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Effect of diets fortified with Nigella sativa, sesame seeds and high calcium on osteoporosis in ovariectomized rats

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Abstract

The study was carried out to assess the effect of diets fortified with Nigella sativa (NS), sesame (SS) seeds and high calcium (Ca) on osteoporosis in ovariectomized (OVX) rats. Fifty six rats were randomized into 8 equal groups (n= 7) and housed individually in metal metabolic cages. Under ether anesthesia, group (1) was SHAM-operated and fed on basal diet, while the other 7 groups were bilaterally OVX-operated. Three weeks after ovariectomy, group (2) was fed on basal diet, while groups (3), (4), (5), (6) and (7) were fed on experimental diets fortified with the following: 10% NS; 10% SS; 2% Ca; 10% NS+2% Ca and 10% SS+2% Ca, respectively for 6 weeks. Group (8) was fed on basal diet and given orally Alendronate (anti-osteoporotic drug) in a daily dose of 3 mg/kg for 6 weeks. During feeding period, initial and final body weights of rats were recorded. At the end of the experiment, the last 24 hr urine samples were collected for estimating Ca, P, pyridinoline (PD) and deoxypyridinoline (DPD) concentrations. Rats were then euthanized by prolonged exposure to anesthetic ether. Blood samples were collected for estimating serum calcium (Ca), phosphorous (P), bone-specific alkaline phosphatase (b-ALP) and osteocalcin (OC) levels. The uteri were dissected out and weighed. Femur bones also were dissected out and used for measurement and analysis of bone ash. The results showed that diets fortified with NS and SS and high calcium prevented the increase in body weight gain and the decrease in uterine weight induced by ovariectomy. These experimental diets normalized serum Ca, P, b-ALP and OC levels and decreased urinary excretion of Ca, P, PD and DPD. There were also significant increases in femur bone mineral density (BMD) and calcium content in bone ash. In conclusion, diets fortified with NS, SS and high calcium have a promising anti-osteoporotic effect in OVX rats. The study recommends enrichment of the bakeries and food stuff with Nigella sativa and sesame seeds and eating meals rich in calcium such as dairy products (milk and yoghurt) may be beneficial for the treatment of postmenopausal osteoporosis in women.

Keywords: Nigella sativa; Sesame seeds; High-calcium diet; Ovariectomy; Osteoporosis; Biochemical analysis; Bone mineralization.

Introduction

Osteoporosis is considered a major public health problem and characterized by bone mass reduction, and decreased bone mineral density leading to an increased risk of bone fragility and fractures ***(McCarron and Heaney, 2004).*** The bone matrix is composed of organic (collagen and glycoprotein) and inorganic (minerals mainly calcium and phosphorus) components. Both organic and inorganic components provide strictness and strength to the bones ***(McNamara et al., 2006).*** Osteoporosis represents a serious problem among postmenopausal women and menopause drastically increases the risk of osteoporosis ***(Vassilopoulou-Sellin, 2003 and Canalis et al., 2007).*** Estrogen deficiency is the main risk factor for postmenopausal osteoporosis because estrogen is most potent inhibitor of osteoclastic bone resorption ***(Gambacciani and Ciaponi, 2000).*** Although long term estrogen replacement therapy has been commonly used to compensate bone loss, estrogen therapy is usually accompanied with severe adverse or side effects ***(Al-Anazi et al., 2011).***

Nigella sativa (NS) seeds have been used for thousands of years for culinary and medical purposes. Many of the biological activities of NS were attributed to thymoquinone in the oil. ***(Houghton et al., 1995).***The seeds of NS are used worldwide to treat various diseases and ailments. Thymoquinone (TQ) is most abundant and active ingredient of NS essential oil that mediates most of therapeutic effects. TQ was reported to produce antioxidant ***(Badary et al., 1997);*** anticancer ***(Gali-Muhtasib et al., 2006);*** nephroprotective ***(Al-Majed et al., 2006);*** anti-inflammatory ***(Ragheb et al., 2009)*** and hypolipidemic and antioxidant effects ***(Ahmad and Beg, 2013).*** Animal studies have shown that combination of NS and human parathyroid hormone was effective for the treatment of diabetes-induced osteoporosis and for promotion of fracture healing ***(Atlan, 2007).*** The antiosteoporotic effect was attributed to the antioxidant and anti-inflammatory activities of NS and TQ because osteoporosis has been linked to oxidative stress and inflammation ***(Shuid et al., 2012).*** Moreover, TQ was found to accelerate osteoblast differentiation and activate bone building ***(Wirries et al., 2013).***

