Effect of Feeding on Diets Supplemented by Some Vegetable Oils on Blood Lipids and Bone Mineral Content in Osteoporotic Rats

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Abstract: The effects of diets supplemented by soybean, flaxseed and sesame oils on blood lipids and bone mineral content in osteoporotic ovariectomized (OVX) rats were investigated. Fifty rats were randomly allocated into 5 equal groups and housed in metabolic metal cages. Rats of group (1) were sham operated (Sham) and of the other 4 groups were OVX and left for 8 weeks for induction of osteoporosis. Group (2) was fed on basal diet (OVX-control) and groups (3), (4) and (5) were fed on experimental diets containing either 5 % soybean, 5 %flaxseed or 5 % sesame oil, respectively for 6 weeks. Blood was collected for estimating serum levels of total cholesterol, triglycerides, phospholipids, calcium (Ca), phosphorous (P), bone - specific alkaline phosphatase (b-ALP) and osteocalcin (OC). Urine samples were collected for determination of Ca and P concentrations. Rats were euthanized, uteri were dissected out and weighed. Femur bones were removed and used for bone analyses. The results showed that feeding of diets supplemented with soybean, flaxseed and sesame oils to OVX rats reversed the decrease in uterine and femur weights and normalized the elevated serum levels of lipids, Ca, P, b-ALP and OC. These oils also decreased urinary Ca and P levels and increased femur bone density and calcium content in bone ash. In conclusion, dietary soybean, flaxseed and sesame oils cause hypolipidemic and anti-osteoporotic activities in osteoporotic rats. Intake of these vegetable oils in foods may be beneficial for the treatment of postmenopausal osteoporosis in women.

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1. Introduction

Bone is a connective tissue which continuously undergoes remodeling. Bone remodeling involves bone formation by osteoblasts and bone resorption by osteoclasts. Bone undergoes continuous remodeling with regular resorption and deposition of calcium into newly deposited bone. Osteoporosis is a bone disease characterized by bone mass reduction, deterioration of bone structure and decreased bone mineral density which leads to an increased risk of bone fractures. The bone matrix is composed of organic and inorganic components. The organic components include collagen and glycoprotein and the inorganic include minerals mainly calcium and phosphorus. Both organic and inorganic components provide strictness and strength to bones **(Annemieke *et al.*, 1997)**. Osteoporosis represents a major public health problem among general populations particularly in postmenopausal women due to loss in serum estrogen level **(Vassilopoulou-Sellin, 2003)**. Adequate calcium intake is recommended for the development of high peak bone mass and for the prevention of osteoporosis **(Kanis, 1999)**. Moreover, consideration should be given not only to the intake of adequate calcium, but also to the absorptive efficiency of the ingested calcium because intestinal calcium absorption is influenced by many factors **(Mjyazawa and Yoshida, 1991)**.

Dietary fats and oils are known as macronutrients which provide concentrated source of energy for the metabolic processes. In addition, fats are the main source of fat-soluble vitamins **(Sanchez-Muniz and Bastida, 2006)**. Dietary fats are composed of different types of fatty acids (FA) which are saturated (SFA), monounsaturated (MUSFA) and polyunsaturated (PUSFA). Evidence has been shown that dietary fats can have important effects on bone health. Studies in animals indicated that high-fat diets could adversely affect bone **(Hoffman *et al.*, 1999)**. Saturated fatty acids may have adverse effects on bone health **(Parhami, 2003)**. Long-chain polyunsaturated fatty acids could influence bone mass in various animal models. Dietary ratio of (n-6) to (n-3) polyunsaturated fatty acids altered biomarkers of bone formation **(Watkins *et al.*, 2000)**. A variety of mechanisms may account for the effects of dietary fats and oils on bone, including alterations in calcium absorption, prostaglandin synthesis, formation of osteoblasts and lipid oxidation **(Haag *et al.*, 2003)**.

Previous studies revealed that consumption of soybeans in foods has been linked to human health benefits such as lowering serum cholesterol so inhibit the development of atherosclerosis **(Nagata and Yamada, 2009)** and protecting against breast and prostate cancer **(Nagata, 2010)**. In addition, dietary soybeans may be beneficial in reducing the risk of osteoporosis **(Byun and Lee, 2010)**.

