Original Article

The Effects of *Nigella sativa* and Curcumin Supplementation on Oxidative Stress Biomarkers in Postmenopausal Women with Primary Osteoporosis or Osteopenia: A Triple-Blind Factorial Randomized Controlled Trial

Amir Mehdi Iranshahi, Azizeh Farshbaf-Khalili[1], Alireza Ostadrahimi[2], Seyed Kazem Shakouri[1], Soraya Babaie[1]

Department of Clinical Nutrition, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, [1]Physical Medicine and Rehabilitation Research Centre, Tabriz University of Medical Sciences, [2]Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

ORCID:

Amir Mehdi Iranshahi: 0000-0001-5164-0479

Alireza Ostadrahimi: 0000-0002-1058-1481

Azizeh Farshbaf-Khalili: 0000-0001-5754-4613

Seyed Kazem Shakouri: 0000-0003-3815-4091

Soraya Babaie: 0000-0003-0259-2866

Abstract

Background: Herbal products with potential antioxidant effects can be used in the management of chronic disorders such as osteoporosis. **Objectives:** The aim of this study was to evaluate the effects of *Nigella sativa* (NS) and curcumin (CUR) supplementation on oxidative stress biomarkers in postmenopausal women with primary osteoporosis or osteopenia. **Methods:** In this randomized controlled trial, conducted from August 2018 to April 2019 using a triple-blind factorial design, 120 postmenopausal women with primary osteoporosis (n = 74) or osteopenia (n = 46) were randomly allocated to 430 person groups, namely the NS, CUR, NS + CUR, and placebo groups. Participants in these groups daily received one 1000 mg NS oil capsule and one CUR placebo capsule, one 80 mg nanomicelle CUR and one NS placebo capsule, one 1000 mg NS oil capsule and one 80 mg nanomicelle CUR capsule, and two placebo capsules, respectively. The intervention lasted 6 months. The serum levels of the superoxide dismutase (SOD), total antioxidant capacity (TAC), and malondialdehyde (MDA) oxidative stress biomarkers were assessed before and after the intervention. **Results:** SOD serum level significantly increased in the NS and the NS + CUR groups and its posttest value in the NS + CUR group was significantly more than the placebo group (mean differences = 100.4, 95% confidence interval = 21.9–178.9; P = 0.013). TAC serum level significantly increased in the NS + CUR group and its posttest value in this group was significantly more than the placebo group (mean difference = 0.23; 95% confidence interval = 0.05–0.41; P = 0.011). No significant change was observed in MDA serum level in any of the study groups (P > 0.05). **Conclusion:** CUR is probably ineffective in significantly reducing oxidative stress, while NS can relatively alleviate oxidative stress and NS + CUR can considerably alleviate oxidative stress in postmenopausal women with primary osteoporosis or osteopenia.

Keywords: *Curcumin, Nigella sativa, Osteopenia, Oxidative stress, Postmenopausal osteoporosis*

Introduction

Osteopenia and osteoporosis are defined as, respectively, a bone mineral density (BMD) of 1–2.5 and more than 2.5 standard deviations (SD) below the mean BMD of the adult population.[1,2] Primary osteoporosis is caused by the decreased levels of sex hormones due to aging and menopause, whereas secondary osteoporosis occurs due to problems such as local infection or inflammation, renal failure, diabetes

Address for correspondence: Prof. Alireza Ostadrahimi, Nutrition Research Center, Tabriz University of Medical Sciences, Golghasht Street, Tabriz, Iran.
E-mail: ostadrahimi@tbzmed.ac.ir

Submitted: 27-Nov-2021 **Revised:** 07-Feb-2023 **Accepted:** 12-Feb-2023
Published: 29-Mar-2023

How to cite this article: Iranshahi AM, Farshbaf-Khalili A, Ostadrahimi A, Shakouri SK, Babaie S. The Effects of *Nigella sativa* and curcumin supplementation on oxidative stress biomarkers in postmenopausal women with primary osteoporosis or osteopenia: A triple-blind factorial randomized controlled trial. Nurs Midwifery Stud 2023;12:7-14.

Access this article online

Quick Response Code:

Website: www.nmsjournal.com

DOI: 10.4103/nms.nms_123_21

mellitus, rheumatoid arthritis, thyroid disorders, Cushing's disease, malnutrition, systemic inflammation, and intake of medications such as corticosteroids and furosemide.[3,4] Dramatic changes in demographic patterns, the high prevalence of osteoporosis, and its debilitating complications have turned osteoporosis into a critical health challenge in Iran.[5,6]

Sex hormones, such as estrogen, can act as antioxidants and protect bones against oxidative stress. Accordingly, postmenopausal decreased level of estrogen is associated with decreased ability of the antioxidant system, the exposure of the organs to oxidative stress, increased bone resorption, and decreased bone formation.[3,7-10] Therefore, pharmacological therapy in addition to healthy eating, regular physical exercise, and avoidance from smoking and alcohol are among the most important strategies for osteoporosis prevention.[11] Pharmacological therapies for osteoporosis prevention and management include anti-resorptive agents such as bisphosphonates and calcitonin, hormone replacement therapy, bone-forming agents such as parathyroid hormone, and selective estrogen receptor modulators such as raloxifene.[12,13] However, these therapies have various side effects and may be associated with treatment failure.[14]