Sesame seeds have been used as a source of healthful foods and sesame oil. Sesame seeds are rich source of many essential minerals as calcium, iron, manganese, phosphorous, magnesium, selenium, and copper. These minerals play a vital role in bone mineralization and health. Sesame oil is one of the most stable vegetable oils contains high content of natural antioxidant lignans (sesamin, sesamolin and sesamol). Sesame oil was reported to prevent bone loss in ovariectomized rats ***(Boulbaroud et al., 2008).*** Sesamin was reported to activate osteoblast differentiation, to produce an osteoprotective effect and may be a promising phytochemical that could be developed for supplementation of osteoporotic therapy ***(Wanachenwin et al., 2012).***

There is an increasing interest in nutrients which influence bone metabolism and health such as calcium and vitamin D ***(Ilich and Kerstetter, 2000 and Gourlay, and Brown, 2004).*** The involvement of oxidative stress in osteoporosis has been shown to be due to insufficient intake of antioxidants, therefore it can increase the risk of hip fracture in smokers ***(Melhus et al., 1999).*** The natural polyphenolic antioxidants and flavonoids have been received much attention for their potential role in preventing osteoporosis induced in rats ***(Draper et al., 1997)*** and in human ***(Potter et al., 1998).*** Flavonoids have been characterized as naturally occurring selective estrogen receptor modulators (SERMs) with beneficial effect on bone heath ***(Brezinski and Debi, 1999).*** Flavonoids have been shown to inhibit bone loss in rats, by inhibiting rat osteoclast formation and differentiation and may be useful for the treatment of bone resorption ***(Jeon et al. 2009; Oka et al.; 2012 and Li et al., 2013).***

The present study, aimed to evaluate the effect of diets fortified with Nigella sativa, sesame seeds and high calcium on serum, urinary and bone markers of osteoporosis in ovariectomized rats.

Materials and Methods

Plant seeds and calcium carbonate

Nigella sativa (Black Seed, Family *Ranunculaceae*) and Sesame (Family *Pelaliaceae*) seeds were purchased from local store of spices, Cairo, Egypt. These seeds were grinded into a fine powder using an electric mixer. Each powder was added to basal diet at a concentration of 10% to formulate experimental diets.

Calcium carbonate is widely used as an inexpensive dietary calcium supplement. It was purchased from El-Gomhoryia Company, Egypt in the form of fine powder packed in brown bottles, of 500 gram each.

Rats

Fifty six mature female Sprague Dawley rats weighing 220-230 g b.wt and 8-10 weeks old were used in this study. The rats were purchased from Laboratory Animal Colony, Helwan, Egypt. The animals were housed individually in metabolic cages under hygienic conditions at room temperature of 25 °C, relative humidity of 50 % and 12 hr light/12 hr dark cycles. The rats were fed on either basal or experimental diets during feeding period and water was provided *ad libitum*.

Standard drug

Alendronate (Fosamax ®, class of bisphosphonates, Merck Sharp and Dohme Company, USA) is widely used for treatment of osteoporosis. It was purchased from a local pharmacy in the form of tablets each contains 70 mg Alendronate sodium. The administered dose 3 mg/kg b.wt of Alendronate was selected according to **Maria *et al.*, (2004).**

Basal and experimental diets

The basal diet was prepared according to the recommended dietary allowances for rats (American Institute of Nutrition, AIN) adjusted by ***Reeves et al. (1993).*** Basal diet consisted of 14 % protein, 10 % sucrose, 5 % corn oil, 0.25% choline chloride, 1% vitamin mixture***(Campbell,1963)*** , 3.5 % salt mixture ***(Hegested et al., 1941)*** and 5% fibers. The remainder was corn starch up to 100 %.

Five experimental diets were formulated as follow:

1- Basal diet fortified with 10% Nigella sativa (NS) seeds powder

2- Basal diet fortified with 10% sesame (SS) seeds powder

3- Basal diet fortified with 2% calcium carbonate (CaCO3) powder

4- Basal diet fortified with 10% NS + 2% CaCO3

5- Basal diet fortified with 10% SS + 2% CaCO3

The above formulated experimental diets were prepared to be contained either 10% NS or 10% SS or 2% CaCO3 or 12% NS and CaCO3 or 12% SS and CaCO3 and the remainder was corn starch up to 100%.

Ovariectomy procedure

Under ether anaethesia, the bilateral ovariectomy was performed in rats by making two dorsolateral incisions using sharp dissecting scissors. The skin and dorsal muscles were then cut and the peritoneal cavity was thus reached. The uterine horn was picked out and the fatty tissue around the ovary was removed. The connection between the Fallopian tube and the uterine horn was clamped by artery forceps and cut was made under the clamped area to remove the ovary. Skin was closed bilaterally with one simple catgut suture. Tincture iodine solution (antiseptic) was applied locally on the skin at both sites of the operation ***(Lasota and Danowska-Klonowska, 2004).*** Similarly sham operation was performed where the ovaries were exposed, but not removed.

