Protective Effect of Anise Fruit (*pimpinella anisum*) Against Osteoporosis in Rat Model

Waleed N Hassan *1, Ahmed M Saed 2

1 Department of Biochemistry, College of Medicine, King Khalid University, Abha, Saudi Arabia
2 Department of Radiology, College of Medical Sciences, King Abdul Aziz University, Jeddah, Saudi Arabia

*Corresponding Author:
Waleed N. Hassan MD, PhD
Biochemistry Department
College of Medicine, King Khalid University
P.O. Box 641
Abha, Saudi Arabia
+966-50-433-467 (M)
E-mail: waleednh@yahoo.com

Received: 2 September 2010; | Revised: 3 October 2010; | Accepted: 6 November 2010

Abstract

Aims: To evaluate the *pimpinella anisum* supplementation in the prevention of induced osteoporosis in rat model.

Methods: Sixty Sprague-Dawley female rats were divided into two groups. Ovariectomised (OVX) group (n = 30) and sham operated group (n =30). Then, each group was further subdivided into control diet (n = 15) and aniseed supplemented diet (n = 15) for 20 weeks.

Results: Bone mineral density (BMD) was lower in OVX control vs. sham control (p<0.05). The OVX aniseed supplemented group has higher BMD vs. OVX control group (p<0.05). The urinary excretion of bone resorption marker deoxypyridinoline (DPD) was higher in OVX control vs. sham control group (p<0.05). The OVX aniseed supplemented group has a lower urinary excretion of DPD vs. OVX control group (p<0.05). The increased level of bone formation markers (alkaline phosphatase and osteocalcin) in OVX control group could be prevented by aniseed supplementation.

Conclusion: Aniseed can prevent the estrogen deficiency-induced osteoporosis in rats.

Keywords: aniseed, osteocalcin, deoxypyridinoline.

1. Introduction

Osteoporosis is considered a major public health problem, and it is characterized by decreased bone density, resulting in skeletal fragility and fractures [1,2]. Decreased estrogen secretion after menopause is an important cause of osteoporosis. So, hormone replacement therapy (HRT) has been used in the prevention and treatment of postmenopausal osteoporosis [3]. The long-term use of HRT is associated with increased...
risk of malignancy in the reproductive tissues [4]. Other agents that can stimulate bone formation (e.g., growth hormone, and anabolic steroids) and antiresorptive agents (e.g., calcitonin) may prevent further bone loss in osteoporosis, but their costs are high for patients in the developing countries [5]. So, it is necessary to develop products, with fewer side effects, that can substitute drugs used currently.

There is an increasing interest in nutrients which influence bone metabolism e.g., calcium and vitamin D [6-8]. The involvement of oxidative stress in osteoporosis has been shown through the insufficient intake of antioxidants can increase the risk of hip fracture in smokers [9]. Free radicals may increase resorption through activation of oxidative stress responsive nuclear factor NF-kB which has been associated with osteoclastogenesis [10-12]. The polyphenolic antioxidants, flavonoids, have received some attention for their potential role in preventing osteoporosis induced by in rats [13,14] and in human [15]. Also, flavonoids have been characterized as naturally occurring selective estrogen receptor modulators (SERMs) with similar beneficial effects to raloxifene on bone [16]. Flavonoids have been shown to inhibit bone loss in rats, both by slowing resorption and by increasing osteoblastic activity, resulting in increased bone strength [17]. Other polyphenols may have a possible role in treatment and prevention of osteoporosis for example; lignans [18] and isotaxiresinol [19] can prevent bone loss in postmenopausal women or rats respectively.

