Osteoprotective effect of soybean and sesame oils in ovariectomized rats via estrogen-like mechanism

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Abstract The purpose of the present study was to investigate the osteoprotective effects of soybean oil (SbO) and sesame oil (SO) in ovariectomized (OVX) rats. The results indicated that the OVX rats exhibited a significant decrease in Ca and P level in both serum and bone, the activities of the antioxidant enzymes SOD and CAT and the antioxidant biomarker GSH accompanied with a marked increase in the oxidative stress markers MDA and PC, the inflammatory indices (TNF- α , CRP levels, WBCs counts and ACP activity) in, both, bone and serum. Supplementating the diet of the OVX rats with SbO (15 % w/w) or SO (10 % w/w) for 2 months to resulted in modulation of the alterations in all tested parameters and succeeded to restore minerals, antioxidant enzymes, antioxidant biomarkers, oxidative stress markers, inflammatory indices, and WBCs counts. It could be concluded that the consumption of diets supplemented with SbO or SO might be useful for preventing bone loss caused by estrogen deficiency in ovariectomy status.

Keywords Ovariectomized rats · Estrogen deficiency · Soybean oil · Sesame oil · Bone loss · Phytoestrogens

Abbreviations

SbO	Soybean oil
SO	Sesame oil
OVX	Ovariectomized
Ca	Calcium
P	Phosphorous
ACP	Acid phosphatase
SOD	Super oxide dismutase
CAT	Catalase
GSH	Glutathione
RBCs	Red blood cells
MDA	Malondialdehyde
PC	Protein carbonyl
TNF	Tumor necrosis factors
CRP	C-reactive protein
WBCs	White blood cells

Introduction

Ovariectomy is often associated a progressive loss of bone mass through a process similar to what occurs during postmenopausal osteoporosis (Muthusami et al. 2005). In this regard, estrogen deficiency has been considered as a major cause for bone loss (Riggs et al. 2003; Hassan et al. 2013). The underlying mechanism may be related to increased generation of oxygen free radicals (OFR) and prevalence of oxidative stress (Lean et al. 2005). Recently, estrogen has been indicated as antioxidant agent, through suppressing

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free radicals, mainly reactive oxygen species and increasing the expression of glutathione peroxidase in osteoclasts (Choi and Song 2009), leading to decreased osteoclasts activity and bone loss. There is evidence that oxidative stress is enhanced in females after gonadectomy and that estrogen deficiency increases peroxidation products and decreases antioxidant defenses in the bone cells (Jagger et al. 2005).

Other investigators have focused on the possible involvement of inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1) and IL-6 in stimulating bone resorption under estrogen deficiency conditions (Yoneda et al. 2004), thus, emphasizing a relation between inflammation and bone loss.

Recently, much attention has been focused on the health benefits of diets rich in phytoestrogens. Phytoestrogens are natural plant derived compounds that structurally mimic mammalian estrogens and have ability of exhibiting estrogenic properties (Potter and Steinmetz 1996; Wu 2007; Hassan et al. 2013). Among the most famous phytoestrogens are isoflavones which are found in abundance in soybeans and their derivatives, such as soybean oil (Messina et al. 1994). Lignans constitute other phytoestrogenic compounds, existing extensively in vegetables, whole grain cereals and oil seeds, such as sesame seed oil (Adlercreutz 2002). Sesame oil (SO) would exert positive effects on bone, through its composition characterized by a low level of saturated fatty acids and the presence of antioxidants such as sesamin, sesamol and sesamol (Suja et al. 2004).

Many women turn to phytoestrogens as an alternative to estrogen replacement therapy (ERT) because of their undesirable side effects, such as increased risk of breast and endometrial cancer and irregular bleeding (Wagner et al. 2001). Recent studies suggested the protective role of phytoestrogens against various diseases, such as atherosclerosis, cardiovascular diseases, osteoporosis, and certain types of cancers (Amir et al. 2010). Many of these actions may occur via inhibiting inflammation and oxidative stress (Ishihara and Hirano 2002), beside induction of apoptosis in cancer cells and suppression of angiogenesis (Zhang et al. 2009). Taken into account the estrogenic properties of soybean and sesame oils, the present study aimed to compare the ability of the two oils to protect against estrogen-deficiency associated bone loss in ovariectomized rats. This was achieved in terms of several bone biomarkers, particularly those related to oxidative stress and inflammation process.