Experiment and feeding of rats

The rats were randomized into to 8 equal groups of 7 animals each and kept individually in metal metabolic cages. Group (1) was sham-operated (SHAM) and fed on basal diet, while the other 7 groups were ovariectomized (OVX) and left for 3 weeks post-operation to ensure almost complete clearance of their bodies from sex hormone residues. Group (2) was kept OVX (Positive control) and fed on basal diet. Groups(3), (4), (5), (6) and (7) were fed on experimental diets fortified with: (**A**) 10% Nigella sativa (NS) powder; (**B**) 10% sesame (SS) seeds powder; (**C**) 2% calcium carbonate (CaCO3) powder ***(Agata et al. 2013)*** ; (**D**) 10% NS + 2% CaCO3 and (**E**) 10% SS + 2% CaCO3 for 6 weeks, respectively. Group (8) was orally administered Alendronate drug (standard antiosteoporotic) in daily dose of 3 mg/kg for 6 weeks and fed on basal diet. During feeding period, the initial and final body weights of rats were recorded and changes in weight gains were calculated. The last 24 hour urine samples and blood samples were collected for biochemical analyses. The rats were then euthanized by prolonged exposure to ether anesthetic and uterine horns were dissected out and weighed. Femur bones were dissected out and prepared for bone analysis.

Biochemical analyses

Blood samples were withdrawn by cardiac puncture, left standing for 10 minutes to clot and centrifuged at 4000 rpm for 15 minutes to separate the serum which kept frozen at – 18°C till biochemical analyses. Urine samples of the last 24 hour were collected, acidified with 12 Mol HCl and kept in the refrigerator till biochemical analyses. Concentrations of calcium ***(Gindler and King, 1972)*** and phosphorus ***(Goodwin, 1970)*** in serum and urine samples were colorimetrically determined using specific diagnostic reagent kits (BioMérieux, France) and measured on UV spectrophotometer. Serum bone-specific alkaline phosphate ***(Nawawi and Girgis, 2002)*** was estimated by colorimetric assay using specific enzyme kits (Sigma-Aldrich Chemical Co., USA). Serum osteocalcin concentration was measured by enzyme-linked immunosorbent assay (Rat Mid™, osteocalcin ELISA kit, USA) according to ***Craciun et al. (2000).*** Urinary pyridinoline (PD) and deoxypyridinoline (DPD) were measured using high performance liquid chromatography (HPLC). Urinary concentrations of PD and DPD were expressed as µmol/Mol creatinine according to ***Ohishi et al. (1993).*** Creatinine concentration was colorimetrically determined using Jaffe reaction according to the method of ***Husdan and Rapoport, (1968).***

Bone analysis

After sacrificing the rats, both femurs were dissected out and the soft tissues were removed. Both femur epiphyses were removed and the length of each femur was measured using Vernier caliper. Femur bone volume and density (BMD) were calculated according to principle of Archimedes ***(Doyle and Cashman, 2003).*** In brief, the femur was cut out at the mid diaphyses and bone marrow washed out. Each femur bone was placed in a vial filled with deionized water and the vial was placed in vacuum desiccator for 90 minutes. The femurs were removed from the vial, dried by blotted paper, weighed, and placed again in another vial containing deionized water. The bone was reweighed and bone volume was measured. Femur bone density (BMD) was calculated using this formula: BMD = femur weight/femur volume. To obtain the ash, femur bones were dehydrated and defatted in acetone and anhydrous ether, dried for 6 hr in an oven at 700°C. The remaining ash was weighed, solubilized with 0.1Mol/L HCl, transferred into volumetric flask and completed to 100 ml with 0.1Mol/L HCl according to ***Yang et al. (2008).*** The final solution was used for estimation of calcium and phosphorus in the ash using colorimetric methods.

Statistical analysis

Data were presented as means ± SE. Statistical analysis was performed using computerized Statistical Package of Social Sciences (SPSS) program with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests according to ***Snedecor and Cochran (1986).***

Results

The result of analysis of body weight of rats revealed that the OVX rats gained more body weight than sham (SHAM) control rats. The percentage of weight gain was 18.00 % in OVX control group versus to 11.96 % in SHAM control group. Ovariectomy in rats caused a significant (P <0.05) decrease in uterine weight when compared with SHAM control group. The mean value ± SE of uterine weight was 0.45±0.01 g in OVX control rats versus to 1.20±0.04 g in SHAM control rats and 0.99±0.02 g in Alendronate-treated rats. Feeding of OVX rats on diets fortified with Nigella sativa, sesame seeds and high calcium significantly reduced weight gain and increased uterine weight when compared to the OVX control group (Table 1).