Given the side effects of pharmacological therapies, nonpharmacological therapies, such as medicinal plants and herbal products, have received special attention in recent years due to their fewer side effects, cost-effectiveness, and easy accessibility. Herbal products can improve bone health and prevent postmenopausal osteoporosis,[14] through mechanisms such as estrogen-like activity, antioxidant and anti-inflammatory effects, and modulation of the signaling pathways.[15]

Nigella sativa (NS) is a useful medicinal plant from the *Ranunculaceae* family with different anticarcinogenic, antioxidant, antihypertensive, antidiabetic, and antiseptic effects. Moreover, some studies reported its protective effects against osteoporosis progression.[16-18] Curcumin (CUR), a herbal derivative found in the rhizomes of Curcuma Longa that belongs to the Zingiberaceae family,[19] is another herbal compound used for therapeutic purposes in patients with neurological, cardiovascular, hepatic, biliary, and renal disorders, coagulopathy, and hypertension. It also has potential anti-inflammatory and antioxidant,[7,20] antiresorptive, and antiosteoporotic effects.[7,19,20]

Since taking care of women throughout their life, including menopause, is one of the duties of midwives, knowing the factors related to the health of this period and optimal care in this field is mandatory. Despite the potential positive effects of NS and CUR on osteoporosis, there are limited studies into their effects on oxidative stress biomarkers in menopause-induced primary osteoporosis and osteopenia. Since, the present study was conducted to narrow this gap.

Objectives

The aim of this study was to evaluate the effects of NS and CUR supplementation on oxidative stress biomarkers in postmenopausal women with primary osteoporosis or osteopenia.

Methods

Design and participants

This is a triple-blind factorial randomized controlled trial that was performed on 120 postmenopausal women with primary osteoporosis (n = 74) or osteopenia (n = 46) selected from healthcare centers in Tabriz, Iran, from August 2018 to April 2019.[21] Inclusion criteria were postmenopausal osteoporosis or osteopenia, age of 50–65 years, BMD T-score of −1 to −2.5 for osteopenia and BMD T-score of <−2.5 for osteoporosis in the lumbar spine or femoral neck, no intake of medications affecting bone metabolism, 25-hydroxy Vitamin D level more than 20 ng/ml, and no history of pathologic fracture, secondary osteoporosis, premature menopause, renal failure, malignancy, peptic ulcer, gallstone, hypercalcemia, hypocalcemia, coagulopathy, or allergic reaction to NS or CUR supplements. Women with BMD T-score <−4 in the lumbar spine or <−3.5 in the femoral neck were not included.

Sample size was determined using the G*Power Version 3.1.9.4 (Germany) and based on the results of a previous study which reported that the mean scores of superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAC) in two groups were 3.86 ± 0.76 and 2.95 ± 0.78, 3.05 ± 0.91 and 3.87 ± 0.98, and 3.84 ± 0.62 and 3.17 ± 0.32, respectively.[22] Accordingly, with a power of 0.80 and a confidence level of 0.95, sample size per group was determined to be 13 for the SOD variable, 22 for the MDA variable, and ten for the TAC variable. With a probable attrition rate of 30%, the final sample size was considered to be 30 per group. The sample size calculation formula was

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

Participants were randomly assigned to four groups through block randomization with a block size of 4 and 8, using the Random Allocation Software, and with an allocation ratio of 1:1:1:1. The four groups of the study were NS, CUR, NS + CUR, and placebo groups. For allocation concealment, a statistician generated

and concealed random allocation sequence, labeled four types of sixty-capsule boxes with random codes generated by the software, provided them to us, and left the study for the purpose of allocation concealment. Based on the generated sequence, the capsule boxes were provided to participants to be taken in 2 months. At the end of the 2-month period, participants referred to the study setting to receive new boxes of capsules for the next 2 months. The study consisted of three 2-month courses, i.e., 6 months in total. The members of the research team, participants, clinical and laboratory staff in the study setting, and statistical data analyst were blind to the allocation sequence until the end of data analysis.

Intervention

Participants in the NS, CUR, NS + CUR, and placebo groups daily received one 1000 mg NS oil capsule and one CUR placebo capsule, one 80 mg nanomicelle CUR and one NS placebo capsule, one 1000 mg NS oil capsule and one 80 mg nanomicelle CUR capsule, and two placebo capsules, respectively. NS oil and NS placebo capsules were prepared by the Barij Essence Pharmaceutical Company (Kashan, Iran). NS oil capsules were standardized based on at least 6.5 mg of thymoquinone and 495–605 mg linoleic acid and NS placebo capsules contained 1000 mg microcrystalline cellulose and had the same shape, color, odor, and size as NS oil capsules. CUR and CUR placebo capsules were prepared by Sina Company (Mashhad, Iran). CUR capsules were standardized based on 80 mg nanomicelle CUR and CUR placebo capsules contained 1000 mg microcrystalline cellulose with the same shape, color, odor, and size as CUR capsules.

Participants received the allocated interventions in three consecutive 2-month courses. At the beginning of each 2-month course, one sealed opaque envelope containing two thirty-capsule boxes was provided to each participant based on the allocation sequences and the participant was asked to take two capsules per day. Besides, the participant was provided with a checklist to document the daily use of the capsules and was asked to refer to the study setting at the end of the 2-month course in order to deliver the checklist and the boxes and receive a new two-box envelope and a new checklist. The delivered boxes and the checklists were used to assess the level of compliance with the intervention through counting the number of the remaining capsules. Besides, monthly telephone contacts were made with participants in order to ensure their intake of the capsules.