Materials and methods

Animals

Three-month-old virgin female Wistar rats (170 ± 5 g) were obtained from the Institute of Ophthalmic Disease Research (Cairo, Egypt). Rats were housed in stainless steel cages at 22–25 °C and 12-h light/dark cycle with free access to standard food and water for 1 week of acclimation prior to treatment. All animals received humane care in compliance with the guidelines of the Mansoura University, and the protocol conformed to the guidelines of the National Institutes of Health.

Experimental diets

The standard diet was supplemented with soybean oil (SbO) at a dose of 15 % w/w or sesame oil (SO) at a dose of 10 % w/w as described by Shuid et al. (2007) and Boulbaroud et al. (2008), respectively. Mixtures were made into pellets form to be used as experimental diets. Both, SbO and SO were purchased from a local market at Mansoura city, Egypt.

Study design

After the acclimation period, rats were divided into six groups (6 rats/group). The first was considered as normal control (NC) group fed on standard diet without any supplementation. The second group consisted of normal rats fed on standard diet supplemented with SbO (15 %, w/w). The third group consisted of normal rats fed on standard diet supplemented with SO (10 %, w/w). In the forth group, rats were bilateral ovariectomized (OVX) and received standard diet without any supplementation. Rats in fifth and sixth groups were OVX supplied with standard diet plus SbO (OVX + SbO) or SO (OVX + SO), respectively, as described in the above groups. Bilateral ovariectomy was done according to the method described by Lien et al. (2009). Dietary treatments were started 2 weeks post-ovariectomy and continued for 2 months.

Samples collection

At the end of the study period, all rats were fasted overnight then two blood samples were collected from the jugular vein. The first was taken on EDTA as anticoagulant for determination of hematological parameters. The second blood sample was collected in

chilled non-heparinized tubes and centrifuged at $860\times g$ for 20 min at 4°C , and the sera were kept at -20°C until used for further analysis. Animals were anesthetized by diethyl ether, sacrificed and dissected. Both left and right femora were immediately washed using chilled saline solution. The left femur was weighed, minced and homogenized in ice-cold saline solution using a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at $860\times g$ for 20 min at 4°C , and the supernatant was collected for biochemical analysis. While the right femur was weighed and used for the determination of bone mineral density (BMD) according to Archimedes principle (Lee et al. 2004b).

Biochemical analysis

Malondialdehyde (MDA) and protein carbonyl (PC) levels were determined according to the methods of Ohkawa et al. (1982) and Smith et al. (1991), respectively. Reduced glutathione (GSH) was assessed based on the method of Prins and Losse (1969). Superoxide dismutase (SOD) and catalase (CAT) activities were determined as described by Niskikimi et al. (1972) and Bock et al. (1980), respectively. Estradiol was estimated according to Bock et al. (1980) using Immulite analyzer Kit (Paris, France). Tumor necrosis factor (TNF)- α level was estimated using an ELISA Kit (Diagnostic Products Corp., Los Angeles, CA, USA) as described by Aggarwal et al. (1985), while C-reactive protein (CRP) level was determined by the Turbox plus analyzer (Peltola et al. 1983). Calcium (Ca) and phosphorus (P) levels were estimated according to Gindler and King (1972) and El-Merzabani et al. (1977), respectively, using kits supplied by Bio-diagnostic Co. (Cairo, Egypt). Acid phosphatase (ACP) activity was estimated as described by Kind and King (1954) using ABC diagnostic kits (Cairo, Egypt). Red blood cells (RBCs), white blood cells (WBCs) and hemoglobin (Hb) were estimated using a Sysmex Cell Counter (Sysmex, Kobe, Japan) as described by Dacie and Lewis (1991). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Statistical analysis

Results were expressed as mean $\pm$ SE. Statistical significance was calculated using one-way analysis of

variance (ANOVA) followed by Duncan's multiple range test (Waller and Duncan 1969). All the statistical analysis was carried out with the use of SPSS 12.00 software. Differences were considered significant at $P \leq 0.05$.

