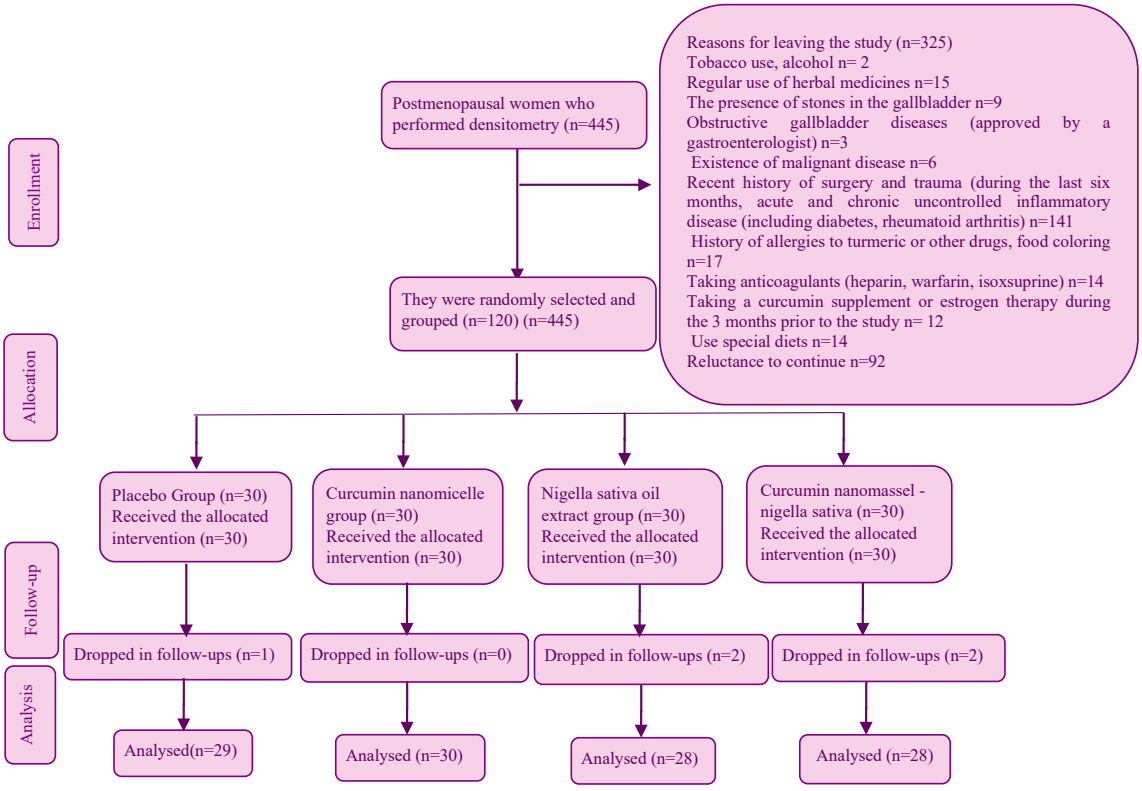


**Figure 1.** Flow diagram of study participants .

Based on ANCOVA adjusted for baseline value and total physical activity, inter-group comparison after intervention showed that in terms of mean FBS, there was not a statistically significant difference between the CN group, NS group, and NS-CN group. When compared to the placebo group. Within-group comparisons were also non-significant. In terms of FI after intervention, the decrease in the NS group was statistically significant, NS group, and NS-CN group compared to the placebo group. There was a statistically significant decrease in insulin resistance after (HOMA-IR) the intervention in the CN group, NS group, and NS-CN group when compared to the placebo group. Within-group comparisons were significant for all groups (Table 2).

Inter-group comparison after intervention using ANCOVA adjusted for baseline values and total physical activity showed that in terms of TG, there was not a significant difference between the CN group, NS group, and NS-CN group in comparison with the placebo group. There was no statistically significant difference in mean TC between the CN group, NS group, and NS-CN group, compared to the placebo group. Significant differences were not shown in LDL, and HDL after the intervention, in the CN group, NS, and NS-CN group when compared to the placebo group. Intra-group comparisons indicated that TG decreased significantly in the NS and placebo groups, and TC reduced significantly in the NS, CN, and NS-CN groups. LDL was significantly reduced and HDL increased in the NS, NS-CN, and placebo groups (Table 3).

There was a significant increase in the NS group compared to the placebo but there were not significant differences between the CN group, and the NS-CN group compared to the placebo group in the mean serum 17-β Estradiol after the intervention. The changes in estrogen levels show a greater increase in the NS group than in the other. Within-group comparisons were significant for all groups (Table 4) (Figure 2).

In this study, four people (13.3%) in the CN group, three people (10%) in the NS group, seven people (23.3%) in the combined group, and five people (16.6%) in the placebo group reported side effects (0.53). The reported side effects included nausea: one person in the CN group, two people in each combined and placebo group; vomiting: only one person in the placebo group; belching: one person in each CN, NS, and combined group; headache: two people in the combination group and one in each of the other groups; unpleasant taste: two people in the NS-CN group and one person in each of the other groups.