The fruits of anise plant, *pimpinella anisum* L. are locally known as aniseed and yansoon. In traditional medicine, aniseed has been used for the treatment of nausea, abdominal colic, insomnia and epilepsy [20,21]. The phytotherapeutic applications of aniseed are based on its digestive, carminative, diuretic, antiseptic and expectorating action [22]. The principal constituents of *pimpinella anisum* are the anise oil (1-4%). The major component of anise oil, trans-anethole (75-90%), is responsible for its characteristic taste and smell and it is considered as an active estrogenic agent. Other constituents include coumarins (umbelliferone, umbelliprenine, bergapten, and scopole-tin), lipids (fatty acids, beta-amyrin, and stigmasterol), flavonoids (flavonol, flavone, glycosides, rutin, isoorientin, and isovitexin), protein, carbohydrate and minerals (calcium 646 mg/100 g, phosphorus 440 mg/100 g) [23,24].

The aim of the present study was to evaluate the *pimpinella anisum* supplementation in the prevention of osteoporosis induced by ovariectomy in rats (an established model for postmenopausal osteoporosis) [25,26].

2. Materials and Methods

2.1 Animals and diet

Sixty Sprague-Dawley virgin female rats (body weight 247 ± 14 g, 3 months old) were randomly divided into two groups. One group was ovariectomised (OVX, n = 30) and the other group received sham operation (Sham, n =30), and then each group was further subdivided into control diet (n = 15) and aniseed supplemented (4%) diet (n = 15) for 20 weeks when we can observe a significant decrease in the femur bone mineral density (BMD) [27]. All rats were supplied with distilled water ad libitum and weighted weekly. The dietary supply of vitamins, minerals, and protein was in accordance with the recommended dietary allowances for rats from the American Institute of Nutrition [28]. The animals were housed in the air-conditioned rooms at temperature 24 ± 2 °C. Fluorescent light was applied for 12h/day to create a light/dark cycles. Ovariectomy and sham operations were performed under anesthesia with an intra-peritoneal injection of 8% chloral hydrate solution (0.4 ml/100 g). The animals of operation group received bilateral ovariectomy by dorsal approach and those of sham groups underwent the same surgery procedure with exposing but not removing the ovaries. The success of ovariectomy was confirmed with the failure to detect ovarian tissue and atrophy of the uterine horns at necropsy [25,26].

2.2 Serum and urine chemistry

Serum calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP) concentrations were measured by standard colorimetric methods using kits from Olympus Diagnostica (Clare, Ireland) and an Olympus AU 2700 analyzer (Mishima, Japan). Urine Ca, P and creatinine (Cr)
concentrations were analyzed by the same method used for the serum samples. Serum osteocalcin (OC) concentration was determined using a rat OC ELISA kit (Biomedical Technologies, Stoughton, MA, USA), with a 4% intra and 7% inter assay variability. Urinary deoxypyridinoline (DPD) concentration was assayed using a rat DPD ELISA kit (Quidel, San Diego, USA). The intra- and inter assay variability was 5.5% and 3.1%, respectively. Urinary excretion of Ca and DPD were both expressed as the ratio to Cr concentration (Ca/Cr; DPD/Cr).

2.3 Determination of bone density

Measurements of bone mineral concentration (BMC), bone width (BW) and bone mineral density (BMD = BMC/BW) were made at the middle and epiphysis of femur by dual energy X-ray absorptiometry PIXImus (GE Lunar Co, Wisconsin, USA) at beginning of experiment and 20 weeks after operation.

2.4 Statistical analysis

The statistical significance of differences among the groups was evaluated by ANOVA, using a computer software package (version 9.13, SAS Institute Inc, Cary, NC, USA). Individual comparisons were made by Duncan's multiple range tests using the ANOVA. A P value <0.05 was considered statistically significant. Data are expressed as means ± SD.

3. Results

3.1 Animal Weight

The results of this study indicate that body weight gain was higher in OVX groups than in Sham groups (p<0.05) regardless of the type of diet. There was no statistical difference in food intake in all the groups. Mean food intake was 14.34 ± 2.93, 14.19 ± 2.85, 14.08 ± 2.73 and 13.74 ± 2.98 g/day for the sham control, sham aniseed, OVX control and OVX aniseed groups, respectively. The weight gain for the OVX rats with control diet was not statistically different from the OVX rats with aniseed supplemented diet (Table 1).