Table 1.

Effect of diets fortified with Nigella sativa (NS), sesame seeds (SS) and high calcium carbonate (CaCO3) on body weight gain and uterine weight in ovariectomized (OVX) rats.

Groups	Body weight (g) during feeding period Initial (Week 0)	Final (Week 6)	Weight gain (%)	Uterine weight (g)
Group 1 SHAM control	255.0±7.5[a]	285.5±7.3[c]	11.96	1.20±0.04[a]
Group 2 OVX control	250.0±7.3[a]	295.0±6.1[a]	18.00	0.45±0.01[c]
Group 3 10% NS	254.0±7.6[a]	290.0±7.2[b]	14.17	0.83±0.02[b]
Group 4 10% SS	257.5±6.9[a]	288.0±6.3[b]	11.84	0.84±0.03[b]
Group 5 2% CaCO3	258.0±6.8[a]	286.5±7.6[b]	11.04	0.75±0.01[b]
Group 6 10% NS+2% CaCO3	255.0±7.1[a]	293.0±7.9[a]	14.90	0.88±0.01[b]
Group 7 10% SS+2% CaCO3	255.0±7.1[a]	285.0±7.5[a]	11.76	0.85±0.03[b]
Group 8 Alendronate(3mg/kg)	260.0±4.1[a]	298.0±8.5[b]	14.61	0.99±0.02[b]

Means ± SE with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test, n=7 rats in each group.

Bilateral ovariectomy in rats caused significant ($P < 0.05$) increases in serum levels of calcium (Ca), phosphorous (P), bone-specific alkaline phosphatase (b-ALP) and osteocalcin (OC) when compared with the SHAM control group. Experimental diets fortified with Nigella sativa, sesame seeds and high calcium significantly ($P < 0.05$) decreased the elevated serum levels of Ca, P, b-ALP and OC in OVX rats when compared to the OVX (positive) control group as recorded in Table (2).

Table 2.

Effect of diets fortified with Nigella sativa (NS), sesame seeds (SS) and high calcium carbonate (CaCO3) on serum calcium (Ca), phosphorous (P), bone specific alkaline phosphatase (b-ALP) and osteocalcin (OC) in ovariectomized (OVX) rats.

Groups	Ca (mg/dL)	P (mg/dL)	b-ALP (U/L)	OC (µg/L)
Group 1 SHAM control	10.90 ± 0.3^b	3.65 ± 0.1^b	125.0 ± 4.7^d	10.6 ± 0.01^d
Group 2 OVX control	13.20 ± 0.6^a	6.15 ± 0.2^a	179.5 ± 7.2^a	15.2 ± 0.03^a
Group 3 10% NS	11.50 ± 0.3^b	4.77 ± 0.2^b	158.5 ± 7.4^b	13.6 ± 0.01^b
Group 4 10% SS	11.60 ± 0.2^b	4.80 ± 0.1^b	156.6 ± 7.6^b	13.4 ± 0.02^b
Group 5 2% CaCO3	11.70 ± 0.3^b	4.85 ± 0.3^b	153.6 ± 6.5^b	13.2 ± 0.03^b
Group 6 10% NS+2% CaCO3	10.70 ± 0.5^b	3.66 ± 0.4^b	135.6 ± 5.2^c	11.5 ± 0.03^c
Group 7 10% SS+2% CaCO3	10.77 ± 0.4^b	3.55 ± 0.3^b	138.6 ± 6.2^c	11.3 ± 0.03^c
Group 8 Alendronate (3 mg/kg)	10.65 ± 0.2^b	3.45 ± 0.4^b	135.6 ± 8.5^c	10.8 ± 0.02^c

Means ± SE with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test, n=7 rats in each group.

The analysis of the last 24 hour urine samples showed that ovariectomy in rats caused high significant ($P < 0.001$) increases of urinary excretion Ca and P when compared to the SHAM control group. Experimental diets fortified with Nigella sativa (NS), sesame (SS) seeds and high calcium when fed to OVX rats and oral administration of drug Alendronate to OVX rats significantly ($P < 0.05$ to $P < 0.001$) decreased urinary excretion of Ca and P when compared to OVX rats as demonstrated in Fig. (1). Similarly, diets fortified with NS, SS and high calcium and oral administration of Alendronate significantly ($P < 0.05$ to $P < 0.01$) decreased urinary excretion of pyridinoline (PD) and deoxypyridinoline (DPD) as illustrated in Fig. (2).

