Impact of Black seed (*Nigella sativa*) extract on bone turnover markers in postmenopausal women with osteoporosis

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ABSTRACT

Background and the purpose of the study: Experimental studies have shown that Ns (Nigella sativa) seeds oil can increase bone formation and may have anabolic effects on bone loss. This study was conducted to investigate the beneficial impacts of the oil of Black seeds on bone turnover in osteoporotic postmenopausal women.

Materials and methods: A placebo controlled pilot study was carried out on 15 postmenopausal osteoporotic women of 48-74 years old. In addition to Calcium-D supplements (2 tablets per day) all participants were randomly received Ns extract (3ml, 0.05 ml/kg/day p .o.) or placebo for 3 months. In all subjects hematological tests were performed and hepatic enzymes, BUN, Cr, Ca, P and plasma bone formation and resorption markers including osteocalcin, bone alkaline phosphatase (Bone-ALP) and carboxy terminal cross linked telopeptide (CTX) was determined before and after 12 weeks of treatment.

Results: Twelve participants completed the entire 12 weeks study course of which 5 and 7 women were belonged to Ns and placebo groups respectively. Women in placebo group were significantly older than women in Ns group. There were not significant differences between BMIs, BMD results and plasma levels of bone marker in two groups at the baseline and plasma levels of bone markers between Ns and placebo group at the end of 12 weeks. Alterations from baseline in bone markers levels did not differ significantly between two groups. We did not observe any side effects due to Ns therapy.

Conclusion: In this pilot study similar to the previous trial, we failed to show beneficial impact of Ns extract administration for a short time on bone turnover so we don't suggest it for medicinal application in the osteoporosis condition. Long time duration studies with larger sample size and usage of a more tolerable dosage forms of Black seeds oil should be emphasized for further clarification of its useful anabolic effects on bone metabolism.

Keywords: Black seed (Nigella sativa), Bone markers, Osteoporosis

INTRODUCTION

Osteoporosis is an important public health problem in the world and is the most prevalent skeletal disease that has affected approximately 6 percent of women aged greater than 50 years old in Iran (1- 3).

Recently a number of medications have been approved for the treatment of this condition, which act by various mechanisms, essentially by antiresorptive action (1, 4, 5).

In the previous study, we failed to demonstrate beneficial effects of Nigella sativa (Ns) seeds extract on bone metabolism by monitoring biochemical bone markers in osteopenic postmenopausal women (6).

Black seeds extract has been shown for many years to be safe, having therapeutic properties in different medical conditions including respiratory, digestive and rheumatologic diseases (7-9). There is a belief in Islamic Arabic countries that black seed is a universal healer that treats all illnesses but is not capable to protect people against aging or death (10).

From an animal model study in Iran it was shown that Black seed essential oil in both local and systemic applications had anti-inflammatory properties and a strong pain relieving effect (11).

Results of another study has shown that the anti-inflammatory effect of Ns oil may results from suppression of inflammatory mediators such as prostaglandins and leukotrines (7). Biochemical markers of bone turnover are validated means to assess the patient response for a therapeutic medication after short course duration of therapy (12).

Recently two experimental animal model studies have exhibited that Ns seeds oil and its active ingredients mainly Thymoquinone (TMQ), can increase bone

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formation and may express helpful effects in diabetic osteopenia (13, 14).

Regarding the high prevalence of osteoporosis in Iran and other geographic regions and its important debilitating nature and with respect to the safety of Ns seeds extract and its beneficial effects (15), this study was designed to investigate the probable therapeutic effects of black seeds oil on bone loss in osteoporotic postmenopausal women by assessment of the plasma levels of biochemical bone markers.

SUBJECTS AND METHODS

A single- blind, placebo controlled, pilot study was carried out on 15 postmenopausal osteoporotic women (according to the WHO classification system, osteoporosis is defined as *T*-scores lower than −2.5 in any of the lumbar spine or hip areas) aged 48-74 years old (16). The patients were selected from the Bone densitometry center of Shariati hospital. BMD of the lumbar spine and total hip was evaluated by dual energy X-ray absorptiometry (DEXA) using a Lunar (DPX) densitometer (Lunar 7164, GE, Madison, WI, USA). Precision error for BMD measurements was 1–1.5% and 2–2.5% in the lumbar spine and femoral (Total hip) areas respectively.

Exclusion criteria were history of the bone fracture within the last three years, history of active or chronic hepatic, renal or cardiac disorders (including myocardial infarction), uncontrolled hypertension, gastrointestinal disorders (such as Crohn's disease, ulcerative colitis, celiac disease, chronic diarrhea, active gasteroduodenal ulcer that is under treatment and/or with a previous history of gasterointestinal bleeding, malignancies, hyper- or hypo- thyroidism, hypocalcaemia, osteomalacia, rheumatoid arthritis and taking any medications that influence bone metabolism (thiazides, antiepileptic agents, steroidal agents, calcitonin and bisphosphonates).