Data collection instruments

Participants' demographic characteristics were assessed at baseline using a demographic questionnaire with items on age, menopause age, gravidity, parity, duration of breastfeeding, and duration of menopause. Weight was measured with minimal clothing and using a weight scale (Seca, Germany) with a precision of 0.1 kg. Height was also measured without shoes and in a standard position using a stadiometer (Seca, Germany) with a precision of 0.1 cm. Then, body mass index (BMI) was calculated through dividing weight (in kilogram) by height (in meters squared). Dietary intake was also assessed both at baseline and at the end of the study using a 3-day food record and the data were analyzed in terms of total energy, macronutrients, fiber, vitamins, and minerals with antioxidant properties via the Nutritionist IV software (San Bruno, Canada) modified based on Iranian foods. Moreover, physical activity was assessed through the Persian International Physical Activity Questionnaire. This questionnaire has acceptable validity and reliability.[23] For oxidative stress biomarker assessment, a 10-mL venous blood sample was taken from each participant after 12 h of overnight fasting both at baseline and at the end of the intervention. The serums of the samples were obtained through centrifugation at 2500 rpm for 10 min at a temperature of 25°C and were stored at −80°C until the final assay at the end of data collection. The levels of serum TAC, SOD activity, and MDA were measured using Naxifer™ kits at 593 nm, NASDOX™ kits at 405 nm, and Nalondi™ kits at 550 nm, respectively. The potential side effects of the interventions were assessed during and after the intervention using a checklist with items on nausea, vomiting, belching, headache, and unpleasant taste as well as an open question about any other side effects.

Ethical considerations

The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran, approved this study (code: IR.TBZMED.REC.1397.1032) and the study was registered in the Iranian Registry of Clinical Trials (code: IRCT20131009014957N4). All participants were informed about the study aim, data confidentiality, and their freedom to voluntarily withdraw from the study and their personal written informed consent was obtained.

Data analysis

The data were analyzed using the SPSS software (v. 23.0, Chicago, IL, USA), based on the intention to treat principle, and at a significance level of <0.05. The kurtosis, skewness, and SD measures were used for normality testing and the data were presented using mean, SD, median, range, absolute frequency, and relative frequency. The one-way analysis of variance and the Kruskal–Wallis test were used for group comparisons and the analysis of covariance was used for group comparisons adjusted for the

confounding effects of the baseline measures of variables such as age, BMI, and physical activity. Moreover, the paired-sample *t* and the Wilcoxon signed-rank test were performed for within-group comparisons.

Results

Capsule count at the end of each 2-month course of the intervention revealed that compliance with the intervention was over 90% in all groups. Totally, 5 participants were excluded from the study groups, and finally, the data obtained from 85 participants (28, 30, 28, and 29 participants in the NS, CUR, NS + CUR, and placebo groups, respectively) were analyzed [Figure 1].

The total means of participants' age, BMI, lumbar spine BMD, and femoral neck BMD were respectively 57.79 ± 3.76 years, 28.18 ± 3.82 kg/m^2, 0.77 ± 0.09 g/cm^2, and 0.76 ± 0.09 g/cm^2 and the median (Minimum [Min], Maximum [Max]) of their total physical activity was 396 (50, 4765.5) MET-min/week. There were no significant differences between the four groups respecting participants' age, menopause age, gravidity, parity, durations of breastfeeding and menopause, weight, height, BMI,

Table 1: Group comparisons respecting participant's characteristics at baseline

Characteristics	Groups, mean ± SD or median (minimum-maximum)				*P*
	Placebo (*n* = 30)	NS (*n* = 30)	CUR (*n* = 30)	NS+CUR (*n* = 30)	
Age (years)	58.43 ± 3.41	57.31 ± 4.37	58.00 ± 3.50	57.43 ± 3.80	0.640[a]
Menopause age (years)	48.13 ± 3.73	47.89 ± 4.40	48.76 ± 3.64	48.60 ± 3.82	0.816[a]
Gravidity (*n*)	5.10 ± 2.11	4.03 ± 2.11	3.85 ± 1.93	4.96 ± 2.59	0.074[a]
Parity (*n*)	4.06 ± 1.43	3.37 ± 1.61	3.35 ± 1.72	4.17 ± 2.10	0.149[a]
Duration of breastfeeding (months)	69.10 ± 42.05	60.00 ± 33.57	59.42 ± 39.27	75.59 ± 46.96	0.409[a]
Duration of menopause (years)	10.21 ± 4.7	9.41 ± 4.5	9.23 ± 4.41	8.83 ± 4.6	0.770[a]
Weight (kg)	70.10 ± 10.10	67.47 ± 10.97	64.90 ± 8.94	65.63 ± 8.26	0.165[a]
Height (cm)	156.08 ± 5.42	155.48 ± 6.44	153.13 ± 6.95	147.87 ± 30.02	0.188[a]
BMI (kg/m^2)	28.77 ± 3.81	27.89 ± 4.07	27.73 ± 3.81	28.33 ± 3.68	0.723[a]
BMD (g/cm^2)					
Lumbar spine	0.76 ± 0.08	0.76 ± 0.09	0.76 ± 0.09	0.78 ± 0.09	0.887[a]
Femoral neck	0.77 ± 0.10	0.77 ± 0.08	0.75 ± 0.08	0.76 ± 0.11	0.840[a]
Total physical activity (MET-min/week)	359.25 (0.00-4426.50)	346.50 (132.00-4158.00)	792.00 (66.00-2559.00)	749.25 (99.00-4765.50)	0.012[b]