The medication adherence rate was more than 90% for all groups based on the gathered checklists of daily medication use and used drug containers.

**Table 2.** Post-intervention serum lipid profile in study groups compared to placebo

| Serum lipid profile | Mean (SD) | | Intra group | Post-intervention comparison with placebo | |
|---|---|---|---|---|---|
| | Baseline | After intervention | *P*[a] | AMD (95% CI) | *P* |
| Triglyceride (mg/dL) | | | | | |
| Curcumin (n=30) | 116.17(26.87) | 108.66(18.81) | 0.18 | 6.63 (-2.13 to15.40) | 0.14 |
| *Nigella sativa* (n=28) | 130.18 (30.57) | 105.11(17.23) | <0.001* | 1.50 (-7.43 to10.44) | 0.74 |
| Curcumin & *Nigella sativa* (n=28) | 122.30 (26/76) | 111.42(10.52) | 0.05 | 9.18 (-0.27 to 18.09) | 0.43 |
| Placebo (n=29) | 116.6(25.7) | 102.5(19.0) | 0.02* | - | - |
| Total cholesterol (mg/dL) | | | | | |
| Curcumin (n=30) | 208.17 (45.01) | 166.44(30.41) | <0.001* | -6.06 (-20.26 to 8.13) | 0.34 |
| *Nigella sativa* (n=28) | 190.40 (37.65) | 164.11(19.79) | <0.001* | -9.68 (-23.62 to 4.25) | 0.17 |
| Curcumin & *Nigella sativa* (n=28) | 201.65 (36.04) | 169.00(24.24) | <0.001* | -2.82 (-11.42 to7.05) | 0.70 |
| Placebo (n=29) | 183.51(55.41) | 171.50(32.32) | 0.25 | - | - |
| LDL cholesterol (mg/dL) | | | | | |
| Curcumin (n=30) | 141.05(48.45) | 137.33(43.88) | 0.080 | 4.24 (-0.69 to 9.18) | 0.09 |
| *Nigella sativa* (n=28) | 121.70(36.14) | 117.46(33.14) | 0.01* | 1.27 (-3.58 to 6.13) | 0.60 |
| Curcumin & *Nigella sativa* (n=28) | 134.99(37.55) | 128.89(34.26) | <0.001* | 0.67 (-4.28 to 5.63) | 0.79 |
| Placebo (n=29) | 117.94(58.42) | 112.77(55.03) | 0.03* | - | - |
| HDL cholesterol (mg/dL) | | | | | |
| Curcumin (n=30) | 43.88(5.84) | 47.60(9.39) | 0.08 | 0.024 (-4.62 to 4.67) | 0.99 |
| *Nigella sativa* (n=28) | 42.66(5.81) | 46.90(6.39) | 0.01* | -0.65 (-5.28 to 3.97) | 0.78 |
| Curcumin & *Nigella sativa* (n=28) | 42.20(7.22) | 48.30(7.64) | <0.001* | 1.36 (-3.31to 6.03) | 0.57 |
| Placebo (n=29) | 42.23(6.45) | 47.40(11.23) | 0.03* | - | - |

AMD: adjusted mean difference. ANCOVA was used for the post-intervention comparisons adjusted for the baseline values and total physical activity (using Sidak to adjust for the multiple comparisons).
*Significant level <0.05; [a]Within-group comparisons using paired samples t-test.

**Table 3.** Post-intervention serum glycemic control parameters in study groups compared to placebo

| Glycemic control parameters | Mean (SD) | | Intra group | Post-intervention comparison with placebo | |
|---|---|---|---|---|---|
| | Baseline | After intervention | *P* | AMD (95% CI) | *P* |
| Fasting blood sugar (mg/dL) | | | | | |
| Curcumin (n=30) | 116.05(18.37) | 106.26(29.50) | 0.11 | -2.573 (-24.41to19.26) | 0.85 |
| *Nigella sativa* (n=28) | 113.11(19.05) | 94.68(31.32) | 0.005* | -4.798 (-26.67 to17.07) | 0.66 |
| Curcumin & *Nigella sativa* (n=28) | 119.40(31.86) | 107.20(26.18) | 0.15 | -3.727 (-27.32 to 19.86) | 0.75 |
| Placebo (n=29) | 110.95(20.35) | 106.85(26.07) | 0.50 | - | - |
| Fasting insulin (uIU/mL) | | | | | |
| Curcumin (n=30) | 8.51(7.57) | 3.30(1.33) | <0.001* | - | <0.001* |
| *Nigella sativa* (n=28) | 5.60(5.05) | 2.40(0.85) | <0.001* | - | <0.001* |
| Curcumin & *Nigella sativa* (n=28) | 7.65(4.75) | 3.42(3.97) | <0.001* | - | <0.001* |
| Placebo (n=29) | 4.30(4.60) | 3.40(2.15) | 0.002* | - | - |
| HOMA-IR | | | | | |
| Curcumin (n=30) | 2.19(2.08) | 0.78(0.46) | <0.001* | - | <0.001* |
| *Nigella sativa* (n=28) | 1.30(1.27) | 0.51(0.20) | <0.001* | - | <0.001* |
| Curcumin & *Nigella sativa* (n=28) | 2.17(1.32) | 0.82(0.22) | <0.001* | - | <0.001* |
| Placebo (n=29) | 1.04(1.11) | 0.77(0.48) | 0.001* | - | - |

AMD: adjusted mean difference. The values presented as mean (SD) unless otherwise identified. *Significant level<0.05.
ANCOVA was used for the post-intervention comparisons adjusted for the baseline values and total physical activity (using Sidak to adjust for the multiple comparisons). Within-group comparisons were done using paired t-test.
For variables without normal distribution, between and within group comparisons were done using Kruskal-Wallis and Wilcoxon tests.

