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The effect of *Nigella sativa* on serum levels of insulin-like growth factor and its binding proteins in postmenopausal women with low bone density: A triple-blind randomized controlled trial

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Animal studies have shown that *Nigella sativa* (*NS*) seed oil can increase Insulin-like growth factor (IGF-1) serum levels. This study aimed to investigate the effect of oral capsule *NS* on serum levels of IGF-1 and its binding proteins (IGFBP-1, IGFBP-3) in postmenopausal women with bone loss density. Sixty postmenopausal women of 50 to 65 years with bone loss density randomly received a soft capsule of *NS* oil 1000 mg or placebo once daily for six months with a 1:1 allocation ratio. DEXA method was used to measure bone density. Serum concentrations of IGF-1, IGFBP-1 and-3, ALT, AST, ALP, Cr, and urea were measured at baseline and after the intervention. There were no significant differences in serum levels of IGF-1, IGFBP-1, urea, Cr, ALT, AST, and ALP between the two groups at the end of six months. However, a significant increase has been shown in IGFBP-3 between groups after the intervention (Adjusted mean difference: 95% CI: 1.65: 0.36 to 2.97; p=0.013). We observed a significant increase in IGFBP-3 serum levels without any side effects. Additional research with an increased number of participants may be needed for further clarification of its beneficial anabolic effects on the GH system.

Keywords: IGF-1, IGFBPs, Menopause, *Nigella sativa*, Osteopenia, Osteoporosis

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Osteoporosis is a systemic skeletal disorder characterized by decreased bone strength and increased bone fracture risk[1]. The World Health Organization (WHO) adds to the above definition and defines osteoporosis as a bone mineral density (BMD) that lies 2.5 standard deviations or more below the average value for adults[2]. Osteoporosis, which is due to estrogen deficiency or the normal aging process, is called primary osteoporosis[3]. Idiopathic osteoporosis in younger people can be more due to reduced bone formation than increased bone resorption. Impaired growth hormone (GH) or insulin-like growth factor-1 (IGF-1) may contribute to its pathogenesis[4].

IGF-1 is a polypeptide hormone that contributes to cell differentiation, proliferation, and death. Synthesis of circulating IGF1 takes place mainly in the liver, and osteoblasts play a part in IGF-1 production[5]. IGF-1 has potentially stimulating effects on the synthesis of bone protein and proliferation of osteoblasts in *in-vitro* cells and organ culture[6]. It has been shown that IGF-1 content in the cortical part of the bone decreases with age, which may be associated with a higher prevalence of osteoporosis in the elderly[7]. The activity of the hypothalamic–GH–IGF1 axis declines with age and some catabolic changes in old age such as decreased bone mass and muscle atrophy are attributed to the reduction in this GH[8].

IGF binding proteins (IGFBPs) comprise a group of secreted proteins that act as carrier proteins for insulin-like growth factors (IGFs) with a high binding affinity that regulates their bioavailability and performance. They consist of six IGFBPs named IGFBP1-IGFBP6[9]. Proteins exhibiting diminished binding affinity were erroneously designated as IGFBP7, IGFBP8, IGFBP9, etc. As a consequence of their conserved protein structure and elevated binding affinity to IGFs, exclusively IGFBP1- IGFBP6 are regarded as authentic IGFBPs[10]. The major part of IGF-1 is bound to IGFBP3, which is quantitatively the dominant circulating IGFBP. Serum IGFBP3 levels

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are assumed to be regulated by GH or IGF-1. It is suggested that IGFBP3 may potentiate the effect of IGF1 on bones[11]. In addition, IGF-1 probably contributes to diabetes-induced metabolic abnormalities and disorders[12]. In individuals afflicted with diabetes mellitus, osteoporosis represents the main metabolic disorder of the skeletal system[13]. Insulin and IGF-1 may contribute to the pathophysiology of bone loss in diabetes, owing to their anabolic effects[14].