[a]The results of the one-way analysis of variance, [b]The results of the Kruskal-Wallis test. BMI: Body mass index, BMD: Bone mineral density, SD: Standard deviation, MET: Metabolic equivalent, CUR: Curcumin, NS: *Nigella sativa*

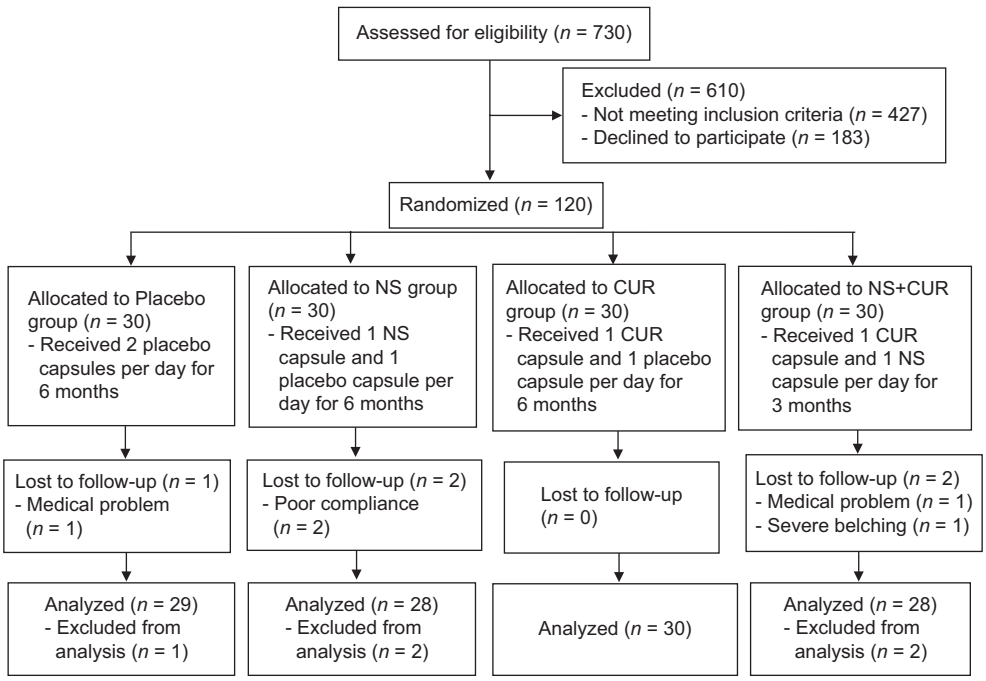

Figure 1: The flow diagram of the study

lumbar spine BMD, and femoral neck BMD ($P > 0.05$). However, the between-group difference respecting total physical activity was significant ($P = 0.012$) [Table 1].

At baseline, the medians (Min, Max) of energy, carbohydrate, and vitamin D intake were 1792.53 (1208.6, 3283.5) kcal/day, 245.96 (140.3, 631.1) g/day, and 1.60 (0.12, 5.14) µg and the means of protein, fat, and calcium intake were 53.70 ± 13.42, 64.48 ± 17.56, and 758.72 ± 205.85 g/day, respectively. Between-group and within-group differences respecting dietary intake were not significant ($P > 0.05$) [Table 2].

There were no significant differences between the groups respecting the baseline levels of oxidative stress biomarkers. The level of SOD significantly increased in

Table 2: Within - and among-group comparisons respecting pretest and posttest dietary intake