**Table 4.** Post-intervention serum estrogen levels in study groups compared to placebo

| Estrogen (pg/mL) | Curcumin (n=30) | *Nigella sativa* (n=28) | Curcumin & *Nigella sativa* (n=28) | Placebo (n=29) |
|---|---|---|---|---|
| Baseline | 11.02 (3.97) | 9.25 (1.49) | 9.38 (0.99) | 9.37 (1.26) |
| End of Intervention | 14.60 (5.73) | 15.34 (5.11) | 11.98 (5.31) | 12.73 (6.30) |
| Mean difference (95% CI) | 3.57 (6.01 to 1.14) | 6.08 (8.00 to 4.17) | 2.59 (4.60 to 0.58) | 3.35 (0.96 to5.75) |
| Within-group comparison (P) | 0.005* | 0.001* | 0.013* | 0.008* |
| Post-intervention comparison with Placebo | | | | |
| AMD (95% CI) | 1.406 (4.46 to-1.65) | 2.63 (5.58 to -0.31) | -0.92 (2.06 to -3.90) | - |
| *P* | 0.36 | 0.045* | 0.54 | - |

AMD: adjusted mean difference. The values presented as mean (SD) or number (percent) unless otherwise identified. *Significant level<0.05
Between groups comparisons were conducted using ANCOVA adjusted for baseline value and physical activity.
Within-group comparisons were done using paired t test.

## Discussion

The present study was designed to study the effect of NS oil and CN soft capsules on some serum metabolic biomarkers in menopause such as lipid profile, glycemic control indicators, and serum level of Estrogen. In the current study, consumption of NS caused a significant reduction in TG, TC, LDL, and HDL compared to the baseline values, although there were no significant changes compared to the placebo. Ibrahim et al studied the effects of NS on lowering blood lipids in postmenopausal women and found that the consumption of powdered NS (1 g/day) during a two-month intervention significantly decreased TG, TC, LDL, and increased HDL levels.[12] Kaatabi et al investigated the optimal effect of NS on lipid profile in type-2 diabetes patients and according to the results was observed that the group receiving capsules containing NS powder at a dose of two grams per day for 12 weeks reduced TC, TG, LDL.[42] The results of these studies were in accordance with the current study.

The probable mechanism of the hypolipidemic action of NS as seen in similar studies was likely arising from an up-regulation of LDL molecules receptor-mediated through endocytosis.[43] Actually, the mechanism of NS action in lipid reduction is by decreased cholesterol absorption in dietary, stimulation of bile acid synthesis, and accelerating its excretion through feces were possibly contributed from its dietary soluble fibers and sterols.[44]

Another mechanism for the effects of lipid reduction is non-enzymatic lipid peroxidation through the antioxidant activity of NS by increasing the density of LDL receptors in the liver and binding to apolipoprotein apo B, which accelerates the removal of cholesterol from the blood by the liver cells.[45]

Based on the findings, consumption of NS decreased significantly serum levels of FBS, FI, and insulin resistance when compared to baseline values. In the study by Altan et