The chemical structures of the essential oil and oil extracted from fennel flower (*Nigella sativa* (*NS*) which belongs to the Ranunculaceae family. The main constituents of *NS* oil include linoleic (55.6%), oleic (23.4%), and palmitic acids (12.5%)[15]. *NS* seeds have traditionally been utilized for the treatment of diverse ailments[16]. Animal research has illustrated the impact of *NS* in the treatment of postmenopausal osteoporosis, osteoporosis caused by diabetes, and fracture healing[17]. It was reported in a study that the application of *NS* seed oil resulted in enhancements to the structural and physiological characteristics of the femur in diabetic male rats to the same level as those treated with parathyroid hormone[18]. Another histological evaluation showed that using *NS* improves diabetes-induced bone changes in rats[19]. The aforementioned findings indicate that *NS* possesses the capacity to be employed in the management of osteoporosis. Its mechanism of action in osteoporosis may be related to the osteogenic effects of its active constituent thymoquinone[20]. It was postulated that the potential of thymoquinone to exert an antioxidative and anti-inflammatory impact might have implications in the management of osteoporosis because this bone disorder is associated with oxidative and inflammation[18]. However, the results of a study conducted on 30 healthy men to investigate the effect of *NS* powder (1 mg/day) on insulin secretion and lipid profile did not show any modification in fasting blood sugar and lowering lipid concentration. Probably too low a dose and a small sample size were not able to detect the effect of *NS* on secreting insulin and lowering lipid concentration[21].

The risk of osteoporosis increases with age. The prevalence of primary osteoporosis among Iranian postmenopausal women was estimated at 24.5% in 2019[22]. The negative consequences of osteoporosis are anticipated to escalate considerably in the forthcoming years due to the rising life expectancy and aging population. Therefore, there is a compelling need for osteoporosis treatment to 1) make a balance between bone formation and bone resorption 2) enhance bone strength 3) impose no side effects or minimum side effects among the various treatments 4) be affordable[23]. It is, therefore, necessary to take a closer look at herbal agents to ameliorate age- and menopause-induced reduction of bone density and conduct relevant studies on their mechanisms of action. No study has been conducted on the effect of *NS* oil on serum levels of IGF-1 and IGFBPs in osteoporosis, and the conducted research has been on the effect of *NS* on IGF-1 in diseases other than osteoporosis in rats. The present study was conducted to investigate the effect of oral *NS* capsules on serum concentrations of IGF-1 and proteins binding to it (IGFBP1, IGFBP3) compared to the placebo in postmenopausal women with primary bone loss.

Material and Methods

This study was reported following the CONSORT 2010 Statement.

Study design

This study was conducted as a component of a large-scale project that received approval from Tabriz University of Medical Sciences. The project, titled "Assessment of primary osteoporosis status and the effect of three interventions of Curcumin, *NS*, and combined Curcumin-*NS* on cellular-molecular and clinical outcomes in postmenopausal women of Tabriz". The study was conducted between April 2019 and November 2019. To ensure ethical considerations, the trial received approval from the Ethics Committee of Tabriz University of Medical Sciences (IR TBZMED.REC.1398.901) and was registered with the Iranian Registry of Clinical Trials on 16/07/2018 (www.irct.ir/IRCT20131009014957N4). All methods employed in this study adhered to relevant guidelines and regulations.

Participants

The trial participants included 60 postmenopausal women with primary osteoporosis and osteopenia aged 50 to 65 and were specifically selected from healthcare facilities located in Tabriz, Iran.

Inclusion criteria

1- Cessation of menstruation for a minimum duration of twelve consecutive months 2- Low bone density (T-score≤-2.5) in the femoral or neck lumbar spine 3- Having the ability to take care of oneself 4- Living in Tabriz city 5- The ability to make verbal

communication to the extent of responding to questions.

Exclusion criteria

1- A T-score equal to or less than -4 in the lumbar spine or a T-score equal to or less than -3.5 in the femoral neck. 2- A history encompassing instances of pathologic fractures. 3- The existence of secondary osteoporosis or bone disorders other than osteoporosis. 4- Untimely onset of menopause. 5- The utilization of medications that influence bone metabolism, such as intravenous bisphosphonate in the last five years, oral bisphosphonate in the preceding six months, oral bisphosphonate for over three years or over one month within a six to twelve-month timeframe prior to the study, parathyroid hormone or strontium analogs in the previous twelve months, and hormonal drugs or corticosteroids. 6- Having diseases such as renal failure, mental illness as self-reported, malignancy as self-reported, peptic ulcer and gallstones 7- 25-(OH) vitamin D serum level less than 20 ng/mL 8- Suffering from existing hypercalcemia or hypocalcemia, disorders related to blood clotting. 9- Utilizing anticoagulant medications. 10- Previous instances of allergic reactions towards the recommended dietary supplements.