Results

As shown in Tables 1, 2 and 3 the recorded data did not show any significant changes between normal control (NC) rats and normal rats fed SbO or SO supplemented diets, indicating safety and nontoxic effect of these oils. However, a significant decrease in serum minerals (Ca, P) and estradiol levels, but increase in ACP activity, as well as inflammatory markers (TNF- α and CRP) were recorded in OVX rats compared to NC group. However, feeding OVX rats on SbO or SO supplemented diets for 2 months caused significant improvement in these parameters (Table 1). Additionally, the current results showed significant decreases in bone mineral density (BMD), minerals (Ca, P), enzymatic and non-enzymatic antioxidants (SOD, CAT and GSH), accompanied with significant increase in oxidative stress biomarkers (MDA and PC), as well as ACP activity in OVX rats compared to the NC group. In contrast, administration of SbO or SO supplemented diets to OVX rats showed significant amelioration in the bone changes, indicating their osteoprotective effects (Table 2). Besides, a significant reduction in the RBCs count and the RBCs indices (Hb, MCV, MCH and MCHC) accompanied with a significant increase in the WBCs count was recorded in OVX rats compared to the NC group. However, administration of SbO or SO supplemented diets to OVX rats exhibited marked improvement in these changes, but no significant differences were found among the two oil-supplemented groups, regarding these indices (Table 3), as well as almost all tested parameters (Tables 1, 2).

Discussion

Ovariectomy has shown to increase risk of osteoporosis as occurs with post menopausal women. Osteoporosis is a metabolic bone disease characterized by loss of bone mass thus making bone more susceptible to fractures (Sakai et al. 1998). In the present study,

Table 1 Serum estradiol, minerals (Ca, P), TNF- α and CRP levels and ACP activities in the normal control and the different experimental groups

Parameters	Animal groups					
	NC	SbO	SO	OVX	OVX + SbO	OVX + SO
Estradiol (pg/ml)	34.60 $\pm$ 0.9 ^a	34.71 $\pm$ 0.84 ^a 0.31	34.62 $\pm$ 0.5 ^a 0.05	17.91 $\pm$ 0.5 ^b −48.23	25.83 $\pm$ 0.7 ^c −25.34	24.83 $\pm$ 0.7 ^c −28.23
Ca (mg/dl)	8.84 $\pm$ 0.05 ^a	8.97 $\pm$ 0.05 ^a 1.47	8.84 $\pm$ 0.13 ^a 0.00	7.06 $\pm$ 0.05 ^b −20.13	7.93 $\pm$ 0.02 ^c −10.29	7.91 $\pm$ 0.11 ^c −10.52
P (mg/dl)	3.77 $\pm$ 0.04 ^a	3.79 $\pm$ 0.03 ^a 0.53	3.82 $\pm$ 0.03 ^a 1.32	3.10 $\pm$ 0.05 ^b −17.77	3.48 $\pm$ 0.03 ^c −7.69	3.47 $\pm$ 0.02 ^c −7.95
ACP (K.A.U/dl)	4.21 $\pm$ 0.07 ^a	4.21 $\pm$ 0.06 ^a 0.00	4.20 $\pm$ 0.06 ^a −0.23	11.29 $\pm$ 0.06 ^b 168.17	8.12 $\pm$ 0.07 ^c 92.87	8.35 $\pm$ 0.09 ^d 98.33
TNF- α (pg/dl)	9.33 $\pm$ 0.15 ^a	9.25 $\pm$ 0.17 ^a −0.85	9.32 $\pm$ 0.15 ^a −0.10	17.24 $\pm$ 0.29 ^b 84.78	11.24 $\pm$ 0.14 ^c 20.47	11.57 $\pm$ 0.22 ^c 24.00
CRP (mg/dl)	5.25 $\pm$ 0.11 ^a	5.10 $\pm$ 0.05 ^a −2.85	5.18 $\pm$ 0.08 ^a −1.33	43.82 $\pm$ 1.38 ^b 734.66	24.55 $\pm$ 0.33 ^c 367.61	25.38 $\pm$ 0.51 ^c 383.42