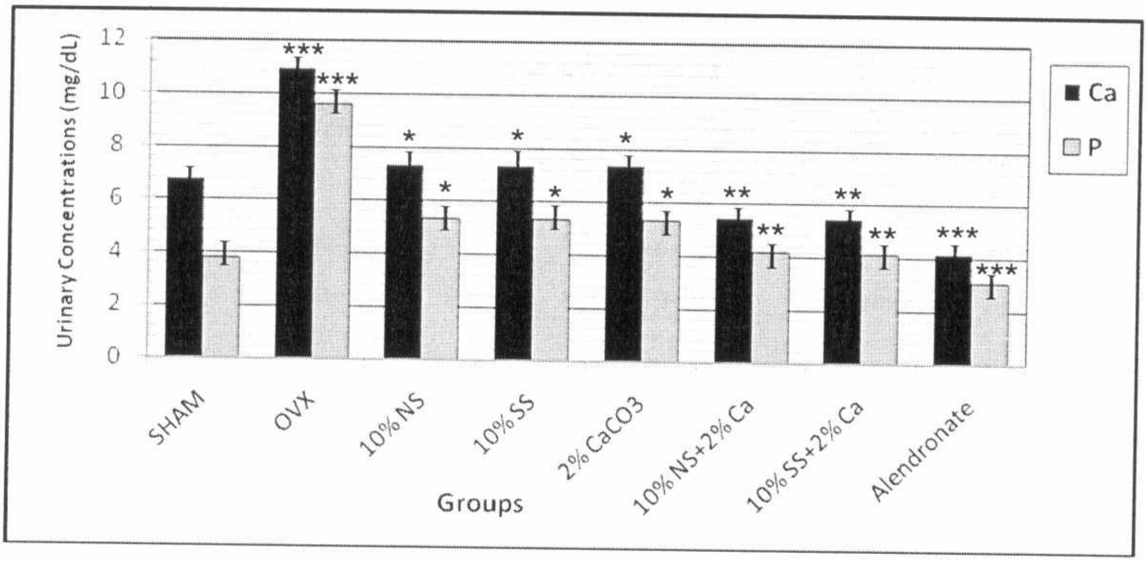

Fig. (1). Effect of diets fortified with Nigella sativa (NS), sesame seeds (SS) and high calcium carbonate (CaCO3) on urinary excretion of calcium (Ca) and phosphorus (P) in ovariectomized (OVX) rats.

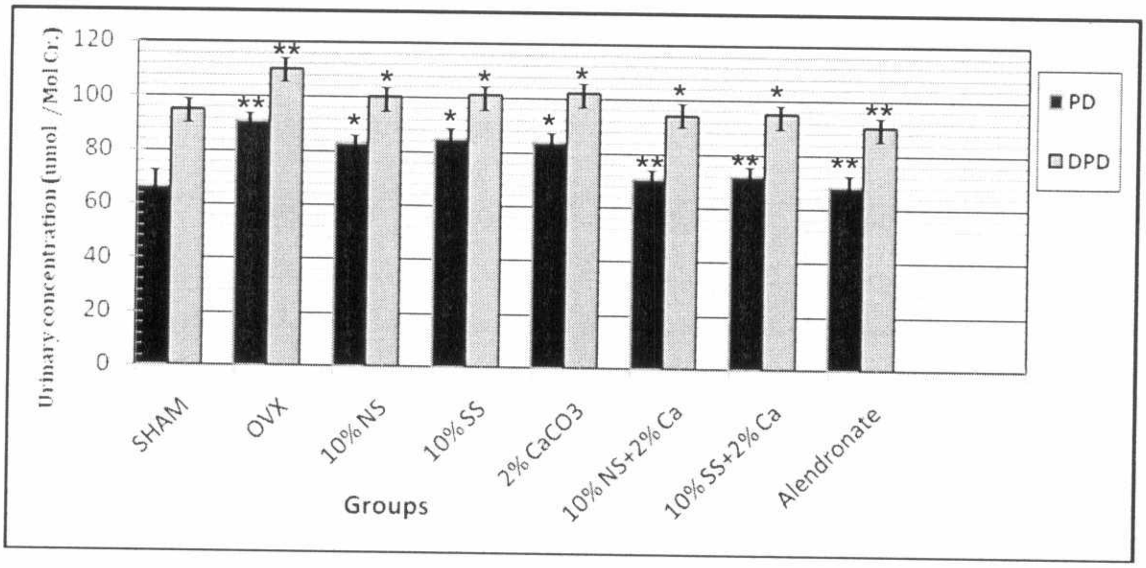

Fig. 2. Effect of diets fortified with Nigella sativa (NS), sesame seeds (SS) and high calcium carbonate (CaCO3) on urinary excretion of pyridinoline (PD) and deoxypyridinoline (DPD) in ovariectomized (OVX) rats.