All participants received 70 mg alendronate tablets once a week and calcium-vitamin D supplements (containing 500 mg of calcium+400 IU of vitamin D) daily as the standard treatment protocol. At baseline, demographic characteristics (including age, menopause age, gravidity, and parity), anthropometric data (including weight, height, and BMI), physical activity, and bone mineral density were collected. Also, a table of dietary intake, calcium, and vitamin D contents of different foodstuffs along with additional explanations was provided to all of the participants at baseline and after the intervention. Moreover, biochemical measurements (including IGF-1, IGFBP-1, IGFBP-3, urea, creatinine, AST, ALT, and ALP) were conducted before and after the intervention). Finally, some side effects reported in each group were mentioned.

The determination of the sample size was conducted through the utilization of G-Power software (version 3.1.2), which was based on a previous study[24]. The parameters taken into consideration were m1=0.92, representing the mean lumbar spine BMD, and a default 20% increase denoted as m2=0.104. Additionally, sd1=sd2=0.178, α= 0.05, 95% confidence interval, and a power of 95% were applied in the calculation. Consequently, the sample size was determined to be 26 individuals in each group. In order to account for a dropout rate of 15%, a total of 30 individuals were eventually assigned to each group.

After obtaining written informed consent from all participants, the participants were allocated into two groups through the utilization of the block randomization technique, employing a block size of 4 and 8. The random allocation software (RAS) was employed for this purpose, with an allocation ratio of 1:1. The intervention lasted for a period of six months, during which the *NS* group was administered a daily dosage of one capsule containing 1000 mg of *NS* oil., and the placebo group also received one placebo capsule daily.

The intervention duration was divided into three periods of two months. Three sealed envelopes, which were not transparent, were included in the package. Each envelope contained two boxes of 60 capsules (intervention or placebo) to be used for two months. At the beginning of the study, each participant was furnished with one of the envelopes, accompanied by a checklist for documenting the utilization of the supplement. To evaluate adherence to the supplementation regimen, participants were instructed to visit the healthcare facilities every two months and provide a checklist outlining their supplement consumption over the preceding two-month period, whether the boxes were empty or full. Consequently, the estimation of compliance with supplementation was determined by tallying the number of remaining capsules. Upon the culmination of each period, after observing the participants' utilization of the supplements and receiving the checklist documenting their two-month consumption, they were issued a fresh envelope and checklist, and this procedure persisted until the conclusion of the sixth month. Moreover, the investigator monitored the status of supplement consumption monthly by telephone. Participants were instructed to persist with their habitual dietary practices and exercise routines and report any adverse reactions associated with the consumption of supplements to the researcher.

The act of producing a random allocation sequence and hiding the allocation was carried out by an individual who was not involved in the research. In addition, the researchers, subjects, clinical and laboratory personnel, and even statistical specialists maintained blindness until the completion of data analysis to guarantee the precision of randomization, allocation, and intervention.

Primary outcomes included serum levels of IGF-1 and its binding proteins (IGFBP-1, IGFBP-3) and secondary outcomes included serum levels of urea, creatinine, ALT, AST, and ALP.

Characteristics of supplements

The production of *NS* oil capsules was carried out by Barij Essence Pharmaceutical Company, located in Kashan, Iran. As indicated by the manufacturer, each individual capsule was composed of a minimum of 6.5 mg of thymoquinone in addition to a range of 495 to 605 mg of linoleic acid. *NS* placebo capsules were made of microcrystalline cellulose (MCC) by this company in the same shape, color, scent, and size.