Results are means $\pm$ SE of six determinations, with % of change compared to the NC group. Values with different superscripts (a–d) in each vertical column differ from each others significantly ($P \leq 0.05$)

NC normal control, SbO soybean oil, SO sesame oil, OVX ovariectomized

Table 2 Bone mineral density (BMD), minerals, antioxidant and oxidative stress biomarker levels and acid phosphatase (ACP) activity in the normal control and the different experimental groups

Parameters	Animal groups					
	NC	SbO	SO	OVX	OVX + SbO	OVX + SO
BMD (g/cm ²)	3.05 $\pm$ 0.16 ^a	3.07 $\pm$ 0.10 ^a 0.65	3.16 $\pm$ 0.14 ^a 3.60	2.05 $\pm$ 0.03 ^b −32.78	2.72 $\pm$ 0.11 ^c −10.81	2.55 $\pm$ 0.03 ^c −16.39
Ca (mg/mg)	153.33 $\pm$ 2.7 ^a	154.00 $\pm$ 2.7 ^a 0.43	153.83 $\pm$ 2.9 ^a 0.32	91.08 $\pm$ 6.82 ^b −40.59	119.16 $\pm$ 3.1 ^c −22.28	113.83 $\pm$ 5.3 ^d −25.76
P (mg/mg)	77.24 $\pm$ 1.64 ^a	78.39 $\pm$ 1.08 ^a 1.48	77.98 $\pm$ 1.13 ^a 0.95	50.90 $\pm$ 1.36 ^b −34.10	62.68 $\pm$ 1.06 ^c −18.85	63.69 $\pm$ 0.72 ^c −17.54
ACP (K.A.U/g)	0.17 $\pm$ 0.004 ^a	0.17 $\pm$ 0.006 ^a 0.00	0.17 $\pm$ 0.006 ^a 0.00	0.36 $\pm$ 0.006 ^b 111.76	0.27 $\pm$ 0.007 ^c 58.82	0.29 $\pm$ 0.006 ^c 70.58
MDA (nmol/g)	13.69 $\pm$ 0.22 ^a	13.24 $\pm$ 0.26 ^a −3.28	13.21 $\pm$ 0.34 ^a −3.50	25.99 $\pm$ 0.91 ^b 89.84	17.75 $\pm$ 0.49 ^c 29.65	17.76 $\pm$ 0.48 ^c 29.73
P (μ mol DNP/g)	0.33 $\pm$ 0.01 ^a	0.32 $\pm$ 0.02 ^a −3.03	0.32 $\pm$ 0.02 ^a −3.03	0.61 $\pm$ 0.01 ^b 84.84	0.46 $\pm$ 0.02 ^c 39.39	0.45 $\pm$ 0.02 ^c 36.36
GSH (mg/g)	0.52 $\pm$ 0.01 ^a	0.59 $\pm$ 0.01 ^a 13.46	0.52 $\pm$ 0.01 ^a 0.00	0.41 $\pm$ 0.01 ^b −21.15	0.49 $\pm$ 0.01 ^c −5.76	0.49 $\pm$ 0.01 ^c −5.76
SOD (U/g)	115.25 $\pm$ 0.49 ^a	115.75 $\pm$ 0.59 ^a 0.43	115.58 $\pm$ 0.66 ^a 0.28	87.74 $\pm$ 1.55 ^b −23.87	100.40 $\pm$ 0.38 ^c −12.88	98.14 $\pm$ 1.49 ^c −14.84
CAT (μ mol/sec/g)	13.74 $\pm$ 0.48 ^a	13.81 $\pm$ 0.38 ^a 0.50	13.95 $\pm$ 0.27 ^a 1.52	5.54 $\pm$ 0.18 ^b −59.68	10.55 $\pm$ 0.50 ^c −23.21	9.79 $\pm$ 0.25 ^c −28.74

Results are means $\pm$ SE of six determinations, with % of change compared to the NC group. Values with different superscripts (a–d) in each vertical column differ from each others significantly ($P \leq 0.05$)

