Anti-osteoporotic effect of medical herbs and calcium supplementation on ovariectomized rats

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KEYWORDS
Ovariectomized rats; Estrogen deficiency; Osteoporosis; Sage; Rosemary; Thyme

Abstract Estrogen deficiency and insufficient calcium in diet in postmenopause create serious problems with resultant osteoporosis and fractures. In the present study, forty adult albino rats weighting 200–220 g were used and divided into five groups. Group 1, control group included non-ovariectomized untreated rats. Group 2, ovariectomized (OVX) rats. Group 3, OVX rats daily received CaCO3 (27 mg/kg b.w.). Group 4, OVX rats daily received a mixture of herbs (sage, rosemary and thyme) (30 g/kg b.w.). Group 5, OVX rats daily received both herb mixture and CaCO3.

The results showed significant decrease in serum calcium (Ca), inorganic phosphorus (P), total proteins (TP), calcitonin, bone mass density (BMD), reduced glutathione (GSH) and catalase (CAT) in OVX rats compared to the control group. While a significant increase in serum parathyroid hormone (PTH), osteocalcin, alkaline phosphatase (ALP) and acid phosphatase (ACP) were recorded. Also the recorded data showed increases in femur, tibia and vertebral lipid peroxidation (MAD) content and the activities of acid phosphatase and alkaline phosphatase in the ovariectomized rats.

On the other hand, when OVX rats were fed on sage, rosemary and thyme supplemented diets or CaCO3, in a single or in combination, the data recorded a significant improvement in all the above mentioned parameters. Finally, the results of this study indicated that the combination of common herbs (sage, rosemary and thyme) with calcium carbonate, was more effective in reduction and prevention of osteoporosis in ovariectomized rats. So, it can be concluded that consumption of mixed herbs supplemented diets might be considered as a functional food for retarding risks of osteoporosis associated with estrogen deficiency in OVX states.

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Abbreviations: OVX, ovariectomized rats; Ca, calcium; P, phosphorus; ACP, acid phosphatase; ALP, alkaline phosphatase; BMD, body mass density; CAT, catalase; GSH, reduced glutathione; MAD, malondialdehyde; PTH, parathyroid hormone; OCN, osteocalcin; TP, total protein

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Introduction

Osteoporosis that is associated with ovarian hormone deficiency following menopause (postmenopausal osteoporosis) is by far the most common cause of age-related bone loss. A sharp decrease in ovarian estrogen production is the predominant cause of rapid hormone related bone loss during the first decade after menopause (Gruber et al., 1984). Traditional therapies for postmenopausal Osteoporosis have emphasized agents that inhibit bone resorption such as estrogen (Centrella and Canalis, 1985), calcitonin (Canalis et al., 1988; Rudman et al., 1981) and bisphosphonates (Bennet et al., 1984). Although the most effective method to reduce the rate of postmenopausal bone loss is estrogen replacement therapy, it may be accompanied by side effects (Genant et al., 1989) and thus recommended only for women who are at high risk of osteoporosis and who have no contraindications. Therefore, it would be most helpful to study a naturally occurring substance that minimizes bone loss in postmenopausal women. Also, recent moves away from hormone replacement therapy suggested calcium as the simplest and cheapest strategies to treat and prevent osteoporosis. (Wallace et al., 2004).

Calcium is an essential element in bone mineralization and formation being the key component of hydroxyapatite and its use as a monotherapy for osteoporosis are reviewed (Blanch and Pros, 1999; Flynn, 2003). Optimizing the dietary intake of calcium is the nutritional goal to prevent osteoporotic fractures in postmenopausal women (Deprez and Fardellone, 2003). Adding three servings of yoghurt to the daily diet of older women resulted in a significant reduction in bone resorption (Shaker et al., 2005).