Dietary intakes	Time	Group, mean ± SD or meadian (minimum-maximum)				*P*
		Placebo (*n* = 29)	NS (*n* = 28)	CUR (*n* = 30)	NS+CUR (*n* = 28)	
Energy (kcal)	Pretest	1785.3 (1454.18-2454.90)	1844.4 (1436.39-2921.41)	1776.4 (1342.95-3483.49)	1790.9 (1423.83-3238.72)	0.420[a]
	Posttest	1823.8 (1300.18-3043.54)	1853.1 (1417.85-3433.19)	1855.8 (1229.26-3483.49)	1820.3 (1285.93-3020.72)	0.531[a]
	P[c]	0.319	0.679	0.251	0.493	-
Carbohydrate (g)	Pretest	245.9 (149.50-393.09)	243.2 (140.27-422.60)	241.4 (183.25-631.11)	251.2 (164.50-436.59)	0.992[a]
	Posttest	244.5 (163.37-398.56)	244.4 (182.27-422.60)	248.7 (167.90-431.63)	269.3 (146.46-436.59)	0.967[a]
	P[c]	0.858	0.308	0.959	0.674	-
Protein (g)	Pretest	52.8 ± 13.680	53.0 ± 12.3	53.4 ± 14.6	55.6 ± 13.5	0.835[b]
	Posttest	52.9 ± 15.1	53.5 ± 9.4	53.8 ± 17.3	55.3 ± 14.9	0.933[b]
	P[d]	0.838	0.804	0.810	0.798	-
Total fat (g)	Pretest	65.1 ± 17.0	65.6 ± 19.5	63.4 ± 18.3	63.8 ± 16.1	0.956[b]
	Posttest	65.5 ± 19.4	64.7 ± 16.4	64.1 ± 22.0	65.4 ± 19.9	0.991[b]
	P[d]	0.785	0.642	0.592	0.633	-
SFA (g)	Pretest	25.3 (10.26-50.41)	22.9 (9.01-50.92)	21.5 (11.31-55.64)	22.0 (11.78-49.61)	0.838[a]
	Posttest	24.8 (11.54-49.45)	24.2 (17.67-33.74)	22.7 (11.39-61.09)	25.8 (16.36-32.84)	0.822[a]
	P[c]	0.807	0.825	0.878	0.926	-
PUFA (g)	Pretest	13.4 (5.71-40.75)	14.1 (3.66-27.80)	12.0 (8.15-30.80)	13.5 (7.19-43.39)	0.913[a]
	Posttest	13.4 (6.36-28.84)	14.1 (3.76-25.80)	11.9 (5.74-33.51)	13.9 (6.88-24.59)	0.661[a]
	P[c]	0.501	0.556	0.794	0.979	-
MUFA (g)	Pretest	24.1 ± 7.2	24.6 ± 10.3	24.3 ± 8.3	24.6 ± 6.9	0.994[b]
	Posttest	24.3 ± 7.8	24.5 ± 7.9	24.6 ± 8.3	24.5 ± 7. 8	0.999[b]
	P[d]	0.535	0.939	0.861	0.748	-
Total fiber (g)	Pretest	30.9 ± 10.8	30.6 ± 10.4	30.5 ± 11.5	30.8 ± 8.2	0.998[b]
	Posttest	30.6 ± 6.5	30.4 ± 5.9	30.8 ± 6.2	30.2 ± 6.2	0.985[b]
	P[d]	0.789	0.866	0.805	0.620	-
Vitamin E (mg)	Pretest	11.7 ± 2.3	11.7 ± 2.3	11.7 ± 2.4	11.8 ± 2.4	0.994[b]
	Posttest	11.8 ± 2.0	11.9 ± 2.6	11.7 ± 2.8	11.81 ± 3.2	0.990[b]
	P[d]	0.674	0.582	0.973	0.885	-
Vitamin C (mg)	Pretest	155.2 (63.71-291.06)	157.9 (93.27-384.31)	162.6 (37.11-321.96)	160.6 (70.81-458.31)	0.982[a]
	Posttest	154.7 (95.05-294.06)	147.8 (94.96-406.26)	160.9 (27.10-321.96)	169.0 (43.67-461.47)	0.967[a]
	P[c]	0.433	0.733	0.831	0.958	-
Vitamin D (µg)	Pretest	1.4 (0.34-5.13)	1.6 (0.12-2.70)	1.6 (0.38-3.38)	1.6 (0.20-2.68)	0.566[a]
	Posttest	1.6 (0.90-3.86)	1.7 (0.12-3.18)	1.5 (0.38-4.88)	1.7 (0.37-2.97)	0.883[a]
	P[c]	0.697	0.732	0.999	0.911	-
Calcium (mg)	Pretest	758.4 ± 223.7	754.8 ± 206.3	761.6 ± 201.9	760.2 ± 201.2	0.999[b]
	Posttest	763.0 ± 186.39	759.1 ± 145.2	764.3 ± 260.0	767.6 ± 269.7	0.999[b]
	P[d]	0.908	0.909	0.940	0.881	-
Selenium (µg)	Pretest	70.5 (32.44-156.16)	72.3 (37.78-136.93)	64.4 (24.82-168.20)	69.4 (43.49-99.67)	0.856[a]
	Posttest	70.6 (34.34-156.16)	68.1 (47.74-122.56)	66.7 (49.18-147.70)	66.1 (39.51-97.83)	0.919[a]
	P[c]	0.929	0.648	0.245	0.336	-
Zinc (mg)	Pretest	7.2 ± 1.5	6.8 ± 1.6	6.9 ± 2.2	7.1 ± 2.1	0.895[b]
	Posttest	7.3 ± 2.2	6.8 ± 1.5	6.9 ± 1.9	7.0 ± 1.6	0.720[b]
	P[d]	0.681	0.994	0.977	0.728	-

[a]The results of the Kruskal-Wallis test, [b]The results of the one-way analysis of variance, [c]The results of the Wilcoxon signed-rank test, [d]The results of the paired-sample *t*-test. SFA: Saturated fatty acid, PUFA: Poly unsaturated fatty acid, MUFA: Mono unsaturated fatty acid, CUR: Curcumin, NS: *Nigella sativa*, SD: Standard deviation

the NS ($P = 0.004$) and the NS + CUR ($P = 0.048$) groups and the posttest level of SOD in the NS + CUR group was significantly more than the placebo group (adjusted mean difference = 100.39; 95% confidence interval = 21.87–178.90; $P = 0.013$). Moreover, TAC level in the NS + CUR group significantly increased ($P = 0.005$) and its posttest level in the NS + CUR group was significantly more than the placebo group (adjusted mean difference = 0.23; 95% confidence interval = 0.05–0.41; $P = 0.011$). The level of MDA did not significantly change in the study groups and no significant difference was observed among the groups respecting its posttest level ($P > 0.05$) [Table 3].