After explanation of study protocol to participants, written informed consent was obtained and all of subjects filled questionaire forms. Medical history was taken and physical examination was performed.

The Black seeds (*Nigella sativa* seeds) were obtained from an herbal store in Esfahan city of Iran. The extraction process was as follows: 30 grams of the powdered Ns seeds was suspended in 250 ml of n- hexane and extracted by suxhelat apparatus, then filtered and concentrated under reduced pressure (at 50˚C) to give 5.3 ml of the oily extract. The extract was added to 100 ml distilled water and its essence was obtained by Clevenger- type apparatus. The oil was dried over anhydrous sodim sulphate and kept in brown vials at 4˚C.

GC-MS analyses were performed on an Agilent Technologies 6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP1-MS capillary fused silica column (30 m; 0.25 mm I.D.; 0.25 μm film thicknesses, methyl polysiloxane). The temperature program initiated at 40 ºC, for 1 minute then raised at 3 ºC min^{-1} to 250 ºC, and held for 20 minutes. Other operating conditions were as follows: carrier gas, helium (99.999%); flow rate, 1 $ml.min^{-1}$; injector temperature, 250 ºC; split ratio, 1:50. Mass spectra were taken at 70 eV. Mass range was 20–500 amu. An Enhanced ChemStation G1701 DA version D.00.01.27 was used for collection of data and processing.

The suitable dose for Ns seeds extract in human was estimated based on its active component (Thymoquinone) amount and considering the results of studies on animal models (for assessment of effective doses of TMQ in rats for the remedy of rheumatoid arthritis which were 2.5 mg/kg/day po and 5 mg/kg/day po respectively) (17).

Using NOVEL (No observed adverse effect level), HED (human equivalent dose) containing approximately 0.6 mg/kg/day po of Thymoquinone was calculated (18).

Therefore, 0.05 ml/kg/day po of Ns extract (equivalent to 0.6 mg/kg/day po of Thymoquinon) for women in Ns gronp was administered in the study.

Before beginning the study protocol, ethics committee of endocrinology and metabolism research center of Shariati hospital evaluated ethical views of this study and approved it.

Fifteen osteoporotic women aged 48-74 years were randomized to 3- months Ns (oil) treatment (3ml, 0.05 ml/kg/day p.o.) or placebo. All participants received calcium-D supplements (2 tablets per day) throughout the study for 3 months period. In all subjects after an overnight fasting venous blood samples were obtained in the morning between 8.00 and 11 AM. CBC diff, liver function test (ALT, AST, ALP), kidney function test (BUN, Cr), serum levels of calcium and phosphorus and plasma levels of bone markers including carboxy terminal cross linked telopeptide (CTX), osteocalcin and bone alkaline phosphatase (Bone-ALP) were measured at the baseline (before treatment) and after 3 months of administration. All biochemical tests (BUN, Cr, Ca, P, ALT, AST and ALP) employed calorimetric assay (Pars Azemoon kits). The intra-assay and inter-assay coefficients of variance (CV) were less than 3% for BUN, Cr, ALT and AST levels and less than 2% for Ca, P and ALP levels respectively. Bone markers assessment were performed by ELISA method and with commercial kits that were obtained from IDS company of Germany (CV<10%). Skin and mucosal examination was performed in all participants at the end of the study and any observed side effects of Ns were recorded during the course of the study.

All data are shown as mean ± standard deviation (SD) between two study groups at the baseline and after 3 months of administration. Initially all parameters were tested for normality of distribution by Kolmogorov-Smirnov and Shapiro-Wilk tests. Then all parameters with normal distribution were analysed for equality of means by independent

t- tests and non parametric Mann - Whitney U test was performed for comparison the differences of parameters that did not have normal distribution (including osteocalcin and creatinine) between two study groups. Chi-square and Fisher exact tests were also performed to compare the percent of participants in each group that were taking calcium and /or vitamin D supplements before beginning of the study. Paired t-test was applied to compare changes in the bone markers levels and other parameters at the baseline (before intervention) and after 3 months of study period in both study groups. Finally resulting changes in bone markers levels during the study course (differences between weeks of 0 and 12) were compared between Ns and placebo groups using Willcoxon signed Ranks and T-tests. Data were analyzed by Statistical Package for Social Science (SPSS version 11.5). Differences were considered significant at the p value <0.05.

RESULTS

The main constituents of Ns essence were o-cymene (30.5%), thymoquinone (22%) and α- thujene (14.7%).

Twelve participants completed the 3 months study period (5 in the Ns and 7 in the placebo group) and 3 women discontinued the study (one woman was excluded because of hypercalcemia and hypercalciuria that further evaluations revealed the diagnosis of hyperparathyroidism and two women withdrew because of poor compliance).None of the subjects stopped the study because of adverse drug reactions. Women in placebo group were significantly older than women in Ns group (p= 0.026).There weren't significant difference between BMIs in Ns and placebo group (Table 1).