There is an increasing interest in phytochemicals as new sources of natural antioxidant and antimicrobial agents. The use of synthetic antioxidants in the food industry is severely restricted as to both application and level (Tawaha et al., 2007). Currently, there is a strong debate about the safety aspects of chemical preservatives, since they are considered responsible for many carcinogenic and teratogenic attributes, as well as residual toxicity. Plant-derived polyphenols receive considerable attention because of their potential antioxidant and antimicrobial properties (Moreira et al., 2005). Phenolic compounds exhibit a considerable free-radical scavenging (antioxidant) activity, which is determined by their reactivity as hydrogen or electron donating agents, the stability of the resulting antioxidant derived radical, their reactivity with other antioxidants and, finally, their metal chelating properties (Genena et al., 2008).

Salvia officinalis L., (sage, common sage, garden sage or Dalmatian sage) Rosmarinus officinalis L., (Rosemary) and Thymus vulgaris L., (thyme) are medicinal and aromatic plants of the Lamiaceae (Labiateae) family, native to Mediterranean countries which today are cultivated all over the world (Gali-muhtasib et al., 2000). Sage is a popular herb commonly used as a culinary spice for flavoring and seasoning that has also been used for centuries in folk medicine for the treatment of a variety of ailments; also it is found that sage strongly inhibits bone resorption. Sage is a herbrich “essential oils are a class of volatile oils obtained from plants, possessing the odor and other characteristic properties of the plant”. Monoterpenes, the major components of essential oils, belong to the group of isoprenoids containing 10 C-atoms. Monoterpenes are widely distributed in the plant kingdom and are present in some herbs commonly used in human nutrition. As essential oils and monoterpenes have a pleasant odor and taste when used at low concentration, they have been extracted since ancient times also from many plants, both edible and inedible, and are used today as food additives. As essential oils and monoterpenes are lipophilic compounds, they readily cross cell membranes and are therefore absorbed through the skin and lung there; therefore, there is a long history of use of essential oils and monoterpenes for many medical applications in ointments, and bath additives to be used in the relief of head and chest colds as well as muscle pain (Abdallah et al., 2010).

Rosemary (R. officinalis L.) and T. vulgaris L., (thyme) are also spice and medicinal herbs widely used around the world. The main compounds are α-pinene, bornyl acetate, camphor and 1,8-cineole (Pintore et al., 2002).

Thyme is employed to season and suppress offensive odors, such as trimethylamine odor, in foods. The essential oil is well recognized for its medicinal properties in the treatment of bronchitis, whooping cough and tooth-ache. It is possible that the flavonoids present may be important. It was found that the main components of the essential oil were thymol and carvacrol (Porte and Godoy, 2008).

In the light of these findings, the present study was undertaken to evaluate weather intake of phytoestrogenic herbs (sage, rosemary and thyme) could play a positive role in reducing development of osteoporosis and bone loss associated with estrogen deficiency in OVX rats.

Materials and methods

Experimental animals

Forty adult female albino rats (Rattus rattus) weighing 200–220 g were used for experimentation. Animals were provided with a balanced standard diet and water ad-libitum. The animals were randomly divided into 5 groups each of 8 rats:
Group I: Sham-operated group (control rats group), included non-ovariectomized untreated rats.

Group II: Ovariectomized rats group (OVX), included rats which were ovariectomized on day 1 of the experiment and sacrificed 2 months after ovariectomy.

Group III: OVX rats treated with CaCO₃ (27 mg/kg b.w.), included rats which were ovariectomized on day 1 of the experiment and received the recommended amount of calcium for one month (from day 30 to day 60) and then sacrificed.

Group IV: Ovariectomized rats treated with herbal mixture (sage, rosemary and thyme) in a diet (30 g/kg b.w.) included rats which were ovariectomized on day 1 of the experiment and received the doses of mixture herbs for one month (from day 30 to day 60) and then sacrificed.

Group V: Ovariectomized rats treated with CaCO₃ + mixture herbs, included rats which were ovariectomized on day 1 of the experiment and received the recommended amounts of CaCO₃ + mixture herbs for one month (from day 30 to day 60) and then sacrificed.