Table 3: Within - and among-group comparisons respecting pretest and posttest oxidative stress biomarkers

Biomarkers	Time	Groups (mean ± SD)				*P*
		Placebo (*n* = 29)	NS (*n* = 28)	CUR (*n* = 30)	NS + CUR (*n* = 28)	
SOD activity (U/mL)	Pretest	235.8 ± 61.4	231.9 ± 80.9	241.6 ± 69.9	236.36 ± 85.66	0.970[a]
	Posttest	232.2 ± 92.4	308.2 ± 106.5	278.8 ± 136.3	308.43 ± 159.16	0.082[b]
	$AMD_{pre\ to\ post}$ (95% CI)	−3.5 (−50.4-43.3)	76.3 (26.8-125.8)	37.2 (−23.2-97.5)	72.06 (0.80-143.32)	-
	$P_{pre\ to\ post}$[c]	0.878	0.004	0.217	0.048	-
	$AMD_{NS/P, P}$	-	62.9 (−12.6-138.4), 0.101[d]	-	-	-
	$AMD_{CUR/P, P}$	-	-	41.6 (−33.8-117.0), 0.276[d]	-	-
	$AMD_{NS + CUR/P, P}$	-	-	-	100.4 (21.9-178.9), 0.013d	-
	$AMD_{CUR/NS, P}$	-	21.3 (−57.9-100.5), 0.595[d]	-	-	-
	$AMD_{NS/NS + CUR, P}$	-	-	-	37.49 (−45.03-120.02), 0.369d	-
	$AMD_{CUR/NS + CUR, P}$	-	-	-	58.76 (−22.46-139.98), 0.154[d]	-
TAC (mmol/mL)	Pretest	1.48 ± 0.32	1.42 ± 0.47	1.42 ± 0.42	1.41 ± 0.48	0.924[a]
	Posttest	1.49 ± 0.29	1.61 ± 0.31	1.65 ± 0.30	1.72 ± 0.28	0.081[b]
	$AMD_{pre\ to\ post}$ (95% CI)	0.007 (−0.18-0.19)	0.18 (−0.03-0.40)	0.22 (−0.01-0.46)	0.30 (0.10-0.51)	-
	$P_{pre\ to\ post}$[c]	0.937	0.103	0.056	0.005	-
	$AMD_{NS/P, P}$	-	0.12 (−0.05-0.30), 0.160[d]	-	-	-
	$AMD_{CUR/P, P}$	-	-	0.13 (−0.04-0.31), 0.134[d]	-	-
	$AMD_{NS + CUR/P, P}$	-	-	-	0.23 (0.05-0.41), 0.011[d]	-
	$AMD_{NS/CUR, P}$	-	-	0.01 (−0.17-0.19), 0.915[d]	–	-
	$AMD_{NS/NS + CUR, P}$	-	-	-	0.11 (−0.07-0.29), 0.244[d]	-
	$AMD_{CUR/NS + CUR, P}$	-	-	-	0.10 (−0.08-0.28), 0.286[d]	-
MDA (nmol/mL)	Pretest	2.76 ± 0.85	2.72 ± 0.68	2.69 ± 1.44	2.71 ± 1.20	0.993[a]
	Posttest	2.72 ± 0.90	2.72 ± 0.75	2.40 ± 0.51	2.46 ± 0.61	0.369[b]
	$AMD_{pre\ to\ post}$ (95% CI)	−0.04 (−0.48-0.39)	0.00 (−0.42-0.42)	−0.29 (−0.90-0.32)	−0.25 (−0.85-0.34)	-
	$P_{pre\ to\ post}$[c]	0.835	0.997	0.341	0.383	-
	$AMD_{NS/P, P}$	-	−0.06 (−0.45-0.33), 0.755[d]	-	-	-
	$AMD_{CUR/P, P}$	-	-	−0.34 (−0.74-0.05), 0.093[d]	-	-
	$AMD_{NS + CUR/P, P}$	-	-	-	−0.16 (−0.57-0.25), 0.438[d]	-
	$AMD_{NS/CUR, P}$	-	-	−0.28 (−0.69-0.13), 0.184[d]	-	-
	$AMD_{NS/NS + CUR, P}$	-	-	-	−0.09 (−0.52-0.32), 0.648[d]	-
	$AMD_{NS + CUR/CUR, P}$	-	-	−0.18 (−0.60-0.24), 0.399[d]	-	-

[a]The results of the one-way analysis of variance, [b]The results of the analysis of covariance adjusted for BMI, age, energy intake and total physical activity, [c]The results of the paired-sample *t*-test, [d]The results of the *post hoc* Tukey's test. P_T: Total *P*. SOD: Superoxide dismutase, TAC: Total antioxidant capacity, MDA: Malondialdehyde, AMD: Adjusted mean difference, CI: Confidence interval, SD: Standard deviation, CUR: Curcumin, NS: *Nigella sativa*

Three participants from the NS group (10%), four participants from the CUR group (13%), seven participants from the NS + CUR group (23%), and five participants from the placebo group (17%) reported side effects such as mild nausea, vomiting, belching, headache, and unpleasant taste sensation in the 1st days of the study intervention.

Discussion

Findings revealed that NS supplementation significantly increased SOD level and NS + CUR supplementation significantly increased SOD and TAC levels but CUR supplementation had no significant effects on SOD and TAC levels and none of the interventions had significant effects on MDA level. These findings imply that NS is effective in significantly increasing SOD level and NS + CUR supplementation is effective in significantly increasing SOD and TAC levels but CUR supplementation has no significant effects on SOD and TAC levels.