It is known that the amount and type of fats and oils in the diet have important effects on blood lipids and bone health. Therefore, the objectives of the present study were to investigate the effects of supplemented diet by soybean, flaxseed and sesame oils on serum lipids and bone mineralization in ovariectomized osteoporotic rats.

2. Material and Methods

2.1. Vegetable oils

Soybean, flaxseed and sesame oils were purchased from Agricultural Seeds, Medicinal Plants and Herbs Company, Cairo, Egypt. These oils were added at 5% concentration during preparation of experimental diets instead of fats of the basal diet.

2.2. Biochemical kits

Kits for biochemical analysis of serum lipids, calcium, phosphorous, bone- specific alkaline phosphatase and osteocalcin were purchased from Gamma Trade Company for Pharmaceuticals and Diagnostic Chemicals, Dokki, Egypt,

2.3. Rats

Fifty adult female rats of Sprague Dawley strain weighing 210-220 g body weight and 14-16 weeks old were used in this study. The rats were obtained from the Laboratory Animal Colony, Helwan, Egypt. The animals were housed individually under hygienic conditions in metabolic cages. The rats were kept at a room temperature of 25 ± 2 °C with relative humidity of 50–60% and on 12 hr light/12 hr dark cycles in the Animal House at the Agricultural Research Center, Giza, Egypt.

2.4. Preparation of basal and experimental diets

Basal diet was prepared according to **Reeves *et al.* (1993)**. It consists of 20 % protein (casein), 10 % sucrose, 5 % fat, 3.5 % salt mixture, 1% vitamin mixture, 2.5% choline chloride, and 5% fibers (cellulose). The remainder was maize starch up to 100 %. Three experimental diets were prepared by substituting the fat content in basal diet by 5% of soybean or flaxseed or sesame oil.

2.5. Ovariectomy procedure

. Ovariectomy was performed in rats by bilateral incisions at both right and left flanks under ether anesthesia. The periovarian fatty tissue was identified and exteriorized. For prevention of subsequent hemorrhage, the arterial blood vessels were clamped by an artery forceps. Above the clamped area, both ovaries were removed out by cutting using a scalpel. Muscles and skin were stitched separately **(Shalaby, 1977)**. Similarly, sham operation was performed in rats where the ovaries were exposed but not removed. Povidone-iodine (antiseptic solution) was applied locally on the skin at both sites of the surgical operation.

2.6. Experiment and grouping of rats

After complete surgical recovery, the operated rats (n = 50) were randomly distributed into 5 equal groups of 10 rats each. Group (1) was sham - operated (SHAM) and the other four groups were ovariectomized (OVX) and left after surgical operation for 8 weeks to induce osteoporosis as a result of estrogen hormone loss **(Coxam, 2005)**. At start and end of the experiment, the rats were weighed and the changes in body weight gain were calculated. Group (2) was left as positive OVX control and fed on basal diet, while groups (3), (4) and (5) were fed on experimental diets containing 5% of soybean, flaxseed or sesame oil, respectively for 6 weeks. At end of the experiment, blood and urine samples were collected for biochemical analyses. Both uterine horns of each rat were removed and weighed. The femur bones were dissected out and taken for bone analyses. The experiment was carried out according to rules and guidelines for animal experimentation which approved by the Institutional Animal Care and Use Committee, NRC, Dokki, Egypt.

2.7. Biochemical analyses

Blood samples were collected from portal hepatic vein into clean dry centrifuge tubes and centrifuged at 3000 rpm for 10 minutes to separate the serum which was stored in a refrigerator till biochemical analyses. Urine samples were collected then acidified with 12 Mol. HCL and stored in a refrigerator till analyses. Total cholesterol and triglycerides were estimated by the method of **Zlatkis *et al*. (1953)** and **Foster and Dunn (1973)** respectively. Phospholipids were analyzed by the method of **Zilversmit** and **Davis (1950)**. Concentrations of calcium **(Gindler and King, 1972)** and phosphorus **(Goodwin, 1970)** in both serum and urine samples were determined spectrophotometrically using specific diagnostic reagent kits (BioMérieux, France). Serum bone-specific alkaline phosphate **(Roy, 1970)** was estimated by colorimetric assay using specific enzyme kits (Sigma Company, St. Louis, USA). Serum osteocalcin concentration was measured by an enzyme-linked immunosorbent assay (Rat Mid™, Osteocalcin ELISA kit, IDS Inc., Fountain Hills, Arizona, USA) according to the manufacturer's instructions.