Calcium supplementation: The recommended daily dose of CaCO₃ in women is 1500 mg (Wallace et al., 2004). The recommended dose calculated in rats was 27 mg daily (Paget and Barnes, 1964) given as calcium carbonate. Twenty seven grams of calcium carbonate was dissolved in 1000 ml water and each rat received 1 ml of the suspension daily by an oral tube.

Preparation of the dose of herbs (sage, rosemary and thyme): The dried leaves of sage, rosemary and thyme were purchased from Ibn Sina center which is considered a specialized center for medical herbs in Mansoura, Egypt. The herbs were identified by Prof. Dr. I. Mashaly professor of plant Taxonomy, Department of Botany, Faculty of Science, Mansoura University. The dried leaves were ground in a mixer and mixed with the basal diet as 30 g/kg b.w. (10 g of each type) and were fed to the rats daily for 1 month (Mühlbauer et al., 2003).

At the end of the experimental period (2 months), animals were then sacrificed, blood samples were collected and the non-hemolyzed sera were separated for later analysis. Thereafter, the femur, tibia and vertebrae were cleaned from surrounding soft tissue, and specimens were removed, weighed, crushed and homogenized in cool distilled water and then centrifuged at 3000 r.p.m. the supernatant was then used for biochemical determinations.

Calcium and phosphorus levels were quantified using kits supplied by Bio-diagnostic Co (Cairo, Egypt). Total protein (TP) content was determined by the method described by Doumas (1975). Reduced glutathione (GSH) was estimated according to the method of Prins and Losse (1969). Acid and alkaline phosphatase activities and the level of lipid peroxidation product of malondialdehyde (MDA) were estimated according to the method described by Kind and King (1954) and Ohkawa et al. (1982) respectively. Serum levels of calcitonin, parathyroid and osteocalcin were measured using ELISA Germany kits. Catalase activity was estimated according to the method Aebi (1983). BMD was measured according to Archimedes principle (Kalu, 1991).

Statistical analysis

All results obtained in the present study were evaluated by One-way ANOVA test and post comparison was carried out with Tukey-test. The values were expressed as means ± SE and values of $P < 0.05$ were considered statistically significant (Snedecor and Cochron, 1989).

Results

As shown in Table 1, the data recorded a significant increase in serum PTH, osteocalcin, acid phosphatase and alkaline phosphatase. While there was a significant decrease in calcium, phosphorus, calcitonin and total proteins content in the ovariectomized rats compared to the untreated control rats group. These changes started to ameliorate when the OVX were treated with CaCO₃ or mixture herbs, but the most amelioration was obtained when the rats received both CaCO₃ and mixture herbs.

Also, the recorded data in Table 2 indicated a significant increase in femur lipid peroxidation, acid phosphatase and alkaline phosphatase in the OVX rats group. Meanwhile a significant decrease in femur bone mass density (BMD), calcium, phosphorus, total proteins, GSH and catalase, was observed compared to the control rat group. On the other hand, a treatment with mixture herbs or CaCO₃ caused a significant amelioration of the most examined parameters. The best amelioration was observed in the group which received the combination of CaCO₃ and mixture herbs. In OVX rats, the values of MAD, ALP and ACP levels were significantly increased in the tibial and vertebral tissues compared to the control rat group. On the other hand, tibial and vertebral BMD, Ca, P, GSH, CAT and TP levels were significantly decreased in the same OVX group. The treated groups had significantly normalized levels in most parameters, but the group which was treated with the mixture of herbs and CaCO₃ had the best normalization. (Tables 3 and 4).