Dietary intake, anthropometric indices, and physical activity assessments

At the commencement of the investigation, weight was assessed employing a lever scale (Seca, Hamburg- Germany) with a precision of 0.1 kg, while ensuring minimal wear. Furthermore, height was evaluated using a wall-mounted stadiometer (Seca, Hamburg- Germany) with an accuracy of 0.1 cm, devoid of footwear, and in a standardized posture (inclusive of the posterior region of the cranium, shoulders, pelvis, posterior region of the lower extremities, and heel coinciding with the wall). Body Mass Index (BMI) was computed by dividing the weight in kilograms by the height in meters squared. The researcher also evaluated physical activity using the International Physical Activity Questionnaire (IPAQ), which has been verified for validity and reliability in Iran[25]. The total physical activity was quantified in MET-min/week. Moreover, the Bone Mineral Density (BMD) of the lumbar spine and femoral neck was measured using dual X-ray absorptiometry (DEXA, Lunar DPX-Plus).

Dietary intake was evaluated by a three-day food record questionnaire (two non-consecutive weekdays and one weekend day) at baseline and the end of the study. The analysis encompassed total energy, macronutrients, fatty acids, fiber, calcium, and vitamin D through the utilization of Nutritionist IV software, which was adapted to accommodate Iranian foods.

Blood sampling and biochemical measurements

After a period of twelve hours of fasting during the night, a quantity of ten milliliters of blood from the veins was gathered from every individual at the beginning, as well as after the intervention. Samples of serum were extracted from the entirety of the blood through the process of centrifugation, utilizing a speed of 2500 revolutions per minute (rpm), for ten minutes, while maintaining a room temperature environment. Subsequently, these samples were promptly stored at a temperature of -80°C until the time of assessment. According to manufacturers' instructions, the quantitative analysis of serum IGF-1 (DRG Diagnostics™, Germany), IGFBP-1 (Shanghai Crystal Day Biotech Co., China), and IGFBP-3 (DRG Diagnostics™, Germany) was performed by ELISA reader (Bio Tek Instruments, Inc, USA) utilizing the ELISA method. Urea and creatinine concentrations were measured via spectrophotometric means, utilizing the Mindray BS-200 Chemistry Analyzer. This was accomplished by employing Pars Azmoon TM detection kits and following their respective protocols. Regarding the determination of serum concentrations for ALT, AST, and ALP, a spectrophotometry method (Mindray BS-200 Chemistry Analyzer) appling corresponding commercial kits (Pars Azmoon Co., Iran) according to the manufacturer's instructions was employed.

Statistical analysis

All data underwent analysis utilizing the software program SPSS (version 23.0), developed by SPSS, and situated in Chicago, IL, USA. The analyses were conducted employing the intention-to-treat (ITT) methodology. A p-value of less than 0.05 was regarded as indicative of statistical significance. Measures of central tendency, such as skewness, kurtosis, and standard deviation (SD), were employed to evaluate the normality of the data distribution. Normally and non-normally distributed quantitative data were presented by mean±SD and median (range), respectively. Side effects were also described by number (percent) and percentage. The statistical methods of one-way analysis of variance (ANOVA) and independent samples Kruskal-Wallis test were utilized to conduct a comparison of normally and non-normally distributed data between the various groups. To further examine the differences inter the groups, the analysis of covariance (ANCOVA) was employed while also taking into account baseline measures and confounding factors such as total physical activity after the intervention. Additionally, the paired t-test and related samples Wilcoxon signed-rank test were applied to assess the disparities within the groups for both parametric and non-parametric variables.

Results

Participants included 60 postmenopausal women with primary osteoporosis (n=19 and n=18 in placebo and *NS* groups, respectively) and osteopenia (n=11 and n=12 in placebo and *NS* groups, respectively).

In total, 56 participants (28 participants in both placebo and *NS* groups) completed the trial and were analyzed. Two individuals in the placebo group (one person due to a medical condition and one person due to the lack of adherence to the trial procedures) and two individuals in the *NS* group due to the lack of adherence to the trial procedures withdrew from the study (Fig. 1).