2.8. Femur bone weight, length, volume and density

The soft tissues around the right femur bone were removed and the femur was weighed. Femur length was measured with vernier caliper and femur volume and density were calculated using Archimedes' principle according to **Doyle and Cashman (2003)**. In brief, the femur was cut out at the mid diaphyses and bone marrow was washed out. Each bone was placed in an unstoppered vial filled with deionized water, and the vial was placed for 90 minutes in a vacuum desiccator. The desiccator was agitated periodically to ensure that the trapped air completely diffused out. The bone was removed from the vial, dried by blotted paper, weighed, and placed again in the vial containing deionized water. The bone was reweighed in a suspended vessel and should be not completely immersed in water before equilibrated at room temperature. Femur bone density (bone weight/bone volume) was then calculated.

2.9. Bone mineral content

The left femur bone was dried overnight at 100^{o}C. The femur was then incinerated for 12 hrs at 1000^{o}C in Muffle apparatus to obtain the ash. The ash was weighed, solubilized with 6 N HCL **(Yang *et al.*, 2008)** and quantitatively transferred into volumetric flask then completed to 100 ml with 6 N HCL. The solutions were used for analysis of calcium content in bone ash using atomic absorption spectrophotometer. The phosphorus content in bone ash was determined using spectrophotometer.

2.10. Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. Differences were considered significant at P <0.05 according to **Snedecor and Cochran (1986)**.

3. Results

The overall body weight analysis revealed that ovariectomized (OVX) positive control rats had a significant (P <0.05) increase in body weight gain. The body weight gain was 27.56% for OVX rats versus to 12.78% for sham (SHAM) control group. Ovariectomy caused a significant (P <0.05) decrease in uterine weight when compared with SHAM control rats. The mean uterine weight of rats was 0.19±0.01 g in OVX control group versus to 0.38±0.04 g in the SHAM group. Feeding of experimental diets containing 5% of soybean, flaxseed or sesame oils to OVX rats decreased body weight gain but increased uterine weight when compared with the OVX control positive group as shown in Table (1).

The bilateral ovariectomy in rats resulted in significant (P < 0.05) increases in serum levels of total cholesterol, triglycerides and phospholipids when compared with the SHAM group. Feeding of experimental diets containing 5% of soybean oil, flaxseed or sesame oil to OVX- rats for 6 weeks normalized the elevated serum levels of total cholesterol, triglycerides and phospholipids when compared to the OVX control group as recorded in Table (2).

Table 1. Effect of feeding diets containing soybean oil (SBO), flaxseed oil (FSO) and sesame oil (SEO) on body and uterus weights of ovariectomized (OVX) rats.

Groups	Body weight (g) at		Weight gain (%)	Uterine weight (g)
	Week 0	Week 8		
Group (1) SHAM (-ve control)	210.00±9.85^a	236.85±5.32^c	12.78	0.38±0.04^a
Group (2) OVX (+ ve control)	220.65±7.17^a	281.46±6.17^a	27.56	0.19±0.01^c
Group (3) 5 % SBO	218.82±6.62^a	252.71±4.25^b	15.49	0.26±0.03^b
Group (4) 5 % FSO	216.54±9.25^a	250.52±2.33^b	15.69	0.24±0.01^b
Group (5) 5 % SEO	219.59±8.12^a	262.42±2.53^b	19.50	0.23±0.04^b

Means ± SD with different superscripts in the same column are significant at P < 0.05 using one way ANOVA test N= 10 rats/group.

Table 2. Effect of feeding diets containing soybean oil (SBO), flaxseed oil (FSO) and sesame oil (SEO) on serum total cholesterol (TC),triglycerides (TG) and phospholipids (PL) in ovariectomized (OVX) rats.