Discussion

Ovariectomy has shown an increased risk of osteoporosis as occurs with postmenopausal women. Osteoporosis is a metabolic bone disease characterized by loss of bone mass thus making the bone more susceptible to fractures (Sakai et al., 1998). In the present study, ovariectomized rats developed bone changes similar to those seen in osteoporotic women as indicated by a decrease in BMD of the femur, tibia and vertebrae, a finding that matches with that of Riggs and Melton (1986), who found that menopause results in elevated bone turnover, an imbalance between bone formation and bone resorption and net bone loss, and this is attributable to the cessation of ovarian function and tapering off estrogen secretion (Heshmati et al., 2002; Elwakil et al., 2014). 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 herbs (sage, rosemary and thyme) exhibited positive effects on BMD. The obtained results were in agreement with the findings of Mühlbauer et al. (2003), who found that bone resorption was inhibited by the essential oils extracted from these herbs. The monoterpenes and essential oil extracts act directly on bone cells via inhibition of the mevalonate pathway and the prenylation of small G-proteins such as Ras, Rho and Rac to inhibit bone resorption. (Mühlbauer et al., 2003). Also it was found that the positive effects of flavonoids on BMD
and bone formation (Alekel et al., 2000; Setchell and Lydeking-Olsen, 2003). Flavonoids are a class of phytoestrogens; plant-derived chemicals, that when absorbed via the gut mimic the actions of estrogen (Messina and Messina, 2000) and that have been found to increase bone morphogenetic protein 2 (BMP2) gene transcription (Zhou et al., 2003). Sage, thyme and rosemary contain phenolic constituents, which have the potential to bind to the estrogen receptor (Fetrow and Avila, 2001). These findings confirm the observations of other investigators that bone loss in ovariectomized rats is prevented by estrogen administration, and that estrogen can also suppress the ovariectomized

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Ovx</td>
</tr>
<tr>
<td>Ca, mg/dl</td>
<td>8.35 ± 0.05</td>
</tr>
<tr>
<td>P, mg/dl</td>
<td>3.24 ± 0.49</td>
</tr>
<tr>
<td>PTH, pg/ml</td>
<td>16.86 ± 0.15</td>
</tr>
<tr>
<td>OCN, ng/ml</td>
<td>3.68 ± 0.08</td>
</tr>
<tr>
<td>Calcitriol, pg/ml</td>
<td>4.66 ± 0.087</td>
</tr>
<tr>
<td>ACP, K.A.U./dl</td>
<td>28.62 ± 0.34</td>
</tr>
<tr>
<td>ALP, K.A.U./dl</td>
<td>47.66 ± 0.44</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td>7.22 ± 0.076</td>
</tr>
</tbody>
</table>

Data are mean ± SE of 8 rats in each group. Significance at P < 0.05. Ovx = ovariectomized group.

a Significant difference compared with control group.

b Significant difference between Ovx treated groups and Ovx group.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Femur biochemical marker levels in control and different treated rat groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Animal groups</td>
</tr>
<tr>
<td>BMD, g/cm²</td>
<td>Control</td>
</tr>
<tr>
<td>Ca, mg/g</td>
<td>150.4 ± 3.7</td>
</tr>
<tr>
<td>P, mg/g</td>
<td>8.11 ± 0.142</td>
</tr>
<tr>
<td>ACP, K.A.U./g</td>
<td>37.78 ± 0.32</td>
</tr>
<tr>
<td>ALP, K.A.U./g</td>
<td>25.66 ± 0.44</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td>7.22 ± 0.076</td>
</tr>
</tbody>
</table>

Data are mean ± SE of 8 rats in each group. Significance at P < 0.05. Ovx = ovariectomized group.
a Significant difference compared with control group.
b Significant difference between Ovx treated groups and Ovx group.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Tibial biochemical marker levels in control and treated groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Animal groups</td>
</tr>
<tr>
<td>BMD, g/cm²</td>
<td>Control</td>
</tr>
<tr>
<td>Ca, mg/g</td>
<td>120.3 ± 3.6</td>
</tr>
<tr>
<td>P, mg/g</td>
<td>76.56 ± 0.14</td>
</tr>
<tr>
<td>ACP, K.A.U./g</td>
<td>32.02 ± 0.39</td>
</tr>
<tr>
<td>ALP, K.A.U./g</td>
<td>27.90 ± 0.42</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td>7.22 ± 0.076</td>
</tr>
</tbody>
</table>