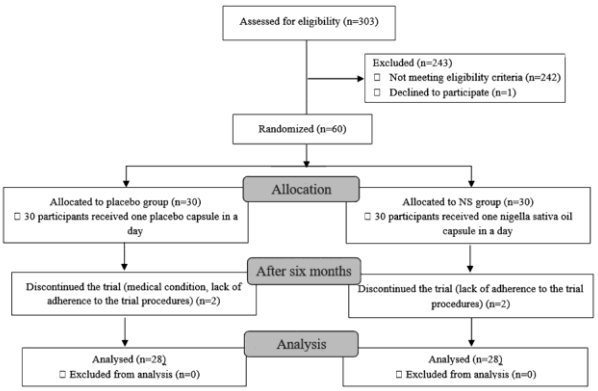

Fig. 1 — Study participants flow diagram

Baseline characteristics of the participants are presented in Table 1. No baseline differences were observed between the two groups.

Table 2 shows the results of dietary intake at baseline and post-intervention. No significant between-group and within-group differences were observed.

As presented in Table 3, no significant differences were observed between *NS* and placebo groups at baseline except for creatinine. After the intervention, no statistically significant difference was observed in IGF1 and Urea levels between or within the groups. IGFBP1 levels significantly decreased in the placebo group [MD (95% CI) =-18.74 (-37.38, -0.11)] compared to baseline values (p=0.049); However, its changes were not significant in the *NS* group compared to the baseline. There was no significant difference in IGFBP1 levels between *NS* and placebo groups at the end of the trial. IGFBP3 levels did not differ significantly in both groups compared to the baseline; However, the between-group analysis indicated a significant increase in the *NS* group [adjusted MD: aMD (95% CI) =1.65 (0.36, 2.97)] compared to the placebo group (p=0.013) at the end of the trial. Besides, Creatinine levels significantly decreased in *NS* and placebo groups [MD (95% CI) =-0.22 (-0.33, -.011) and MD (95% CI) =-0.26

Table 1 — Baseline characteristics of the study participants

Variable	Placebo group (n=30)	NS group (n=30)	p-value
Age (year)* 58.43±3.41		57.31±4.37	0.275†
Menopause age (year)*	48.13±3.73	47.89±4.40	0.823†
Gravidity (number)*	5.10±2.11	4.03±2.11	0.059†
Parity (number)*	4.06±1.43	3.37±1.61	0.091†
Weight (kg)*	70.10±10.10	67.47±10.97	0.343†
Height (cm)*	156.08±5.42	155.48±6.44	0.700†
BMI (kg/m^2)*	28.77±3.81	27.89±4.07	0.394†
Physical activity (MET-min/ week)**			
Mild	346.50 (1386.00)	346.50 (4138.20)	0.557††
Moderate	0.00 (1680.00)	0.00 (320.00)	0.064††
Severe	0.00 (3360.00)	0.00 (160.00)	0.114††
Total	359.25 (4426.50)	346.50 (4026.00)	0.378††
BMD (gr/cm^2)*			
Lumbar spine	0.76±0.08	0.76±0.09	0.998†
Femoral neck	0.77±0.10	0.77±0.08	0.787†
T–score*			
Lumbar spine	-2.56±0.79	-2.55±.084	0.963†
Femoral neck	-1.39±0.79	-1.41±0.71	0.946†
Z–score*			
Lumbar spine	-1.18±0.79	-1.31±0.83	0.539†
Femoral neck	-0.36±0.87	-0.60±0.63	0.235†

BMI: body mass index, BMD: bone mineral density, * Mean ± SD, ** Median (Range)
† Independent Samples T test, †† Mann-Whitney U test

Table 2 — Comparison of dietary intake between study groups at baseline and after the intervention

Variable	Placebo group (n=28)	NS group (n=28)	p-value
Energy (kcal)*			
Baseline	1923.79 (1000.71)	1853.13 (1485.01)	0.135†
End	1785.29 (1743.36)	1844.39 (2015.33)	0.506†
Pre to post P-value‡	0.250	0.357	-
Carbohydrate (g)*			
Baseline	245.95 (249.21)	243.25 (282.33)	0.976†
End	244.55 (235.34)	244.43 (240.33)	0.824†
Pre to post P-value‡	0.964	0.308	-
Protein (g)**			
Baseline	52.77±13.60	53.02±12.31	0.940††
End	52.98±15.07	53.47±9.38	0.880††
Pre to post P-value‡‡	0.838	0.804	-
Total fat (g)**			
Baseline	65.14±17.00	65.60±19.52	0.923††
End	65.53±19.43	64.71±16.43	0.859††
Pre to post P-value‡‡	0.785	0.642	-
Calcium (mg)**			
Baseline	758.39±223.74	754.76±206.28	0.948††
End	763.05±186.29	759.10±145.17	0.927††
Pre to post P-value‡‡	0.908	0.909	-
Vitamin D (µg)*			
Baseline	1.43 (4.79)	1.59 (2.58)	0.284†
End	1.56 (2.96)	1.70 (3.14)	0.784†
Pre to post P-value‡	0.697	0.732	-