Data in Table (3) showed that bilateral ovariectomy in rats induced significant ($P < 0.05$) decreases in femur weight and bone mineral density (BMD) when compared to the SHAM control group. Feeding of OVX rats on diets fortified with Nigella sativa seeds, sesame seeds and high calcium restored the ovariectomy-induced decreases in femur weight and BMD when compared to the OVX control group. Alendronate drug (standard) increased femur weight and BMD when compared to the OVX control group. No significant changes in femur length and volume between control and experimental groups were observed.

Table 3.

Effect of diets fortified with Nigella sativa (NS), sesame seeds (SS) and high calcium carbonate (CaCO3) on femur weight (Wt), length (L),volume (V) and bone mineral density (BMD) in ovariectomized (OVX) rats.

Groups	Femur Wt. (g)	Femur L (mm)	Femur V (cm^3)	BMD (g/cm^3)
Group 1 SHAM control	1.65±0.01[a]	45.01±3.75[a]	0.68±0.02[a]	2.43±0.06[a]
Group 2 OVX control	0.88±0.03[d]	43.09±3.71[a]	0.67±0.03[a]	1.31±0.02[d]
Group 3 10% NS	1.30±0.01[c]	44.10±3.25[a]	0.66±0.01[a]	1.96±0.03[c]
Group 4 10% SS	1.33±0.03[c]	43.15±3.15[a]	0.67±0.02[a]	1.98±0.02[c]
Group 5 2% CaCO3	1.32±0.02[c]	45.10±3.05[a]	0.68±0.04[a]	1.94±0.03[c]
Group 6 10% NS+2% CaCO3	1.40±0.06[b]	43.10±2.05[a]	0.69±0.03[a]	2.02±0.01[b]
Group 7 10% SS+2% CaCO3	1.45±0.04[b]	44.10±1.90[a]	0.67±0.02[a]	2.12±0.02[b]
Group 8 Alendronate (3mg/kg)	1.55±0.01[b]	45.10±2.55[a]	0.68±0.01[a]	2.28±0.01[b]

Means ± SE with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test, n=7 rats in each group.

Bilateral ovariectomy in rats produced significant ($P < 0.05$) decreases in weights of femur ash and calcium level in bone ash when compared to the SHAM control group. No significant changes in ash phosphorous content between control and experimental groups were found. Experimental diets fortified with Nigella sativa, sesame seeds and high calcium normalized weights of femur, ash, and calcium content in the ash. Alendronate drug also increased weights of femur, ash, and calcium content in the ash when compared to the OVX control group as depicted in Table (4).

Table 4.

Effect of diets fortified with Nigella sativa (NS), Sesame seeds (SS) and high calcium carbonate ($CaCO_3$) on femur ash weight and calcium(Ca) and phosphorous (P) ash levels in ovariectomized (OVX) rats.

Groups	Ash Wt. (g)	Ca (mg/g ash)	p (mg/g ash)
Group 1 SHAM control	0.95 ± 0.03^{a}	12.5 ± 0.02^{a}	7.42 ± 0.12^{a}
Group 2 OVX control	0.60 ± 0.01^{d}	6.5 ± 0.01^{d}	7.41 ± 0.13^{a}
Group 3 10% NS	0.77 ± 0.01^{c}	8.2 ± 0.03^{c}	7.42 ± 0.14^{a}
Group 4 10% SS	0.78 ± 0.03^{c}	9.0 ± 0.01^{c}	7.43 ± 0.11^{a}
Group 5 2% CaC03	0.79 ± 0.03^{c}	9.2 ± 0.02^{c}	7.42 ± 0.10^{a}
Group 6 10% NS+2% CaC03	0.84 ± 0.02^{b}	10.6 ± 0.02^{b}	7.40 ± 0.12^{a}
Group 7 10% SS+2% CaC03	0.88 ± 0.02^{b}	11.9 ± 0.03^{b}	7.41 ± 0.13^{a}
Group 8 Alendronate (3mg/kg)	0.92 ± 0.03^{b}	12.1 ± 0.02^{b}	7.44 ± 0.10^{a}

Means ± SE with different superscript letters in the same column are significant at $P < 0.05$ using one way ANOVA test , n=7 rats in each group.

Discussion

The present study aimed to evaluate the effect of diets fortified with Nigella sativa, sesame seeds and high calcium on serum, urinary and bone markers of osteoporosis in ovariectomized (OVX) rats.