Other demographic characteristics and bone markers of Ns and placebo group have been illustrated in table 1. At the baseline (before beginning of study), plasma levels of bone marker were higher in placebo group but there were not statistically significant differences between Ns and placebo groups (Table 1).

BMD results (including T- Score values of lumbar spine and total hip) were also similar between Ns and placebo groups at the baseline (Table 1).

Hematological and biochemical tests did not differ significantly between Ns and placebo groups at the baseline except for ALP that was significantly higher in placebo group (p =0.001) (Table 2).

There was not any significant difference in plasma levels of bone markers including CTX, Osteocalcin and Bone ALP between Ns and placebo group at the end of 3 months (Table 3).

Also alterations in bone markers levels during the study period were not significantly different between two groups (Table 4).

Hematological and biochemical tests did not change with Ns treatment except for decrease in ALT level at the end of 3 months study course (Table 5). In placebo group, Hb and HCT levels decreased significantly from baseline values at the end of third months. There was any side effect due to Ns therapy in this study.

DISCUSSION

In this study, Ns treatment for a short time had no significant effect on the plasma levels of bone turnover markers and changes of this marker levels was not different from the placebo treatment. In the recent study, we also failed to show anabolic effects of Ns seeds extract on bone turnover in postmenopausal osteopenic women using bone markers assessment (6).

However in an experimental study in Streptozotocin-induced diabetic rats Ns treatment singly or in combination with human parathyroid hormone (hPTH) after 1 month, significantly prevented bone loss resulting from diabetic state and combination therapy led to good results. By applying histomorphometric measures it was demonstrated that administration of both Ns and hPTH excerted more anabolic effects on bone than treatment with individual agents by increasing the rate of bone formation and improving biomechanical bone strength. It was pointed out that diabetes is associated with bone loss as a consequence of decrease in bone formation and possibly increase in bone resorption (14).

In another study it was exhibited that sustained release of TMQ (active ingredient of black seed), in male rats that were affected with femoral defect can increase bone healing after 1 month, and did not have side effects on vital organ and gonadal tissues (13). In the present as well as the previous study in osteopenic postmenopausal women it was not observed any side effects due to Ns treatment which confirmed its good safety profile (6). Zaoui et al investigated the acute and chronic toxicity of Ns fixed oil in mice and rats by calculation of LD_{50} values and monitoring biochemical, hematological and histopathological tests. They concluded that because of high LD_{50} levels, there were not changes in liver enzymes and any organ damages, Ns oil in the therapeutic amounts have a wide range of safety but attention should be paid to the rise in hematocrit and hemoglobin levels and decrease in white blood cells and platelet counts (15).

Precautions of Ns seed extract administration are pregnancy and youth periods of life and also diabetes condition (19) and due to its well known hypoglycemic properties diabetic patients should consult with a physician before taking it (14, 20).

Menopausal state is accompanied with acceleration of bone resorption and disrupted bone formation that are arising from estrogen withdrawal. Aside from estrogen deficiency other contributing factors are related to ageing including decrease in paracrine growth factors production, and low blood concentrations of growth hormone and insulin like

Table 1. Demographic characteristics, bone markers, bone mineral densities of subjects at the baseline

	NS (n= 5) Mean ± SD	placebo (n = 7) Mean ± SD	p-value
Age (years)	55.0 ± 6.2	64. 5 ± 6.3	0.026
Height(cm)	161.2 ± 4.7	155.0± 4.2	0.040
Weight (kg)	60.0 ± 14.2	62.4±9.5	0.0729
BMI (kg/m^2)	23.0±5.0	25.8±2.8	0.233
% use of calcium supplement before study	40	28.6	1.000
Bone markers			
Bone – ALP (µg/L)	13.42±4.11	20.38±7.19	0.082
Osteocalcin (ng/ml)	16.48±3.15	25.64±14.21	0.106
CTX (ng/L)	0.64±0.33	0.81±0.54	0.524
Bone mineral density			
T – Score (Lumbar spine)	–2.26±1.32	–2.15±0.69	0.863
T – Score (Total hip)	–2.94±0.51	–2.95±0.88	0.970