NC normal control, SbO soybean oil, SO sesame oil, OVX ovariectomized

Table 3 Hematological indices in the normal control and the different experimental groups

Parameters	Animal groups					
	NC	SbO	SO	OVX	OVX + SbO	OVX + SO
RBCs ($10^6/\mu\text{l}$)	5.53 ± 0.24^a	5.73 ± 0.28^a 3.61	5.78 ± 0.29^a 4.52	4.23 ± 0.15^b −23.50	5.41 ± 0.19^a −2.16	5.35 ± 0.06^a −3.25
Hb (g/dl)	13.60 ± 0.22^a	13.83 ± 0.24^a 1.69	13.93 ± 0.17^a 2.42	8.25 ± 0.16^b −39.33	10.87 ± 0.06^c −20.07	10.61 ± 0.17^c −21.98
Hct %	42.77 ± 1.26^a	43.07 ± 0.77^a 0.70	43.46 ± 0.82^a 1.61	27.30 ± 0.80^b −36.17	38.61 ± 0.96^c −9.72	37.61 ± 0.84^c −12.06
MCV (fl)	75.34 ± 1.26^a	74.16 ± 1.36^a −1.26	74.19 ± 1.34^a −1.52	57.52 ± 1.35^b −23.65	70.13 ± 1.26^c −6.91	70.09 ± 1.14^c −6.96
MCH (pg/ml)	23.59 ± 1.85^a	23.41 ± 1.77^a −0.76	23.00 ± 1.32^a −2.50	18.08 ± 1.59^b −23.35	20.47 ± 1.56^c −13.22	20.63 ± 1.39^c −12.54
MCHC %	32.19 ± 1.99^a	32.91 ± 0.32^a 2.23	33.00 ± 1.36^a 2.51	26.26 ± 2.29^b −18.42	29.99 ± 1.48^a −6.83	30.01 ± 1.11^a −6.77
WBCs ($10^3/\mu\text{l}$)	3.26 ± 0.19^a	2.95 ± 0.06^a −9.50	2.98 ± 0.03^a −8.58	10.78 ± 0.28^b 230.67	6.73 ± 0.32^c 106.44	6.80 ± 0.23^c 108.58

Results are means $\pm$ SE of six determinations, with % of change compared to the NC group. Values with different superscripts (a–c) in each vertical column differ from each others significantly ($P \leq 0.05$)

NC normal control, SbO soybean oil, SO sesame oil, OVX, ovariectomized

OVX rats had shown marked bone loss, as evidenced by significant decreases in femoral bone minerals (Ca and P) and bone mineral density (BMD).

Several studies have indicated a relation between ovariectomy-induced bone loss and estrogen deficiency (Riggs et al. 2003; Lien et al. 2009; Hassan et al. 2013). Estrogen plays an important role in regulating bone metabolism and its deficiency was found to cause negative bone remodeling balance that augments bone loss and increases incidence of osteopenia. A number of mechanisms may contribute to this effect; however increased oxidative stress has a central role (Watkins et al. 2005). This suggestion was further confirmed in the present study through the finding of decreased estrogen level, along with increased levels of malondialdehyde (MDA) and protein carbonyl (PC) in the bone of OVX rats, thus, indicating high level of free radicals generation and oxidative stress due to estrogen deficiency.

Oxidative stress represents an imbalance between production of reactive oxygen species (ROS) and the ability of the biological system to readily detoxify these reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of tissues can cause toxic effects through production of peroxides that damage cell components, including

proteins, lipids, and DNA (Janssen-Heininger et al. 2008). Previous studies supported enhanced oxidative stress in female rats after gonadectomy and further proposed that ovarian sex hormones protect tissues against oxidative stress and decrease the oxidative cell damage (Hassan and Abdel-Wahhab 2012). There is evidence that estrogen deficiency in states of ovariectomy activates ROS, such as H_2O_2 for induction of bone loss through increased osteoclastic activity (Riggs et al. 2003). There is evidence that H_2O_2 not only augments osteoclastic activity, but also is essential for osteoclastic differentiation (Steinbeck et al. 1994). The current findings of increased H_2O_2 , MDA and PC in the femur of OVX rats may indicate that estrogen deficiency stimulates ROS that activates osteoclasts resulting in oxidative damage of bone.