Groups	TC (mg/dL)	TG (mg/dL)	PL (mg/dL)
Group (1) SHAM (-ve control)	96.98 ± 1.4^c	33.43 ± 1.5^b	80.25 ± 1.5^c
Group (2)	125.95 ± 2.6^a	41.02 ± 1.9^a	91.32 ± 1.7^a

OVX (+ ve control)			
Group (3) 5 % SBO	112.97 ± 2.8^{b}	38.60 ± 1.4^{b}	85.65 ± 1.6^{b}
Group (4) 5 % FSO	110.50 ± 6.5^{b}	34.50 ± 1.2^{b}	86.37 ± 1.8^{b}
Group (5) 5 % SEO	109.50 ± 4.2^{b}	36.70 ± 1.4^{b}	84.39 ± 1.6^{b}

Means ± SD with different superscripts in the same column are significant at $P < 0.05$ using one way ANOVA test and those with similar superscripts are non significant. N= 10 rats/group.

The OVX rats had significant ($P < 0.05$) increases in serum levels of calcium, phosphorous, bone-specific alkaline phosphatase (b-ALP) and osteocalcin (OC) when compared with the SHAM group. Feeding of OVX rats on diets supplemented with 5% of soybean, flaxseed or sesame oil for 6 weeks significantly ($P < 0.05$) normalized the elevated serum levels of Ca, P, b-ALP and OC when compared with the OVX control group (Table 3).

Table 3. Effect of feeding diets containing soybean oil (SBO), flaxseed oil (FSO) and sesame oil (SEO) on levels of serum calcium (Ca), phosphorous (P), bone specific alkaline phosphatase (b-ALP) and osteocalcin (OC) in ovariectomized (OVX) rats.

Groups	Serum concentrations of Ca (mg/dL)	P (mg/dL)	b-ALP (U/mL)	OC (μg/L)
Group (1) SHAM (-ve control)	11.21 ±0.9^{b}	3.55±0.3^{b}	155.6±8.3^{b}	0.8±0.05^{c}
Group (2) OVX (+ ve control)	13.35±0.6^{a}	6.26±0.2^{a}	168.6±4.2^{a}	1.9±0.02^{a}
Group (3) 5 % SBO	10.25±0.8^{b}	3.39±0.4^{b}	140.5±5.1^{c}	1.4±0.02^{b}
Group (4) 5 % FSO	10.35±0.6^{b}	3.30±0.2^{b}	144.6±6.3^{c}	1.3±0.01^{b}
Group (5) 5 % SEO	10.66±0.8^{b}	3.55±0.3^{b}	146.6±7.3^{c}	1.5±0.02^{b}

Means ± SD with different superscripts in the same column are significant at $P < 0.05$ using one way ANOVA test.
N= 10 rats/group

The analysis of urine samples of OVX control rats revealed significant ($P < 0.05$) increases in calcium and phosphorous concentrations when compared with SHAM control rats. Feeding of OVX rats on diets containing soybean oil, flaxseed oil or sesame oil significantly ($P < 0.05$) reduced the elevated levels of calcium and phosphorous in the urine when compared to the OVX-control group (Table 4).

As shown in Table (5) the OVX control rats had significant ($P < 0.05$) decreases in femur weight, volume and mineral density when compared with the SHAM control group. Feeding of soybean, flaxseed or sesame oil at 5% concentration in diet to OVX rats significantly ($P < 0.05$) restored the ovariectomy-induced changes in femur weight, volume and mineral density when compared with OVX control rats. There were no changes in the length of femur bone in any of the treated groups.

Data in Table (6) showed that the ovariectomy in rats caused significant ($P < 0.05$) decreases in ash weight of femur bone and calcium content in the ash when compared with SHAM control rats. There were no significant changes in phosphorous content in femur ash of any of groups fed on experimental diets. The decreases in femur ash weight and calcium content in femur ash were significantly ($P < 0.05$) normalized in OVX rats fed on diets containing 5% of soybean, flaxseed or sesame oil.

Table 4. Effect of feeding diets containing soybean oil (SBO), flaxseed oil (FSO) and sesame oil (SEO) on urine concentrations of calcium (Ca) and phosphorus (P) in ovariectomized (OVX) rats.

Groups	Urine concentrations of Ca (mg/dL)	P (mg/dL)
Group (1)	4.67±0.6^{d}	9.55±0.3^{b}

SHAM (-ve control)		
Group (2) OVX (+ ve control)	8.15±0.7[a]	11.24±0.7[a]
Group (3) 5 % SBO	5.45±0.4[c]	8.57±0.2[b]
Group (4) 5 % FSO	5.55±0.7[c]	8.45±0.3[b]
Group (5) 5 % SEO	6.33±0.6[b]	8.35±0.5[b]

Means ± SD with different superscripts in the same column are significant at $P < 0.05$using one way ANOVA test. N= 10 rats/group.