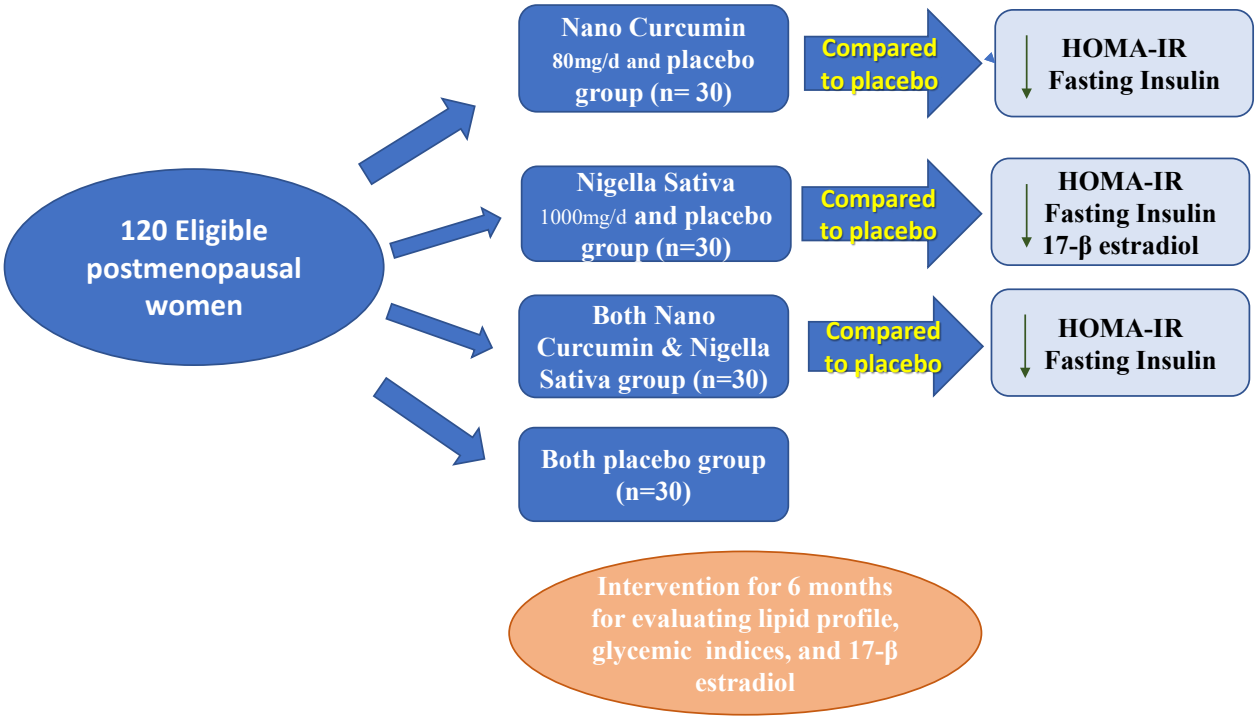


**Figure 2.** The effect of interventions on study biomarkers compared to placebo

al a prescription of 2ml NS per kg per day intraperitoneal alone and also with human parathyroid hormone 2mcg/kg per day in diabetic rats compared to the placebo group for 4 weeks, similar to insulin, it improves blood sugar level.[46] Najmi et al studied the effects of NS on various clinical and biochemical parameters. Consumption of NS with atorvastatin and metformin for 6 weeks in patients with type-2 diabetes led to a significant reduction in TC, FBS, and LDL.[47]

NS mediates its hypoglycemic effects through various pharmacological activities. Alsaif and El-Dakhakhny et al reported that the NS effect on glucose-lowering may be arising from improved insulin sensitivity and further pancreatic actions of insulin in diabetic rats.[48,49] The effects of NS hypoglycemia are due in part to reduced hepatic gluconeogenesis because as Fararh et al showed in their study that giving black seed to hamsters significantly reduced hepatic glucose production from gluconeogenesis precursors (such as alanine, glycerol, and lactate).[50] In addition to the above, the anti-diabetic activity of NS is related to its antioxidant compounds. TQ, one of the main components in NS has antioxidant effects and therefore reduces oxidative stress through preserving pancreatic β-cell integrity and increased insulin levels.[51]

Consumption of NS increased estrogen levels significantly when compared to baseline values and placebo. Parhizkar et al indicated that the estrogenic effects of NS are confirmed by examining vaginal cell cornification, utero-trophic assay, and serum estrogen levels.[52] Most studies have demonstrated that phytoestrogens bind to estrogen receptors and have an estrogenic-like effect. Unsaturated fatty acids of NS may be responsible for its estrogenic effects.[21]

The result of the present study also demonstrated that CN consumption had significant reduction effects on TC, FI, and insulin resistance and incremental effect on estrogen levels compared to baseline values. The findings of this study were coordinated with the results of some studies. Neerati et al evaluated the effectiveness of Curcumin on lipid profile and glycemic status. Eight patients with type 2 diabetes were treated with 475 mg of Curcumin for ten days in addition to standard glyburide treatment. Glucose levels dropped and no patients experienced hypoglycemia. LDL and TG decreased significantly and HDL increased. Concomitant use of curcumin capsules and glyburide may lead to better glycemic control in patients.[36] A study by Kang and Chen reported that Curcumin breaks down insulin resistance. Curcumin increases the activity of PPAR-Y (peroxisome proliferator-activated receptor-y) and PPAR-Y activity suppresses LDL receptors and treats hypercholesterolemia. Curcumin, a compound of turmeric may be helpful in the prevention of hepatic cholesterol-related liver fibrogenesis.[53] Rahimi et al in a double-blind clinical trial in 70 patients with type-2 diabetes reviewed the effect of 80 mg CN once daily for 3 months compared with the placebo on FBS, HbA1c, and lipid profiles. The results showed that in the CN group, there was a significant reduction in FBS, HbA1c, and BMI when compared to the placebo group. In addition, most of the studied indices, including the lipid profile, in the CN group had also a significant decrease.[27] The results of this study are coordinated with the present study.