Estrogen is the most potent inhibitor of osteoclastic bone resorption (loss), so estrogen deficiency is a major risk factor in the pathogenesis of osteoporosis ***(Gambacciani and Ciaponi, 2000).*** Bilateral ovariectomy in rats caused dramatic decreases in uterine weight, bone mineral content, density and biomechanical strength due to estrogen deficiency ***(Mori-Okamoto, et al., 2004; Coxam, 2005; Srikanta et al., 2011 and Zhang et al., 2013).*** Postmenopausal osteoporosis is commonly treated by estrogen replacement therapy and/or by some drugs such as Alendronate (one of Bisphosphonates series) which inhibits osteoclast-mediated bone resorption ***(Srikanta et al., 2011).***

Free radicals are known to be the main cause of oxidative stress which is grossly implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, and osteoporosis. Natural antioxidants have gained much attention from consumers because they are considered safer than synthetic antioxidants. Natural antioxidants derived from fruits, vegetables, spices, and cereals are very effective and can protect the human body from oxidative stress caused by ROS ***(Sreeramulu and Raghunath, 2010).***

Results of the present study showed that diets fortified with Nigella sativa, sesame seeds and high calcium prevented the increase in weight gain and the decrease in uterine weight in ovariectomized rats and turned the changes in body and uterine weights to near the normal weights of SHAM-operated rats. Estrogen was reported to increase the vascularity, growth and weight of the uterus in immature rats and mice ***(Shalaby, 1977).*** The decreased weight in uterus induced by ovariectomy could be attributed to estrogen deficiency in OVX rats. This finding was previously also reported by ***Srikanta et al. (2011)*** who found that bilateral ovariectomy in rats significantly increased body weight and decreased uterine weight. The increases in body weight gain and serum levels of bone- specific alkaline phosphatase (b-ALP) and osteocalcin (OC) in OVX rats, reported in this study, were similar to the previously reported by ***Ke et al. (1997); Tamir et al. (2001); Coxam (2005) and Srikanta et al. (2011)*** who concluded that increases in body weight gain and serum b-ALP and OC are due to estrogen deficiency in OVX rats and mice. Serum b-ALP and OC are commonly used as biochemical markers of bone formation. Serum high levels of b-ALP and OC after feeding diets fortified with Nigella sativa, sesame seeds and high calcium could be possibly due to an increased osteoblastic activity ***(Srikanta et al. (2011).***

Calcium, vitamin D, and parathyroid hormone are critical regulators of bone remodeling **(Lu *et al.*, 2013).** Calcium and phosphorus are widely used as markers for bone formation as they have a vital role in bone mineralization ***(Evans et al., 1990 and Choi and Seo, 2013).*** In the present study, the bilateral ovariectomy increased serum calcium and phosphorus levels as compared to sham-operated rats. The decreases in serum calcium and phosphorous levels were reported to be due to estrogen deficiency in ovariectomized rats ***(Choi and Seo, 2013).*** Diets fortified with Nigella sativa and sesame seed and high calcium reduced serum calcium levels denoting that calcium may be deposited in bones, so increasing bone mineral density and calcium content in bone ash.

The results of urine analysis showed that bilateral ovariectomy in rats increased urinary excretion of calcium, phosphorous, pyridinoline (PD) and deoxypyridinoline (DPD). Feeding of diets fortified with Nigella sativa, sesame seed and high calcium decreased urinary excretion of Ca, P, PD and DPD in OVX rats. Urinary pyridinoline (PD) and deoxypyridinoline (DPD) are commonly used as biochemical markers of bone resorption (loss). Urinary PD and DPD were found to be released into the blood during bone degradation and rapidly excreted in the urine ***(Samma et al. 1997).*** The values of urinary PD and DPD were significantly higher in females than in males and increased in the urine during early postmenopausal period ***(Ohishi et al., 1993).*** In rats fed on diet fortified with 10% sesame seeds, the decreased urinary excretion of PD and DPD, reported in this study, denoted that these seeds could prevent bone loss in OXV rats. This finding was partially similar to that reported by ***Boulbaroud et al., (2008)*** who concluded that sesame oil can prevent bone loss in ovariectomized rats. Sesamin a major lignan compound in sesame oil was reported to produce an osteoprotective effect ***(Wanachenwin et al., 2012).***

The mechanism(s) of anti-osteoporotic activity of Nigella sativa seeds could be due to antioxidant and anti-inflammatory effects of thymoquinone partly via modulation of osteoblast maturation because osteoporosis has been positively linked to the oxidative stress and inflammation ***(Shuid et al., 2012).*** Other mechanism may be due to high contents of polyphenolic and flavonoid compounds in Nigella sativa ***(Draper et al., 1997)*** and in human ***(Potter et al., 1998).***

The anti-osteoporotic effect of sesame seeds could be due to presence of high contents of essential minerals especially calcium and phosphorous which play a vital role in bone mineralization. Sesame seeds also contain high contents of natural antioxidant lignans which prevent bone loss in ovariectomized rats **(Boulbaroud *et al.*, 2008)**. In addition, high calcium plus vitamin D3 diet was reported to play a vital role in bone mineralization and so preventing osteoporosis ***(Chen et al., 2013).***