Table 5. Effect of feeding diets containing soybean oil (SBO), flaxseed oil (FSO) and sesame oil (SEO) on femur weight (Wt), length (L),volume (V) and density (D) in ovariectomized (OVX) rats.

Groups	Parameters of femur bones			
	Femur Wt. (g)	Femur L (mm)	Femur V (cm^3)	Femur D (g/cm^3)
Group (1) SHAM (-ve control)	0.75±0.02[b]	40.02±2.75[a]	0.74±0.01[a]	0.84±0.04[a]
Group(2) OVX (+ ve control)	0.66±0.17[c]	40.09±8.71[a]	0.44±0.02[c]	0.64±0.02[c]
Group (3) 5 % SBO	0.80±0.02[a]	39.80±9.25[a]	0.52±0.01[b]	0.75±0.07[b]
Group(4) 5 % FSO	0.81±0.03[a]	39.85±8.55[a]	0.55±0.03[b]	0.77±0.01[b]
Group(5) 5 % SEO	0.74±0.05[b]	39.90±4.05[a]	0.65±0.02[a]	0.80±0.03[a]

Means ± SD with different superscripts in the same column are significant at $P < 0.05$ using one way ANOVA test and those with similar superscripts are non significant. N= 10 rats/group.

Table 6. Effect of feeding diets containing soybean oil (SBO), flaxseed oil (FSO) and sesame oil (SEO) on concentrations of calcium and phosphorous in femur bone of ovariectomized (OVX) rats.

Groups	Femur bone mineral content		
	Ash weight (g)	Calcium (mg/g ash)	Phosphorous (mg/g ash)
Group (1) SHAM (-ve control)	0.67±0.02[a]	11.6±0.22[a]	6.30±0.54[a]
Group (2) OVX (+ ve control)	0.55±0.05[c]	8.5±0.24[c]	6.36±0.46[a]
Group (3) 5 % SBO	0.63±0.05[b]	9.8±0.28[b]	6.47±0.56[a]
Group (4) 5 % FSO	0.62±0.03[b]	10.3±0.25[b]	6.45±0.41[a]
Group(5) 5 % SEO	0.64±0.04[b]	10.5±0.31[b]	6.50±0.50[a]

Means ± SD with different superscripts in the same column are significant at $P< 0.05$ using one way ANOVA test and those with similar superscripts are non significant.

4. Discussion

The aim of the present study was to investigate the effect of supplementation of diets by soybean, flaxseed and sesame oils on blood lipids and bone mineral content in ovariectomized osteoporotic rats.

Ovariectomy causes estrogen deficiency which is one of important risk factors in the pathogenesis of osteoporosis. In this concern, Weitzmann *et al.* (2002) concluded that estrogen inhibits bone resorption and prevents bone loss, so estrogen deficiency will increase bone loss. Previous studies showed that the bilateral ovariectomy in rats resulted in dramatic decreases in the uterine weight, bone mineral content and density and biomechanical strength due to estrogen loss (Vassilopoulou-Sellin, 2003 and Srikanta *et al*., 2011).

In the present study, feeding of soybean, flaxseed and sesame oils, each at 5% concentration in the diet, to ovariectomized rats for 6 weeks produced hypolipidemic effect evident by decreased serum levels of total cholesterol, triglycerides and phospholipids in ovariectomized rats. The hypolipidemic effect of soybean oil was partially similar to that of previous studies which revealed that soybean oil reduced serum total cholesterol, triglycerides and lipoproteins in different aged rats fed on hypercholesterolemic diets (Choi *et al.,*1993) and improved serum lipid profile (Ramadan *et al.,* 2008). The hypolipidemic effect of flaxseed oil, as reported in this study, agreed with that reported by Cintra *et al.* (2006) and Tomaz *et al.* (2011) who found that dietary flaxseed oil improved lipid profile and might be important to prevent cardiovascular disorders. These authors concluded that flaxseed oil is promising for dietary manipulation of hyperlipidemia. The hypolipidemic effect of sesame oil, as reported in this study, was in accordance with that recorded by Reena and Lokesh (2007) and Arumugam *et al.* (2011) who suggested that feeding oils with balanced amounts of fatty acids obtained by blending and interesterification of coconut oil with rice bran oil or sesame oil lowered serum and liver lipids in rats.