The proposed mechanism of curcumin's anti-diabetic effect is inhibiting insulin resistance. It increases insulin sensitivity through three mechanisms. Firstly, curcumin stimulates glucokinase activity in the liver and improves glucose hemostasis. Secondly, it stimulates lipid metabolism by increasing the activity of lipoprotein lipase to decrease triglycerides. Thirdly, curcumin induces the glucose transporter type 4 (GLUT4) expression to increase glucose absorption. In this way, it independently affects the insulin pathway.[54] In addition, curcumin is a phytoestrogen,[55] so it can interact with the endocrine system and affect the hypothalamus-hypophysial-ovarian axis activity. Also, it can downregulate androgen receptors and upregulate 3-β-hydroxysteroid dehydrogenase.[56]

Consumption of CN-NS significantly reduced the level of total TC, LDL, FI, and insulin resistance and significantly increased serum HDL-C and estrogen levels compared to baseline values. Amin et al studied the effect of NS (1.5 g/d) alone and simultaneously with curcumin (900 mg/d and 1.5 g/d respectively) on 250 patients who had metabolic syndrome. The results demonstrated that at 4 weeks, when compared to baseline, NS and Turmeric alone caused improvement in BMI, waist circumference (WC), and body fat percent (BF%). Combined consumption improved all parameters except HDL-cholesterol with lower FBG and LDL-cholesterol as compared to placebo. At 8 weeks, when compared to placebo, NS decreased lipids and FBG, while Turmeric reduced LDL and C-reactive protein (CRP). This is interesting, combination group with a 60% dose of the individual herbs caused an improvement in all parameters from baseline. When compared to placebo, it decreased body fat percent (BF%), FBG, cholesterol, TG, LDL, CRP, and raised HDL.[32] The results of this study are consistent with the present study.

The findings of our research showed that in terms of serum FI levels and insulin resistance, there was a statistically significant difference between the intervention groups of NS, CN, and the combined group compared with the placebo. In a study by Heshmati et al it was stated that consumption of 3 gr of NS oil per day for 12 weeks caused a slight decrease in BMI, insulin level, and insulin resistance, as well as an increase in HDL-C. In type-2 diabetes patients, it was shown that the levels of FBS, TG, LDL, and HbA1c were significantly reduced in the intervention group when compared to the placebo group.[34] This discrepancy between FBS, TG, LDL, and HbA1c in our study could be due to differences in subjects in terms of underlying disease, sex, and age. Improvement of the parameters that occurred in the placebo group may be partly responsible for the lack of significance between the groups compared to the placebo.

In a report by Amini et al a total of 70 healthy volunteers with blood cholesterol levels between 200 and 300 mg/dL were randomly divided into two groups of NS oil and placebo. The volunteers of the NS oil and placebo group consumed 2.5 mL of NS oil and placebo twice daily for 8 weeks, respectively. Volunteers in two groups underwent TG, TC, HDL, LDL, alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen, creatinine, FBS, and HbA1c tests before entering the study and then after eight weeks. The results showed that the levels of TC, LDL, and TG in the NS group at the end of the research were significantly reduced when compared to the placebo group. In addition, the level of FBG and HbA1c in the NS oil group at the end of the research was significantly decreased when compared to the placebo group. The results of this study are not coordinated with the current study.[57] Possible causes of inconsistency are due to the conducting study on healthy individuals in this study.

This study was conducted on women aged 50-65 years, the critical age range for initiation of some disorders due to a sudden drop in estrogen level. This research was performed on healthy postmenopausal women and therefore it is recommended that a similar study be performed in people with determined metabolic problems. It is noteworthy that because all groups had received dietary and physical activity advice, so the study serum biomarkers improved also in the placebo group.

## Conclusion

This study revealed that although NS, CN, and NS-CN improved the lipids profiles, serum glycemic indices as well as hormone levels, the improvement in glycemic indices (FI and HOMA-IR) and estrogen serum levels were significant compared to the placebo groups. It seems that NG and CN can have a therapeutic and protective effect on metabolic syndrome during menopause. In addition, NG may be recommended to remedy estrogen deficiency. According to the results of this study, the combination therapy of NS and CN does not seem to be more beneficial in improving the lipid profile, glycemic control indices, and serum estrogen levels compared to the single use of these medicinal plants. Further studies with larger sample sizes are recommended for confirmation.

### Acknowledgments

We appreciate all headquarters and personnel of the Physical Medicine & Rehabilitation Research Center, Nutrition Research Center, the Health Vice-Chancellor, and all the women who participated in this study.

### Authors' Contribution

**Conceptualization:** Azizeh Farshbaf-Khalili, Alireza Ostadrahimi.
**Data curation:** Alireza Ostadrahimi, Minoo Ranjbar.
**Formal analysis:** Azizeh Farshbaf-Khalili, Alireza Ostadrahimi.
**Funding acquisition:** Azizeh Farshbaf-Khalili.
**Investigation:** Azizeh Farshbaf-Khalili, Zeynab Sadeghzadeh, Minoo Ranjbar.
**Methodology:** Azizeh Farshbaf-Khalili, Alireza Ostadrahimi.
**Project administration:** Azizeh Farshbaf-Khalili, Zeynab Sadeghzadeh.
**Resources:** Azizeh Farshbaf-Khalili, Zeynab Sadeghzadeh.
**Software:** Azizeh Farshbaf-Khalili, Alireza Ostadrahimi.
**Supervision:** Alireza Ostadrahimi.

## Research Highlights

**What is the current knowledge?**

- After menopause, a significant decrease in serum estrogen and an increase in serum testosterone is accompanied by an increased incidence of metabolic disorders.
- Some compounds that act through the effect on estrogen receptors can reduce the risk of cardiovascular diseases and diabetes.