Data are mean ± SE of 8 rats in each group. Significance at P < 0.05. Ovx = ovariectomized group.
a Significant difference compared with control group.
b Significant difference between Ovx treated groups and Ovx group.
induced increase in biochemical markers of bone turnover (Kalu, 1984). Also, the present work indicated that the levels of bone Ca and P in ovariectomized rats were significantly lower than the control group. These results are in agreement with Boulbaroud et al. (2008) and Hassan et al. (2013), who recorded that the levels of serum Ca and P were decreased in the ovariectomized rats. The obtained results suggested that ovarian hormone deficiency following ovariectomy, is marked by reduced intestinal calcium absorption and may contribute to the accompanying bone loss. In addition, the level of Calcitonin recorded a significant decrease in ovariectomized rats. These results are in agreement with Zaidi et al., 2002, and may be attributed to the concentration of calcium level which is considered the principal stimulus for the secretion of calcitonin by C-cells. When blood calcium is lowered, the stimulus for calcitonin secretion is diminished. Also the actions of PTH and calcitonin are antagonistic on bone resorption. Further, evidence of osteoporosis resulting from ovariectomy was indicated by the present elevation of PTH level. The elevated level of PTH causes additional loss of BMD in estrogen deficient animals beyond the rapid bone loss associated with ovariectomy. Decreased estrogen level in females increased the sensitivity of bones to the action of PTH, leading to bone resorption with lower BMD (Krivosikov et al., 2010). Moreover, ovariectomies cause hyperparathyroidism which may cause calcium change associated with a compensatory rise in PTH resulting in calcium release from the skeleton thus causing bone loss (Guillemant et al., 1999). Treatment with sage, rosemary and thyme, restored the decreased levels of serum Ca and P to normal values. Phytoestrogenic compounds in sage, rosemary and thyme have structural similarities to estrogen conformation and binding capabilities to estrogen receptors, which may therefore promote calcium absorption through an estrogen receptor pathway within intestinal cells (Arjmandi et al., 2002).

The present study revealed that the raised bone ALP and ACP activity occurring with ovariectomy could contribute to high bone turnover rate, being characterized by an increase in both bone resorption and formation, but bone resorption excedes formation, leading to bone loss (Elwakf et al., 2014) thus, indicating an increase in the osteoelastic and osteoclastic activity, respectively, resulting in an overall net loss of bone with an increase in the excretion of urinary hydroxyproline, as an index of bone turnover (Wu et al., 2008). The positive role of medical herbs supplemented diets was also achieved by the observed improvement of bone metabolic markers ALP and ACP (Chiechi et al., 2002). Phytoestrogenic herbs exhibited comparable effects regarding all tested parameters, indicating almost similar ability of herbs to protect against bone loss (Boulbaroud et al., 2008).

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 toward estrogen receptor ERa and ERb 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 and interact with estrogen receptors (ER) which allow them to act through the same intracellular pathways of estrogens (Toran-Allerand et al., 2002).

Serum osteocalcin (OCN) level is frequently used as the bone formation marker to monitor drug actions (Kyoko et al., 2002a,b; Shirwaikar et al., 2003). OCN, an extra cellular calcium binding protein is expressed by mature osteoblasts in association with organic matrix mineralization (Patterson-Buckendahl et al., 1995). Serum osteocalcin levels are most likely to either reflect newly synthesized protein, as well as that released from bone matrix during resorption (Ducy et al., 1996; Nian et al., 2006). The present investigation showed that ovariectomized rats exhibited high levels of osteocalcin. These results coincided with those of Abdallah et al. (2010), who showed that ovariectomy induced a rise in serum osteocalcin level as a result of compensation of increased bone turnover. In addition, osteocalcin was found to be lowered after sage, rosemary and thyme administration to rats. This suggests that they were able to suppress the increased bone turnover due to estrogen deficiency (Wu, 2007). The present results