Supplementation of diets by soybean, flaxseed and sesame oils when fed to ovariectomized rats induced an antiosteoporotic activity as it revised the alterations caused by bilateral ovariectomy. These vegetable oils maintained the body weight changes; restored the ovariectomy-induced decrease in the uterine weight; normalized the changes in serum levels of calcium, phosphorus, bone – specific alkaline phosphatase and osteocalcin. They also restored the ovariectomy-induced changes in femur weight, and density and calcium content in bone ash and decreased urinary calcium excretion of calcium and phosphorous.

Concerning soybean oil, the obtained results suggested that it induced similar effects to estrogen on the bone. These effects were partially similar with previous studies that showed that soybean oil in the diet has a role in the prevention of osteoporosis as it reduced bone loss and increased bone mineral density (Watkins *et al.,* 1997 and Jie *et al*., 2000). The previous authors attributed the effect of soybean oil due to its high content of polyunsaturated fatty acids which are beneficial in inhibiting the activity of osteoclasts and enhancing the activity of osteoblasts in ovariectomized rats. However, Shuid *et al.* (2007) and Byun and Lee (2010) concluded that recycled palm oil is better than soybean oil in maintaining bone properties in a menopausal syndrome model of ovariectomized rats. Regarding flaxseed oil and sesame oils the present results were in agreement with that of Boulbaroud *et al.* (2008) who reported that flaxseed and sesame oils produced protective effects against bone loss in ovariectomized rats. Moreover, it was reported that 10% of flaxseed oil + low dose of estrogen therapy provided the greatest protection against ovariectomy-induced bone loss in ovariectomized rats (Sacco *et al*., 2009).

Regarding the effect on uterine weight, it is known that estrogen increases vascularity, growth and weight of the uterus in immature rats and mice (Shalaby, 1977). Therefore, the ovariectomized rats had a decreased weight of the uterus due estrogen deficiency. The increased body weight gain and elevated serum levels of bone- specific alkaline phosphatase (b-ALP) and osteocalcin (OC) support the observations of the previous investigations related to increased body weight and elevated serum levels of b-ALP and OC due to ovarian hormone deficiency (Ke *et al .,* 1997; Tamir *et al.*, 2001 and Coxam, 2005).

It is well known that calcium and phosphorus are widely accepted as phenotype markers for bone formation (Evans *et al.,* 1990). In the present study, the results showed that bilateral ovariectomy developed bone changes similar to those seen in the estrogen-deficient osteoporotic women, most markedly is the decrease in bone density (Gao *et al.*, 2011). Feeding diets containing 5% of soybean, flaxseed or sesame oils to ovariectomized rats significantly restored the decreased serum calcium and phosphorus levels induced by ovariectomy to normal levels, while it decreased urinary calcium and phosphorous concentrations. These results suggest that diet supplementation with soybean, flaxseed and sesame oils were more effective in inhibiting bone resorption and in increasing bone formation. These findings were similar to those of Shuid *et al.* (2007), Boulbaroud *et al.* (2008) and Byun and Lee (2010) who reported that vegetable oils contain omega -3 and omega-6 polyunsaturated fatty acids which increased serum calcium and phosphorus concentrations and reduce urinary calcium and phosphorous excretion, thus enhanced bone formation.

The mechanism(s) of anti-osteoporotic activity of soybean, flaxseed and sesame oils could be possibly explained by presence of n-6 and n-3 polyunsaturated fatty acids (PUFAs) which are found in various vegetable oils. It well known that the PUFAs have a critical role in the regulation of a variety of biological processes including bone formation and metabolism (Maggio *et al.*, 2009).

In conclusion, the results suggest that dietary soybean, flaxseed and sesame oils induce hypocholesterolemic and anti-osteoporotic effects in ovariectomized rats. These vegetable oils can effectively ameliorate ovariectomy-induced osteoporosis in rats therefore they are considered promising natural dietary supplements for the treatment of postmenopausal osteoporosis in women. Further studies are needed to determine the mechanism(s) by which a diet supplemented by these oils can modulate bone tissue.

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