**What is new here?**

- We evaluated the effect of *Nigella sativa* (NS), curcumin nanomicelle (CN), and both on lipid profile, glycemic status, and serum 17-β estradiol (ES) levels in postmenopausal women.
- NS, CN, and NS-CN improved the lipid profiles, blood sugar, and hormone levels. However, this improvement was significant in glycemic control indices and estrogen levels compared to the placebo group.

**Validation:** Azizeh Farshbaf-Khalili, Alireza Ostadrahimi.
**Visualization:** Minoo Ranjbar.
**Writing–original draft:** Zeynab Sadeghzadeh, Minoo Ranjbar.
**Writing–review & editing:** Azizeh Farshbaf-Khalili, Alireza Ostadrahimi.

### Competing Interests

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

### Data Availability Statement

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

### Ethical Approval

Ethical approval was obtained from the Institutional Ethics Committee (IR.TBZMED.REC.1397.131).

### Funding

This study was supported financially by Tabriz University of Medical Sciences.

### References

1. Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause. 2006; 13(2): 265-79. doi: 10.1097/01.gme.0000218683.97338.ea
2. World Health Organization (WHO). Menopause: Key Facts [Internet]. Switzerland: WHO; 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/menopause. Accessed October 17, 2022.
3. Ibrahim RM, Hamdan NS, Ismail M, Saini SM, Abd Rashid SN, Abd Latiff L, et al. Protective effects of *Nigella sativa* on metabolic syndrome in menopausal women. Adv Pharm Bull. 2014; 4(1): 29-33. doi: 10.5681/apb.2014.005
4. Speroff L, Glass HR, Kase NG. Clinical Gynecologic Endocrinology and Infertility. 16th ed. USA: Lippincott Williams & Wilkins; 1999.
5. Ko SH, Kim HS. Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women. Nutrients. 2020; 12(1):202. doi: 10.3390/nu12010202
6. Cignarella A, Kratz M, Bolego C. Emerging role of estrogen in the control of cardiometabolic disease. Trends Pharmacol Sci. 2010; 31(4): 183-9. doi: 10.1016/j.tips.2010.01.001
7. Schwartsmann G, Ratain MJ, Cragg GM, Wong JE, Saijo N, Parkinson DR, et al. Anticancer drug discovery and development throughout the world. J Clin Oncol. 2002; 20(18 Suppl): 47S-59S.
8. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: past history and future perspective. J Herbmed Pharmacol. 2018; 7(1): 1-7. doi: 10.15171/jhp.2018.01
9. Thakur S, Kaurav H, Chaudhary G. *Nigella sativa* (Kalonji): a black seed of miracle. Int J Res Rev. 2021; 8(4): 342-57. doi: 10.52403/ijrr.20210441
10. Forouzanfar F, Fazly Bazzaz BS, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its constituent (thymoquinone): a review on antimicrobial effects. Iran J Basic Med Sci. 2014; 17(12): 929-38.
11. Namazi N, Larijani B, Ayati MH, Abdollahi M. The effects of *Nigella sativa* L. on obesity: a systematic review and meta-analysis. J Ethnopharmacol. 2018; 219: 173-81. doi: 10.1016/j.jep.2018.03.001
12. Ibrahim RM, Hamdan NS, Mahmud R, Imam MU, Saini SM, Rashid SN, et al. A randomised controlled trial on hypolipidemic effects of *Nigella sativa* seeds powder in menopausal women. J Transl Med. 2014; 12: 82. doi: 10.1186/1479-5876-12-82
13. Nickavar B, Mojab F, Javidnia K, Roodgar Amoli MA. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Z Naturforsch C J Biosci. 2003; 58(9-10): 629-31. doi: 10.1515/znc-2003-9-1004
14. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of *Nigella sativa*: a miracle herb. Asian Pac J Trop Biomed. 2013; 3(5): 337-52. doi: 10.1016/s2221-1691(13)60075-1
15. Ciesielska-Figlon K, Wojciechowicz K, Wardowska A, Lisowska KA. The immunomodulatory effect of *Nigella sativa*. Antioxidants (Basel). 2023; 12(7):1340. doi: 10.3390/antiox12071340
16. Majdalawieh AF, Fayyad MW, Nasrallah GK. Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of *Nigella sativa*. Crit Rev Food Sci Nutr. 2017; 57(18): 3911-28. doi: 10.1080/10408398.2016.1277971
17. Razavi BM, Hosseinzadeh H. A review of the effects of *Nigella sativa* L. and its constituent, thymoquinone, in metabolic syndrome. J Endocrinol Invest. 2014; 37(11): 1031-40. doi: 10.1007/s40618-014-0150-1
18. Bamosa AO, Kaatabi H, Lebdaa FM, Elq AM, Al-Sultanb A. Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian J Physiol Pharmacol. 2010; 54(4): 344-54.
19. Hadi S, Mirmiran P, Hosseinpour-Niazi S, Hedayati M, Azizi F. Effect of *Nigella sativa* oil extract on lipid profiles in type 2 diabetic patients: a randomized, double blind, placebo-controlled clinical trial. Iran J Endocrinol Metab. 2015; 16(6): 411-8.
20. Mansour MA, Ginawi OT, El-Hadiyah T, El-Khatib AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of *Nigella sativa* on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Res Commun Mol Pathol Pharmacol. 2001; 110(3-4): 239-51.
21. Parhizkar S, Abdul Latiff L, Abdul Rahman S, Dollah MA, Parichehr H. Assessing estrogenic activity of *Nigella sativa*