### Table 4 Vertebral biochemical markers level in control and treated groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal groups</th>
<th>Control</th>
<th>Ovx</th>
<th>Ovx + CaCO₃</th>
<th>Ovx + Herbs</th>
<th>Ovx + CaCO₃ + Herbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD, g/cm²</td>
<td></td>
<td>0.134 ± 0.002</td>
<td>0.118 ± 0.003</td>
<td>0.123 ± 0.008</td>
<td>0.126 ± 0.007</td>
<td>0.128 ± 0.008</td>
</tr>
<tr>
<td>Ca, mg/g</td>
<td></td>
<td>103.91 ± 3.2</td>
<td>83.98 ± 2.9</td>
<td>92.62 ± 2.8</td>
<td>89.33 ± 5.1</td>
<td>96.52 ± 3.8</td>
</tr>
<tr>
<td>P, mg/g</td>
<td></td>
<td>7.83 ± 0.055</td>
<td>7.02 ± 0.08</td>
<td>7.07 ± 0.057</td>
<td>7.00 ± 0.02</td>
<td>7.22 ± 0.044</td>
</tr>
<tr>
<td>ACP, K.A.U./g</td>
<td></td>
<td>30.16 ± 0.32</td>
<td>49.10 ± 0.50</td>
<td>42.16 ± 0.87</td>
<td>47.88 ± 0.53</td>
<td>36.50 ± 0.38</td>
</tr>
<tr>
<td>ALP, K.A.U./g</td>
<td></td>
<td>27.48 ± 0.35</td>
<td>38.92 ± 0.19</td>
<td>22.70 ± 0.25</td>
<td>20.42 ± 0.18</td>
<td>27.48 ± 0.31</td>
</tr>
<tr>
<td>MAD, nmol/g</td>
<td></td>
<td>7.08 ± 0.235</td>
<td>10.82 ± 0.174</td>
<td>9.05 ± 0.207</td>
<td>9.39 ± 0.155</td>
<td>8.78 ± 0.165</td>
</tr>
<tr>
<td>GSH, mg/g</td>
<td></td>
<td>5.34 ± 0.075</td>
<td>4.42 ± 0.097</td>
<td>5.00 ± 0.089</td>
<td>4.80 ± 0.055</td>
<td>4.98 ± 0.188</td>
</tr>
<tr>
<td>CAT, µmol/min/g</td>
<td></td>
<td>0.502 ± 0.009</td>
<td>0.369 ± 0.001</td>
<td>0.511 ± 0.001</td>
<td>0.438 ± 0.001</td>
<td>0.485 ± 0.002</td>
</tr>
<tr>
<td>TP, mg/g</td>
<td></td>
<td>5.03 ± 0.05</td>
<td>4.77 ± 0.08</td>
<td>5.04 ± 0.06</td>
<td>5.01 ± 0.09</td>
<td>5.05 ± 0.04</td>
</tr>
</tbody>
</table>

Data are mean ± SE of 8 rats in each group. Significance at P < 0.05. Ovx = ovariectomized group.

a Significant difference compared with control group.

b Significant difference between Ovx treated groups and Ovx group.
demonstrated that total proteins were significantly decreased in ovariectomized rats. The decreased level of total protein is concomitant with that observed with Hassan et al. (2013), and this decrease may be attributed to the increase in protein catabolism. 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 increased levels of malondialdehyde (MDA) 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 the 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 ovariectomy 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 instates of ovariectomy activates ROS, such as H$_2$O$_2$ for induction of bone loss through increased osteoclastic activity (Riggs et al., 2003). Furthermore, H$_2$O$_2$ not only augments osteoclastic activity, but also is essential for osteoclastic differentiation (Steinbeck et al., 1994). The current findings of increased H$_2$O$_2$, MDA in the femur, tibia and vertebrae 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 GSH-Px, SOD, CAT and GSH (Clark, 2002). Therefore, the decrease in bone CAT and GSH reported here, supported those findings and indicated the enhancement of oxidative stress as a result of bone tissue damage.

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 donation (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. (Munoz-Castaneda et al., 2005). The natural antioxidant, rosemary has been demonstrated to help in preventing bone loss associate with estrogen deficiency.

References