In conclusion, the results denote that diets fortified with Nigella sativa, sesame seeds and high calcium has an anti-osteoporotic effect in ovariectomized rats. The study suggests that Nigella sativa and sesame seeds appear to be promising natural food supplements for the treatment of osteoporosis. Therefore, the study recommends enrichment of the bakeries and food stuff with Nigella sativa and sesame seeds and eating meals rich in calcium such as dairy products (milk and yoghurt) may be beneficial for the treatment of postmenopausal osteoporosis in women. In addition, isolation of bioactive constituents from Nigella sativa and sesame seeds is necessary to search for safe and bioactive natural substances that could be developed for osteoporotic therapy.

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تأثير الوجبات الغذائية المدعمة بحبة البركة وبذور السمسم وتركيز عال من الكالسيوم على هشاشة العظام في الفئران مستأصلة المبيض

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الملخص العربى

أجريت هذه الدراسة لتقييم تأثير الوجبات الغذائية المدعمة بحبة البركة وبذور السمسم وتركيز عال من الكالسيوم على هشاشة العظام فى الفئران مستأصلة المبيض. فتم توزيع عدد ٥٦ من فئران التجارب عشوائيا إلى ٨ مجموعات متساوية كل منها ٧ فئران ، ووضعت منفردة فى أقفاص الأيض. استخدمت المجموعة (١) كمجموعة ضابطة سالبة (SHAM) حيث أجريت العملية الجراحية للفئران بدون استئصال المبيضين تحت تأثير مخدرالإثير ، وتم تغذيتها على الوجبة الأساسية. و تم استئصال المبيضين فى فئران المجموعات السبعة الأخرى تحت تأثير مخدرالإثير . وبعد ثلاثة اسابيع من إجراء العملية الجراحية (Ovariectomy) ، استخدمت المجموعة (٢) كمجموعة ضابطة موجبة وتم تغذيتها على الوجبة الاساسية ، بينما تم تغذية فئران المجموعات (٣) ، (٤) ، (٥) ، (٦) و (٧) على وجبات غذائية مدعمة بما يلى :- ١٠% حبة البركة ، ١٠ % بذور السمسم ، ٢% كربونات الكالسيوم ، ١٠% بذور حبة البركة + ٢% كربونات الكالسيوم و١٠ % بذور السمسم + ٢% كربونات الكالسيوم على التوالى لمدة ٦ اسابيع. وتم اعطاء المجموعة (٨) دواء الينذرونات (فوساماكس) الذى يستخدم لعلاج هشاشة العظام بجرعة ٣مجم/كجم يوميا عن طريق الفم لمدة ٦ اسابيع وتم تغذيتها على الوجبة الأساسية. وفى بداية ونهاية فترة التغذية تم وزن الفئران ، وفى نهاية فترة التجربة تم تجميع عينات بول آخر ٢٤ ساعة لقياس تركيزات الكالسيوم ، الفوسفور، بيريدينولين و أوكسى بيريدينولين فى البول .وتم تخدير الفئران لإحداث موت رحيم لها وسحبت عينات دم لقياس مستويات الكالسيوم ، الفوسفور ، إنزيم الكالين فوسفاتيز الخاص بالعظم (bone-specificALP) و اوستيوكالسين فى المصل.. ، و استخراج الرحم ووزنه وأخذ عظام الفخذ للقياس والتحليل الكيميائى لرماد العظام . وأظهرت النتائج أن تغذية الفئران مستأصلة المبيض على وجبات غذائية مدعمة بحبة البركة وبذور السمسم , وتركيز عال من الكالسيوم أدت إلى زيادة وزن الرحم وعودة تركيزات القياسات البيوكيميائية السابقة فى المصل والبول إلى مستويات متقاربة من المستوى الطبيعى. كما أدت الى زيادة كثافة المعادن فى عظام الفخذ وزيادة محتوى الكالسيوم فى رماد العظم. وتدل النتائج أن تغذية الفئران مستأصلة المبيض على وجبات غذائية مدعمة بحبة البركة وبذور السمسم وتركيز عال من الكالسيوم أدت الى تأثير مضاد لهشاشة العظام. وتوصي الدراسة بأن تناول المخبوزات و انواع الاطعمة المدعمة بحبة البركة وبذورالسمسم ، مع تناول أطعمة غنية بالكالسيوم مثل منتجات الألبان (الحليب والزبادي) قد يكون مفيدا لعلاج هشاشة العظام عند النساء بعد سن اليأس نتيجة نقص الاستروجين .