Recent studies reported that CUR inhibits osteoclastogenesis and improves BMD and bone strength through increasing apoptosis and inhibiting osteoclast proliferation, inhibiting nuclear factor-kappa B (NF-κB) and activating receptor activator of nuclear factor kappa B ligand, reducing the production of nitric oxide and free radicals, and inhibiting the sysnthesis of inflammatory cytokines.[7,24] NS can also inhibit osteoclastogensis and prevent bone resorption through different mechanisms such as inhibiting cyclooxygenase and lipoxygenase that synthesize prostaglandins and leukotrienes from arachidonic acid and neutralizing free radicals that activate NF-κB and increase bone-resorbing cytokines including interleukin-1 and interlekin-6.[16]

The results of a study into the antioxidant effects of CUR indicated that the daily intake of 80 mg nanomicelle CUR for 10 weeks significantly increased TAC serum level and decreased MDA serum level in infertile men.[25] Another study showed that the daily intake of 1500 mg curcuminoid for 6 months significantly increased SOD level and decreased MDA level in patients with knee osteoporosis.[26] Similarly, a study found that the daily intake of 1000 mg curcuminoid and piperine for 8 weeks increased SOD and TACK serum levels and decreased MDA serum level in patients with type 2 diabetes mellitus.[27] However, our findings revealed the ineffectiveness of CUR in significantly increasing SOD and TAC levels and decreasing MDA level. This contradiction is attributable to differences among studies with respect to CUR dosage, type of CUR supplement, and their participants' health status and characteristics.

Study findings also indicated that NS significantly increased SOD level but had no significant effects on TAC and MDA levels. A previous study showed that the daily intake of 1000 mg NS oil for 8 weeks significantly decreased MDA serum level in women with rheumatoid arthritis.[18] Another study revealed that the daily intake of 2000 mg NS powder for 12 months significantly increased the serum levels of SOD and TAC in patients with type 2 diabetes mellitus.[28] Moreover, a study found that the intake of 2000 garlic pearl and 3000 mg NS powder for 8 weeks decreased MDA and increased SOD serum levels.[29]

One of the strengths of the study was the assessment of bone resorption in early menopause ages that helped provide a basis for timely prevention or management of menopausal osteoporosis and health promotion in older adulthood. Another strength of the study was the acceptably long duration of the intervention, i.e. 6 months. This study also had some limitations such as limited financial resources to select a large sample of women with osteoporosis that forced us to include women with osteopenia in addition to women with osteoporosis.

Conclusion

This study concludes that CUR has no significant effects on SOD, TAC, and MDA levels, NS can significantly increase SOD level but has no significant effects on TAC and MDA levels, and combined NS and CUR can significantly increase SOD and TAC levels and has no significant effect on MDA. Therefore, CUR is probably not effective in significantly reducing oxidative stress, while NS can relatively alleviate oxidative stress and NS + CUR can considerably alleviate oxidative stress in postmenopausal women with primary osteoporosis or osteopenia. Therefore, it is recommended that midwives take the supplemental role of these herbs into consideration in treatment and caring for postmenopausal osteoporosis, especially in dietary recommendations.

Acknowledgments

We would like to thank the Research Administration and the Aging Research Institute of Tabriz University of Medical Sciences, Tabriz, Iran, for their support.

Financial support and sponsorship

The Research Administration of Tabriz University of Medical Sciences, Tabriz, Iran, granted this study (grant number: 62693).

Conflict of interest

There are no conflicts of interest.