In this regard, it was explained that cells maintain their integrity against damaging oxygen free radical with the help of the antioxidant system including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) (Clark 2002). Therefore, the decrease in bone SOD, CAT and GSH reported herein supported those findings and indicated the enhancement of oxidative stress as a result of bone tissue damage.

It was reported that the impact of oxidative stress on bone is bidirectional, affecting not only bone tissue, but also bone vascular hemostasis. This is expected, since excess production of ROS can lead to oxidative hemolysis of RBCs and decreased survival of oxidized RBCs in blood (Chiu and Liu 1997). Oxidized RBCs may activate inflammation of the vascular endothelium (Blum 2009), with consequent reduction in bone blood flow and thereby bone mass. Accordingly, the present hematological alterations may be manifested by increased oxidative stress.

In this context, recent studies have considered oxidative stress as a major contributor for activation of immune functions which may affect cellular inflammatory response (Khansari et al. 2009). According to Fernandes et al. (2003), the disruption in immune response and inflammation may be a responsible factor for bone loss. Only a few years ago the immune system was linked to bone loss as indicated by the elevation in the inflammatory cytokines such as TNF- α , IL-6 and IL-1 in osteoporotic states (Yoneda et al. 2004). Other inflammatory mediators including TNF- α , CRP (Pacifci et al. 1991) and total leukocytes (Kim et al. 2005) were also associated with the risk of osteoporosis. In consistence, Boulbaroud et al. (2008) reported an increased risk of developing osteoporosis in various inflammatory conditions, such as rheumatoid arthritis, hematological diseases and inflammatory bowel disease. In these conditions, certain pro-inflammatory cytokines such as (TNF- α , IL-6 and IL-1) are elevated which may activate osteoclastogenesis by stimulating self-renewal and inhibiting apoptosis of osteoclasts progenitors (Jilka et al. 1998). Additional studies in this concern demonstrated that when the body is subjected to estrogen deficiency as in postmenopausal or OVX females, an increased production of proinflammatory cytokines occurs, which may increase osteoclasts activity and bone resorption (Papanicolaou et al. 1998). However, estrogen can lead to inhibition of cytokines, with stimulation of osteoprotegerin (OPG). OPG is a potent anti-osteoclastogenic factor that inhibits osteoclasts activity (Hofbauer et al. 1999).

Naturally, active osteoclasts reside on the mineralized surface of bone where they release free radicals and lysosomal enzymes for dissolving the minerals and degrading bone matrix (Watkins and Seifert 2000). In the present study, the increased levels of total leukocytes, TNF- α and CRP, along with the

increased activity of the lysosomal enzyme, ACP in the bone tissue of OVX rats enhanced osteoclastic activity with consequent bone loss.

Recently, considerable attention has been given to the nutritional factors that can prevent estrogen deficiency associated bone loss. In the present study, feeding diets supplemented with oils rich in phytoestrogens (SbO or SO) exhibited positive effects on BMD, bone minerals (Ca, P) and other bone biomarkers. However, both phytoestrogenic oils exhibited comparable effects regarding all tested parameters, indicating almost similar ability of both oils to protect against bone loss.

Phytoestrogens are plant derived polyphenolic compounds, which are widely used for treatment and protection against various health problems, including bone diseases (Wilkinson et al. 2002; Hassan et al. 2013). Phytoestrogens are thought to protect from bone loss mainly through estrogen dependent mechanisms as phytoestrogens seemed to directly increase estrogen levels, as seen in the present study and in other investigations (Rice and Whitehead 2008). Phytoestrogens associated estrogen raise may be related to their structural similarity to estrogens at the molecular level which may help to exert estrogen like activities. It is well documented that estrogen has high affinity towards estrogen receptor (ER) α and ER β on osteoblasts (Beral et al. 2002) and activation of ER complex is vital in maintaining bone remodeling processes (Manolagas et al. 2002). Because phytoestrogens have a stable structure and low molecular weight, they can pass through cell membranes (Toran-Allerand et al. 2002) and interact with estrogen receptors (ER) which allow them to act through the same intracellular pathways of estrogens.