in ovariectomized rats using vaginal cornification assay. Afr J Pharm Pharmacol. 2011; 5(2): 137-42. doi: 10.5897/ajpp10.276
22. Liu ML, Xu X, Rang WQ, Li YJ, Song HP. Influence of ovariectomy and 17beta-estradiol treatment on insulin sensitivity, lipid metabolism and post-ischemic cardiac function. Int J Cardiol. 2004; 97(3): 485-93. doi: 10.1016/j.ijcard.2003.11.046
23. El-Saadony MT, Yang T, Korma SA, Sitohy M, Abd El-Mageed TA, Selim S, et al. Impacts of turmeric and its principal bioactive curcumin on human health: pharmaceutical, medicinal, and food applications: a comprehensive review. Front Nutr. 2022; 9: 1040259. doi: 10.3389/fnut.2022.1040259
24. Akazawa N, Choi Y, Miyaki A, Tanabe Y, Sugawara J, Ajisaka R, et al. Curcumin ingestion and exercise training improve vascular endothelial function in postmenopausal women. Nutr Res. 2012; 32(10): 795-9. doi: 10.1016/j.nutres.2012.09.002
25. Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem Toxicol. 2002; 40(8): 1091-7. doi: 10.1016/s0278-6915(02)00037-6
26. Ghorbani Z, Hekmatdoost A, Mirmiran P. Anti-hyperglycemic and insulin sensitizer effects of turmeric and its principle constituent curcumin. Int J Endocrinol Metab. 2014; 12(4): e18081. doi: 10.5812/ijem.18081
27. Rahimi HR, Mohammadpour AH, Dastani M, Jaafari MR, Abnous K, Ghayour Mobarhan M, et al. The effect of nano-curcumin on HbA1c, fasting blood glucose, and lipid profile in diabetic subjects: a randomized clinical trial. Avicenna J Phytomed. 2016; 6(5): 567-77.
28. Su LQ, Wang YD, Chi HY. Effect of curcumin on glucose and lipid metabolism, FFAs and TNF-α in serum of type 2 diabetes mellitus rat models. Saudi J Biol Sci. 2017; 24(8): 1776-80. doi: 10.1016/j.sjbs.2017.11.011
29. Nabati M, Mahkam M, Heidari H. Isolation and characterization of curcumin from powdered rhizomes of turmeric plant marketed in Maragheh city of Iran with soxhlet technique. Iran Chem Commun. 2014; 2(4): 236-43.
30. Mobasheri M, Attar H, Rezayat Sorkhabadi SM, Khamesipour A, Jaafari MR. Solubilization behavior of polyene antibiotics in nanomicellar system: insights from molecular dynamics simulation of the amphotericin B and nystatin interactions with polysorbate 80. Molecules. 2015; 21(1): E6. doi: 10.3390/molecules21010006
31. Shakeri A, Sahebkar A. Opinion paper: nanotechnology: a successful approach to improve oral bioavailability of phytochemicals. Recent Pat Drug Deliv Formul. 2016; 10(1): 4-6. doi: 10.2174/1872211309666150611120724
32. Amin F, Islam N, Anila N, Gilani AH. Clinical efficacy of the co-administration of turmeric and black seeds (Kalongi) in metabolic syndrome - a double blind randomized controlled trial - TAK-MetS trial. Complement Ther Med. 2015; 23(2): 165-74. doi: 10.1016/j.ctim.2015.01.008
33. Takahashi TA, Johnson KM. Menopause. Med Clin North Am. 2015; 99(3): 521-34. doi: 10.1016/j.mcna.2015.01.006
34. Gudmundsdottir SL, Flanders WD, Augestad LB. Physical activity and cardiovascular risk factors at menopause: the Nord-Trøndelag health study. Climacteric. 2013; 16(4): 438-46. doi: 10.3109/13697137.2013.768231
35. Heshmati J, Namazi N, Memarzadeh MR, Taghizadeh M, Kolahdooz F. *Nigella sativa* oil affects glucose metabolism and lipid concentrations in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Food Res Int. 2015; 70: 87-93. doi: 10.1016/j.foodres.2015.01.030
36. Neerati P, Devde R, Gangi AK. Evaluation of the effect of curcumin capsules on glyburide therapy in patients with type-2 diabetes mellitus. Phytother Res. 2014; 28(12): 1796-800. doi: 10.1002/ptr.5201
37. Osteoporosis Research Center, Endocrine and Metabolism Research Institute, Tehran University of Medical Sciences, Osteoporosis Society, National Osteoporosis Research Network. Osteoporosis Clinical Guideline. 2nd ed. Tehran: Pune Publications; 2015.
38. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003; 35(8): 1381-95. doi: 10.1249/01.mss.0000078924.61453.fb
39. Moghaddam MB, Aghdam FB, Jafarabadi MA, Allahverdipour H, Nikookheslat SD, Safarpour S. The Iranian Version of International Physical Activity Questionnaire (IPAQ) in Iran: content and construct validity, factor structure, internal consistency and stability. World Appl Sci J. 2012;18(8):1073-80. doi: 10.5829/idosi.wasj.2012.18.08.754
40. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972; 18(6): 499-502.
41. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004; 27(6): 1487-95. doi: 10.2337/diacare.27.6.1487
42. Kaatabi H, Bamosa AO, Lebda FM, Al Elq AH, Al-Sultan AI. Favorable impact of Nigella sativa seeds on lipid profile in type 2 diabetic patients. J Family Community Med. 2012 Sep;19(3):155-61. doi: 10.4103/2230-8229.102311
43. Bhatti IU, Ur Rehman F, Khan MA, Marwat SK. Effect of prophetic medicine Kalonji (*Nigella sativa* L.) on lipid profile of human beings: an in vivo approach. World Appl Sci J. 2009; 6(8): 1053-7.
44. Talati R, Baker WL, Pabilonia MS, White CM, Coleman CI. The effects of barley-derived soluble fiber on serum lipids. Ann Fam Med. 2009; 7(2): 157-63. doi: 10.1370/afm.917
45. Al-Naqeeb G, Ismail M, Al-Zubairi AS. Fatty acid profile, α-tocopherol content and total antioxidant activity of oil extracted from *Nigella sativa* seeds. Int J Pharmacol. 2009; 5(4): 244-50. doi: 10.3923/ijp.2009.244.250
46. Altan MF, Kanter M, Donmez S, Kartal ME, Buyukbas S. Combination therapy of *Nigella sativa* and human parathyroid hormone on bone mass, biomechanical behavior and structure in streptozotocin-induced diabetic rats. Acta Histochem. 2007; 109(4): 304-14. doi: 10.1016/j.acthis.2007.02.006
47. Najmi A, Nasiruddin M, Khan RA, Haque SF. Effect of Nigella sativa oil on various clinical and biochemical parameters of insulin resistance syndrome. Int J Diabetes Dev Ctries. 2008; 28(1): 11-4. doi: 10.4103/0973-3930.41980
48. Alsaif MA. Effect of *N. sativa* oil on impaired glucose tolerance and insulin insensitivity induced by high-fat-diet and turpentine-induced trauma. Pak J Biol Sci. 2008; 11(8): 1093-9. doi: 10.3923/pjbs.2008.1093.1099
49. El-Dakhakhny M, Mady N, Lembert N, Ammon HP. The hypoglycemic effect of *Nigella sativa* oil is mediated by extrapancreatic actions. Planta Med. 2002; 68(5): 465-6. doi: 10.1055/s-2002-32084
50. Fararh KM, Shimizu Y, Shiina T, Nikami H, Ghanem MM, Takewaki T. Thymoquinone reduces hepatic glucose production in diabetic hamsters. Res Vet Sci. 2005; 79(3): 219-23. doi: 10.1016/j.rvsc.2005.01.001
51. Hamdy NM, Taha RA. Effects of *Nigella sativa* oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. Pharmacology. 2009; 84(3): 127-34. doi: 10.1159/000234466
52. Parhizkar S, Abdul Latiff L, Parsa A. Effect of *Nigella sativa* on