SFA: saturated fatty acid, PUFA: poly unsaturated fatty acid, MUFA: mono unsaturated fatty acid
* Median (Range), ** Mean ± SD, † Mann-Whitney U test, †† Independent Samples T test
‡ Related Samples Wilcoxon Signed Rank test, ‡‡ Paired t-test

Table 3 — Comparison of biochemical parameters between study groups at baseline and after the intervention

Variable	Placebo group (n=28)	NS group (n=28)	Adjusted MD (95% CI)	P-value
IGF 1 (ng/ml)				
Baseline*	11.91±4.19	11.85±5.51	-	0.967†
End*	10.21±5.11	10.70±4.14	0.67 (-1.85, 3.19)	0.599††
MD (95% CI)	-1.69 (-3.96, 0.58)	-1.15 (-3.25, 0.93)	-	
Pre to post P-value†††	0.139	0.268	-	-
IGF BP 1 (µg/l)				
Baseline*	828.42±93.28	814.01±75.98	-	0.514†
End*	809.67±68.14	798.30±41.23	-6.59 (-13.56, 26.74)	0.515††
MD (95% CI)	-18.74 (-37.38, -0.11)	-15.71 (-39.30, 7.88)	-	
Pre to post P-value†††	0.049	0.184	-	-
IGF BP 3 (µg/ml)				
Baseline*	11.40±3.20	11.84±2.63	-	0.553†
End*	11.32±2.50	12.77±2.69	1.65 (0.36, 2.97)	0.013††
MD (95% CI)	-0.07 (-1.20, 1.05)	0.93 (-0.1, 1.99)	-	
Pre to post P-value†††	0.893	0.084	-	-
Urea (mg/dl)				
Baseline*	26.04±9.25	27.72±5.65	-	0.089†
End*	23.28±5.59	25.09±5.19	2.59 (-2.13, 7.31)	0.272††
MD (95% CI)	-2.76 (-5.31, 1.79)	-2.63 (-5.73, 0.46)	-	
Pre to post P-value†††	0.220	0.092	-	-
Creatinine (mg/dl)				
Baseline*	0.92±0.10	1.00±0.13	-	0.016†

(*Contd*.)

Table 3 — Comparison of biochemical parameters between study groups at baseline and after the intervention (*Contd*.)

Variable	Placebo group (n=28)	NS group (n=28)	Adjusted MD (95% CI)	P-value
End*	0.66±0.17	0.77±0.26	0.12 (-0.03, 0.27)	0.124††
MD (95% CI)	-0.26 (-0.34, -0.18)	-0.22 (-0.33, -.011)	-	
Pre to post P-value†††	<0.001	<0.001	-	-
AST (U/l)				
Baseline*	26.94±7.28	30.36±11.56	-	0.218†
End*	25.11±7.52	24.22±8.65	-1.40 (-7.17, 4.36)	0.622††
MD (95% CI)	-1.83 (-6.11, 2.44)	-6.13 (-11.15, -1.12)	-	
Pre to post P-value†††	0.379	0.019	-	-
ALT (U/l)				
Baseline*	15.16±6.70	14.61±7.62	-	0.840†
End*	10.83±5.00	9.00±4.34	-0.59 (-3.79, 2.60)	0.708††
MD (95% CI)	-4.33 (-8.01, -0.64)	-5.61 (-8.81, -2.42)	-	
Pre to post P-value†††	0.024	0.002	-	-
ALP (U/l)				
Baseline*	187.95±34.70	199.23±51.96	-	0.433†
End*	180.29±61.97	172.03±46.64	-2.34 (-33.15, 28.45)	0.878††
MD (95% CI)	-7.66 (-32.62, 17.29)	-27.19 (-44.76, -9.61)	-	
Pre to post P-value†††	0.531	0.004	-	-