Table 2. Hematological and biochemical parameters of subjects at the baseline

	Ns (n = 5) Mean ± SD	Placebo (n = 7) Mean ± SD	p -value
Hematology			
WBC(Cu.mm)	5240 ± 1297	6342 ± 2532	0.395
Hb (g/dl)	13.38 ± 1.18	14.12 ± 1.07	0.280
Hct (%)	41.10 ± 3.43	44.65 ± 3.47	0.110
Plt (Cu.mm)	187200± 56565	256571 ± 60527	0.072
Biochemical tests			
Ca (Mg/dl)	9.74 ± 0.38	9.81 ± 0.39	0.753
P (Mg/dl)	3.90 ± 0.15	3.98± 0.69	0.794
BUN (Mg/dl)	14.70 ± 4.66	16.21 ± 6.71	0.675
Cr (Mg/dl)	0.94 ± 0.08	1.10 ± 0.29	0.272
AST (IU/L)	18.40 ± 8.64	24.42 ± 3.30	0.119
ALT (IU/L)	17.40 ± 5.85	17.00 ± 7.72	0.925
ALP (IU/L)	142.00 ± 24.92	227.42 ± 38.34	0.001

Table 3. Plasma levels of bone markers at baseline and week 12 in Ns and Placebo treated subjects (comparison within groups)

	Ns (n =5)			Placebo (n = 7)		
	Base line	after 12 weeks	*p*-value	Base line	after 12 weeks	*p* -value
BoneALP(µg/L)	13.42 ± 4.11	12.80 ± 4.53	0.772	20.38 ± 7.19	17.62 ± 6.07	0.230
CTX (ng/L)	0.64± 0.33	0.60± 0.29	0.789	0.81 ± 0.54	0.74 ± 0.35	0.630
Osteocalcin (ng/ml)	16.48 ± 3.15	16.00 ± 3.68	0.677	25.64 ± 14.21	27.00 ± 13.66	0.335

growth factor 1 (IGF1) (21) .

Negative results of this study in exhibiting of anabolic effects of Ns extract on bone metabolism may be related to the different age group (women in placebo group had significantly higher age than women in Ns group), short duration of Ns therapy, inadequate sample size or seasonal factors (participants in this study we enrolled in different seasons of year from winter to autumn). The

Table 4. Changes in bone markers between groups over the course of the study (differences between 012- weeks).

	Ns (n = 5) Mean ± SD	Placebo (n = 7) Mean ± SD	*p*-value
Diff Bone- ALP(µg/L)	-0.62 ± 4.46	-2.75 ± 5.45	0.489
Diff Osteocalcin (ng/ml)	-0.48 ± 2.39	1.35 ± 3.42	0.329
Diff CTX (ng/L)	-0.40 ± 0.31	-0.71 ± 0.37	0.881

Table 5. Hematologic and biochemistry parameters of subjects at baseline and Week 12 in Ns treated and Placebo treated group.

	Ns(n=5)			Placebo (n=7)		
	Baseline	Week 12	*p* -value	Baseline	Week 12	*p* -value
WBC (Cu.mm)	5240 ± 1297	6030 ± 1548	0.269	6342 ± 2523	6671 ± 994	0.683
Hb (Gr/dl)	13.38 ± 1.18	13.16 ± 1.15	0.276	14.12 ± 1.07	13.27 ± 0.87	0.013
Hct (%)	41.10 ± 3.43	39.58 ± 3.35	0.112	44.65 ± 3.42	41.00 ± 2.46	0.001
Plt (Cu.mm)	187200 ± 56567	176000 ± 92614	0.829	256571 ± 60527	257142 ± 43113	0.979
Ca (mg/dl)	9.74 ± 0.38	9.70 ± 0.32	0.688	9.81 ± 0.39	9.81 ± 0.45	1.000
P (mg/dl)	3.90 ± 0.15	3.82 ± 0.40	0.729	3.98 ± 0.69	3.88 ± 0.36	0.744
BUN (mg/dl)	17.40 ± 4.66	12.32 ± 4.47	0.229	16.21 ± 6.71	16.52 ± 6.91	0.859
Cr (mg/dl)	0.94 ± 0.89	0.98 ± 0.13	0.374	1.10 ± 0.29	1.05 ± 0.26	0.200
AST (IU/L)	18.40 ± 8.64	22.60 ± 4.33	0.129	24.42 ± 3.30	24.57 ± 3.35	0.881
ALT (IU/L)	17.40 ± 5.85	10.10 ± 5.77	0.001	17.00 ± 7.72	13.85 ± 4.45	0.170
ALP (IU/L)	142.00 ± 24.92	150.20 ± 19.86	0.400	227.42 ± 38.34	206.14 ± 43.96	0.069

Values are mean and standard deviation

limitations of this study were small sample size, short duration and unfavorable greasy taste of Ns extract and as a consequence low compliance of some patients in completing the three months study period.

CONCLUSION

In summary, based on the results of this study Ns extract therapy for osteoporotic women is not recommended. However these data will not suffice to exclude the beneficial effects of Ns on bone turnover reliably. Future long term studies with larger sample size and by applying more tolerable forms of Ns seeds extract such as tablets, capsules or oral vials for improvement of patients' compliance seem necessary to clarify the anabolic effects of Ns seed extract on bone metabolism.

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