reproductive system in experimental menopause rat model. Avicenna J Phytomed. 2016; 6(1): 95-103.

53. Kang Q, Chen A. Curcumin suppresses expression of low-density lipoprotein (LDL) receptor, leading to the inhibition of LDL-induced activation of hepatic stellate cells. Br J Pharmacol. 2009; 157(8): 1354-67. doi: 10.1111/j.1476-5381.2009.00261.x
54. Jiménez-Osorio AS, Monroy A, Alavez S. Curcumin and insulin resistance-molecular targets and clinical evidences. Biofactors. 2016; 42(6): 561-80. doi: 10.1002/biof.1302
55. Mohajeri M, Bianconi V, Ávila-Rodriguez MF, Barreto GE, Jamialahmadi T, Pirro M, et al. Curcumin: a phytochemical modulator of estrogens and androgens in tumors of the reproductive system. Pharmacol Res. 2020; 156: 104765. doi: 10.1016/j.phrs.2020.104765
56. Tiwari-Pandey R, Ram Sairam M. Modulation of ovarian structure and abdominal obesity in curcumin- and flutamide-treated aging FSH-R haploinsufficient mice. Reprod Sci. 2009; 16(6): 539-50. doi: 10.1177/1933719109332822
57. Amini M, Fallah Huseini H, Mohtashami R, Sadeqhi Z, Ghamarchehre M. Hypolipidemic effects of *Nigella sativa* L. seeds oil in healthy volunteers: a randomized, double-blind, placebo-controlled clinical trial. J Med Plants. 2011; 10(40): 133-8. [Persian].