IGF 1: insulin-like growth factor 1, IGF BP 1: insulin-like growth factor binding protein 1, IGF BP 3: insulin-like growth factor binding protein 3, AST: aspartate amino transferase, ALT: alanine amino transferase, ALP: alkaline phosphatase, MD: mean difference, CI: confidence interval

* Mean ± SD, † Independent Samples T test, ††† Paired t-test

†† Analysis of Covariance (ANCOVA) test adjusted for baseline measures (baseline biochemical parameters, total MET, age, energy intake, lumbar spine BMD and femoral neck BMD)

(-0.34, -0.18), respectively] compared to baseline values (p<0.001 in both groups), between-group analysis also did not show any significant difference between *NS* and placebo groups at the end of the trial. AST and ALP levels significantly decreased in the *NS* group [MD (95% CI) =-6.13 (-11.15, -1.12) and MD (95% CI) =-27.19 (-44.76, -9.61), respectively] compared to baseline values (p=0.019 and p=0.004, respectively); However, its changes were not significant in the placebo group compared to the baseline. Between-group analysis did not show any significant difference in AST and ALP levels between *NS* and placebo groups at the end of the trial. ALT levels also significantly decreased in *NS* and placebo groups [MD (95% CI) =-5.61 (-8.81, -2.42) and MD (95% CI) =-4.33 (-8.01, -0.64), respectively] compared to baseline values (p=0.002 and p=0.024, respectively); However, between-group analysis did not show any significant difference between *NS* and placebo groups at the end of the trial.

A total of 11 participants (n=8 (64.26%) and n=3 (9.99%) in placebo and *NS* groups, respectively) reported side effects due to the supplement use. Only one participant (3.33%), in the placebo group, reported very severe side effects. Other participants reported very mild to moderate side effects (Table 4).

Discussion

In the present study, no statistically significant difference was observed in serum IGF-1 and IGFBP1 levels between the placebo group and the group receiving *NS* capsules. However, there was a statistically significant difference between the two groups in IGFBP-3 concentration after adjusting for base values and the confounding variables of age, lumbar vertebrae and femoral neck BMD, physical activity, calorie intake, and the basic biochemical parameters.

Most studies on IGFBPs are related to IGFBP-3 and confirm a positive relationship between serum IGFBP-3 levels and BMD values in different skeletal areas[26,27]. Elevated levels of this protein have even been observed in osteoporotic individuals[28]. Studies on the relationship between IGF-1 and BMD are quite inconsistent. The paradoxical relationships between serum IGF-1, bone, and fat may be involved in the development of idiopathic osteoporosis in premenopausal women[29]. The qualitative bone properties are probably more closely related to the GH system than the quantitative ones[30].

Over 50% of *NS* oil consists of α-linoleic acid. Numerous studies have shown that dietary linoleic acid increases bone formation, inhibits bone

Table 4 — Reported side effects of the intervention

Side effects	Placebo group (n=30)	NS group (n=30)	Total
Nausea*	2 (6.66)	0	2 (6.66)
Very mild	0	0	0
Mild	1 (3.33)	0	1 (3.33)
Moderate	1 (3.33)	0	1 (3.33)
Severe	0	0	0
Very severe	0	0	0
Vomiting*	1 (3.33)	0	1 (3.33)
Very mild	0	0	0
Mild	0	0	0
Moderate	1 (3.33)	0	1 (3.33)
Severe	0	0	0
Very severe	0	0	0
Headache*	1 (3.33)	1 (3.33)	2 (6.66)
Very mild	1 (3.33)	0	1 (3.33)
Mild	0	1 (3.33)	1 (3.33)
Moderate	0	0	0
Severe	0	0	0
Very severe	0	0	0
Unpleasant taste*	1 (3.33)	1 (3.33)	2 (6.66)
Very mild	0	0	0
Mild	0	0	0
Moderate	0	1 (3.33)	1 (3.33)
Severe	0	0	0
Very severe	1 (3.33)	0	1 (3.33)
Other side effects*	3 (9.99)	1 (3.33)	4 (13.32)
Total*	8 (26.64)	3 (9.99)	11 (36.63)

* Number (Percent)

resorption[31-33], and increases IGF biosynthesis[31,34,35]; some studies, however, reported no positive effect on bone metabolism[36,37]. If linoleic acid is considered to induce fat mass reduction and weight loss, a reduction in bone density will also be expected. However, conservation of bone mass has been observed with linoleic acid supplements. Preclinical data support the positive effect of linoleic acid on bone health, whereas clinical data in humans have a long way to go to report convincing evidence[38].