Phytoestrogens are polyphenolic compounds featuring with 1–3 hydroxyl (–OH) groups that resemble the naturally occurring hydroxyl group on the phenol ring of estrogen. These OH groups attach free radicals through hydrogen electron denotation (Clark 2002) resulting in stable antioxidant state. Phytoestrogens may play an antioxidant role not only by scavenging reactive oxygen species, but also via stimulating activity of the antioxidant enzymes (SOD, GSH-Px and CAT) (Muñoz-Castañeda et al. 2005). According to Sierens et al. (2001), the possible candidates for such effect are a group of chemicals, including isoflavones (Lund et al. 2007) and lignans (Potter and Steinmetz 1996), being found as the main

phytoestrogens in soybeans and sesame seeds and their derivatives, respectively.

Naturally occurring isoflavones that have estrogenic activity are: genistein, glycitein and daidzein. Recently, they attracted considerable attention because of their antioxidant properties (Cena and Steinberg 2011). Soy isoflavones have shown to restore cellular oxidative balance and to protect against diseases associated with oxidative stress, such as osteoporosis (Sies and Stahl 1995).

Interestingly, another phytoestrogen class (lignans) also showed potent antioxidant activities (Sowmya et al. 2009). Sesame seeds contain abundant amounts of lignans and the most well known are sesamin, sesamol, and sesamolol which have antioxidant activity, through scavenging free radicals and reducing oxidative stress (Wu et al. 2009). In another way, sesame may exert an antioxidant influence through its particular composition characterized by the presence of vitamin E that acts as a good antioxidant (Sharma et al. 2000). The current findings of reduced oxidative stress of bone and enhanced antioxidant performance in OVX rats fed on SbO and SO, may be due to the osteoprotective effect of phytoestrogen rich oils.

Additionally, sesame seed lignans or their metabolites may induce beneficial effects on bone by binding to the bone estrogen receptors (ER) and inducing transcription of estrogen-responsive genes, which promote bone strength perhaps through production of bone matrix proteins. However, further investigation is required to determine the binding potential of the sesame seed lignans, sesamin, sesamolol, and sesamolol, to the estrogen receptors (Sacco et al. 2007).

In this context, other mechanisms were suggested to explain the beneficial effects of dietary phytoestrogens on the risk of bone loss. These mechanisms include their efficacy in reducing cellular inflammatory response (Hsu et al. 2007). Evidence of their efficacy may come from the present findings that consumption of SbO and SO was found to be effective in normalizing levels of TNF- α and CRP, as well as total leukocytes count in OVX rats. As reported by Yang et al. (2012), there was a positive effect of soy diet consumption on modulating pro-inflammatory cytokines, such as CRP and TNF- α in OVX rats. Soy supplementation may also have a positive effect on total leukocytes count, as a result of lowering the percent of monocytes and lymphocytes (Ishimi et al.

1999). Such an effect may be attributed to the presence of estrogenic isoflavones which can exert a double positive action, the first one through direct interaction with estrogen receptors and the second one by suppressing the release of pro-inflammatory cytokines from bone cells (Borai et al. 2009).

Similar to soy isoflavones, lignan rich food supplements, such as SO, has generated interest as an anti-inflammatory agent. In this line, Lee et al. (2004a) have shown that consumption of diet supplemented with sesamol, as component of sesame lignans inhibited the production of cytokines such as IL-6. Based on this and the above findings regarding SbO, it can be said that both oils can exert an anti-inflammatory influence, through reducing production of inflammatory cytokines in bone tissues which may help in preventing OVX-induced bone loss.

In conclusion, the present data indicated that feeding SbO and SO was able to modulate bone loss in OVX rats, as represented by the elevation of bone minerals (Ca, P) and bone mineral density. This may occur through similar intracellular pathways, involving the suppression of oxidative stress and modulation of inflammatory response. Thus, both SbO and SO can be used as a natural approach to help in preventing bone loss associated with states of estrogen deficiency.

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