References

1. Vilaca T, Eastell R, Schini M. Osteoporosis in men. Lancet Diabetes Endocrinol 2022;10:273-83.

2. Nourmohammadi H, Abdan Z, Amin Hashemipour SM, Sarokhani D, Abdan M, Fakhri M, *et al.* Prevalence of osteoporosis and osteopenia in people over 60 years in Iran: A systematic review and meta-analysis. Int J Prev Med 2022;13:11.
3. Ahmadian F, Mozaffari-Khosravi H, Azaraein MH, Faraji R, Zavar-Reza J. The effect of consumption of garlic tablet on proteins oxidation biomarkers in postmenopausal osteoporotic women: A randomized clinical trial. Electron Physician 2017;9:5670-5.
4. Murray TÉ, Williams D, Lee MJ. Osteoporosis, obesity, and sarcopenia on abdominal CT: A review of epidemiology, diagnostic criteria, and management strategies for the reporting radiologist. Abdom Radiol (NY) 2017;42:2376-86.
5. Fahimfar N, Noorali S, Yousefi S, Gharibzadeh S, Shafiee G, Panahi N, *et al.* Prevalence of osteoporosis among the elderly population of Iran. Arch Osteoporos 2021;16:16.
6. Mirhashemi S, Kalantar MH, Mirhashemi AH, Mehrvarz S, Danial Z. Osteoporosis in Iran. Hosp Pract Res 2017;2:57.
7. Khanizadeh F, Rahmani A, Asadollahi K, Ahmadi MR. Combination therapy of curcumin and alendronate modulates bone turnover markers and enhances bone mineral density in postmenopausal women with osteoporosis. Arch Endocrinol Metab 2018;62:438-45.
8. Wang L, Ma R, Guo Y, Sun J, Liu H, Zhu R, *et al.* Antioxidant effect of fructus *Ligustri lucidi* aqueous extract in ovariectomized rats is mediated through Nox4-ROS-NF-κB pathway. Front Pharmacol 2017;8:266.
9. Wu Q, Zhong ZM, Pan Y, Zeng JH, Zheng S, Zhu SY, *et al.* Advanced oxidation protein products as a novel marker of oxidative stress in postmenopausal osteoporosis. Med Sci Monit 2015;21:2428-32.
10. Zhou Q, Zhu L, Zhang D, Li N, Li Q, Dai P, *et al.* Oxidative stress-related biomarkers in postmenopausal osteoporosis: A systematic review and meta-analyses. Dis Markers 2016;2016:1-12.
11. Rosen HN. Patient Education: Osteoporosis Prevention and Treatment (Beyond the Basics); 2021. Available from: https://www.uptodate.com/contents/osteoporosis-prevention-and-treatment-beyond-the-basics. [Last accessed on 2023 Feb 01].
12. Thummuri D, Jeengar MK, Shrivastava S, Nemani H, Ramavat RN, Chaudhari P, *et al.* Thymoquinone prevents RANKL-induced osteoclastogenesis activation and osteolysis in an *in vivo* model of inflammation by suppressing NF-KB and MAPK signalling. Pharmacol Res 2015;99:63-73.
13. Bukhari SN, Hussain F, Thu HE, Hussain Z. Synergistic effects of combined therapy of curcumin and fructus *Ligustri Lucidi* for treatment of osteoporosis: Cellular and molecular evidence of enhanced bone formation. J Integr Med 2019;17:38-45.
14. Habibi Ghahfarrokhi S, Reisi R. Effects of medicinal herbs on osteoporosis: A systematic review based on clinical trials. J Shahrekord Univ Med Sci 2019;21:229-36.
15. Wang T, Liu Q, Tjhioe W, Zhao J, Lu A, Zhang G, *et al.* Therapeutic potential and outlook of alternative medicine for osteoporosis. Curr Drug Targets 2017;18:1051-68.
16. Shuid AN, Mohamed N, Mohamed IN, Othman F, Suhaimi F, Mohd Ramli ES, *et al. Nigella sativa*: A potential antiosteoporotic agent. Evid Based Complement Alternat Med 2012;2012:696230.
17. Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H. Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. J Pharmacopuncture 2017;20:179-93.
18. Hadi V, Kheirouri S, Alizadeh M, Khabbazi A, Hosseini H. Effects of *Nigella sativa* oil extract on inflammatory cytokine response and oxidative stress status in patients with rheumatoid arthritis: A randomized, double-blind, placebo-controlled clinical trial. Avicenna J Phytomed 2016;6:34-43.
19. Cho DC, Ryu K, Kim KT, Sung JK. The therapeutic effects of combination therapy with curcumin and alendronate on spine fusion surgery in the ovariectomized rats. Korean J Spine 2017;14:35-40.
20. Sundar Dhilip Kumar S, Houreld NN, Abrahamse H. Therapeutic potential and recent advances of curcumin in the treatment of aging-associated diseases. Molecules 2018;23:835.
21. Hemmati E, Mirghafourvand M, Mobasseri M, Shakouri SK, Mikaeli P, Farshbaf-Khalili A. Prevalence of primary osteoporosis and low bone mass in postmenopausal women and related risk factors. J Educ Health Promot 2021;10:204.
22. Bayat N, Haji Amini Z, Ali Shiri GH, Ebadi A, Hosseini M, Lalouei A. Frequency of osteoporosis and osteopenia in post-menopausal military family's women. Ann Mil Health Sci Res 2008;6:25-30.
23. Mohamed EA, Mohamed MM. Evaluation of antidiabetic and insulintropic potential of *Nigella sativa* seeds water extract and/or Alpha Lipoic Acid in type 2 diabetic rats. Indian J Med Res Pharm 2015;2:26-35.
24. Hatefi M, Ahmadi MR, Rahmani A, Dastjerdi MM, Asadollahi K. Effects of curcumin on bone loss and biochemical markers of bone turnover in patients with spinal cord injury. World Neurosurg 2018;114:e785-91.
25. Alizadeh F, Javadi M, Karami AA, Gholaminejad F, Kavianpour M, Haghighian HK. Curcumin nanomicelle improves semen parameters, oxidative stress, inflammatory biomarkers, and reproductive hormones in infertile men: A randomized clinical trial. Phytother Res 2018;32:514-21.
26. Panahi Y, Alishiri GH, Parvin S, Sahebkar A. Mitigation of systemic oxidative stress by curcuminoids in osteoarthritis: Results of a randomized controlled trial. J Diet Suppl 2016;13:209-20.
27. Panahi Y, Khalili N, Sahebi E, Namazi S, Karimian MS, Majeed M, *et al.* Antioxidant effects of curcuminoids in patients with type 2 diabetes mellitus: A randomized controlled trial. Inflammopharmacology 2017;25:25-31.
28. Kaatabi H, Bamosa AO, Badar A, Al-Elq A, Abou-Hozaifa B, Lebda F, *et al. Nigella sativa* improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: Placebo controlled participant blinded clinical trial. PLoS One 2015;10:e0113486.
29. Mostafa RM, Moustafa YM, Mirghani Z, AlKusayer GM, Moustafa KM. Antioxidant effect of garlic (*Allium sativum*) and black seeds (*Nigella sativa*) in healthy postmenopausal women. SAGE Open Med 2013;1:1-6.