No study was found on the effect of *NS* oil on serum IGF-1 and IGFBP levels concerning osteoporosis in humans. Studies conducted on rats have examined the effect of *NS* on IGF-1 in diseases other than osteoporosis. Most studies have been conducted on the anti-diabetic effect of *NS*. Administration of *NS* supplements has reduced diabetes-induced IGF-1 levels close to normal levels[39]. In addition, increased serum IGF-1 levels in rats due to the administration of this supplement have led to physiological cardiac hypertrophy[40]. The anti-diabetic effect of *NS* has been shown in several studies to be due to up-regulation of the IGF-1 gene and the anti-inflammatory effect and improvement of diabetes-induced oxidative stress by regulation of antioxidants at cellular and molecular levels in diabetic rats[41]. Given the role of oxidative stress and inflammatory processes in bone diseases such as osteoporosis, the anti-osteoporotic activity of *NS* can be attributed to its anti-inflammatory and anti-oxidant properties[15]. Most of the anti-inflammatory property of *NS* is attributed to its active constituent thymoquinone[20].

A human study was conducted to investigate the effect of *NS* supplements on bone markers in postmenopausal women. In this study, taking *NS* supplementation for three months had no significant effect on the levels of bone markers. The limitations of this study, such as the small sample size, the short study period, and the incompliance of participants to regularly use oral *NS* oil, may have affected the results[42]. The studies conducted on the effect of therapeutic doses of *NS* in animals, and previous human studies, reported no undesirable or toxic effects on the liver or kidneys[43,44]. However, caution should be taken regarding increases in hemoglobin and hematocrit as well as decreases in leukocytes and platelets[42]. In the present study, no significant difference was observed in ALT, AST, ALP, BUN, and Cr between the groups after the intervention, which showed its good safety properties. No severe or very severe adverse effects were reported in those taking *NS*. About 3.3% of the participants reported mild headaches and 3.3% complained moderately of the bad taste of *NS* oil, which may have contributed to the lack of full compliance of the participants with the intervention process.

To our knowledge, this is the first human study on the effect of oral *NS* oil capsules on serum levels of IGF-1 and its binding proteins (IGFBP-1, IGFBP-3) in women with primary bone loss. One of the reasons for the lack of significant differences in IGF-1 and IGFBP-1 levels between the two groups is the small sample size in the present study. In addition, the weak correlation between IGFBP-1 and BMD can contribute to the lack of significant difference in this

protein between the two groups. It is suggested to conduct similar studies with a larger sample size. It seems that according to the results of the present study, this herbal medicine may be used to prevent bone loss in the elderly but more clinical trials are required on human participants to clarify further the role of *NS* in improving bone health.

Conclusion

In the present study, a significant increase in IGFBP-3 levels was observed in the *NS* group adjusted for age, BMD, physical activity, and daily calorie intake; however, there was no significant difference in IGFBP-1 levels between the two groups. Contrary to most animal studies, which showed significant differences in IGF-1 levels between the *NS* and the control groups, no significant difference was observed between the two groups even after adjusting for mentioned confounding variables in the present study on human participants.

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Ethics statement

The study protocol has been approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.901) and was registered at the Iranian Registry of Clinical Trials on 16/07/2018 (www.irct.ir/IRCT20131009014957N4). All procedures were conducted ethically following the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all the participants.

Conflict of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Study concept and design: AFKh, SA, AMI, MA. Acquisition, analysis, and interpretation of data: AFKh, SA, AMI, MA. Drafting of the manuscript: AFKh, SA. Critical revision of the manuscript for important intellectual content: AFKh, SA. Statistical analysis: AFKh, AMI. All authors have read and approved the manuscript.

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