Table 1. The effect of diet on body weight in different experimental groups

<table>
<thead>
<tr>
<th>Sh / O</th>
<th>Control</th>
<th>Aniseed</th>
<th>Control</th>
<th>Aniseed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>246.4 ± 13.2</td>
<td>247.2 ± 15.4</td>
<td>250.6 ± 14.7</td>
<td>248.4 ± 13.8</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>283.5 ± 16.3</td>
<td>281.3 ± 17.5</td>
<td>317.4 ± 20.4 *</td>
<td>314.6 ± 21.7 *</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>41.4 ± 7.8</td>
<td>39.8 ± 8.5</td>
<td>77.6 ± 11.6 *</td>
<td>79.8 ± 12.3 *</td>
</tr>
</tbody>
</table>

The data presented as mean ± SD. n = 15 for each group. * Significantly different at p<0.05 vs. Sham control and Sham aniseed groups within a raw. OVX = ovariectomised.

3.2 Serum Ca, P, alkaline phosphatase and osteocalcin

The concentrations of serum Ca and P were not significantly different among the experimental groups (Table 2). The concentrations of bone formation markers (ALP and OC) were higher in OVX control vs. sham control (p<0.05). The OVX aniseed supplemented group has a lower bone formation markers (ALP and OC) vs. OVX control group (p<0.05) (Table 2).

3.3 Urine Ca, P, deoxypyridinoline, creatinine

The corrected values for urinary excretion of Ca and P were higher in OVX control vs. sham
control group (p<0.05). The OVX aniseed supplemented group has a lower urinary excretion values of Ca and P vs. OVX control group (p<0.05) (Table 3). The corrected values for urinary excretion of bone resorption marker DPD was higher in OVX control vs. sham control group (p<0.05). The OVX aniseed supplemented group has a lower urinary excretion corrected values of DPD vs. OVX control group (p<0.05) (Table 3).

Table 2. The effect of diet on serum Ca, P, ALP, and OC in different experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>OVX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Aniseed</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.49 ± 0.13</td>
<td>2.53 ± 0.15</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>1.62 ± 0.37</td>
<td>1.56 ± 0.42</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>165 ± 27.1</td>
<td>154 ± 34.8</td>
</tr>
<tr>
<td>OC (nmol/L)</td>
<td>8.48 ± 0.63</td>
<td>7.61 ± 0.57</td>
</tr>
</tbody>
</table>

The data presented as mean ± SD. n = 15 for each group. * Significantly different at p<0.05 vs. Sham control and ** significantly different at p<0.05 vs. OVX control values within a raw. Ca = calcium, P = phosphorus, ALP = alkaline phosphatase, OC = osteocalcin.

Table 3. The effect of diet on urinary Ca, P, and DPD corrected by creatinine excretion in experimental rat groups

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>OVX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Aniseed</td>
</tr>
<tr>
<td>Ca/Cr (mmol/mmol)</td>
<td>0.21 ± 0.03</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>P/Cr (mmol/mmol)</td>
<td>3.62 ± 0.37</td>
<td>3.67 ± 0.42</td>
</tr>
<tr>
<td>DPD/Cr (nM/mM)</td>
<td>57.5 ± 3.51</td>
<td>60.2 ± 4.57</td>
</tr>
</tbody>
</table>

The data presented as mean ± SD. n = 15 for each group. * Significantly different at p<0.05 vs. Sham control and ** significantly different at p<0.05 vs. OVX control values within a raw.Ca = calcium, P = phosphorus, Cr = creatinine, DPD = deoxypyridinoline.

3.4 Femur BMD, BMC, BMD per weight and BMC per weight

The absolute and ratio values per weight for BMD and BMC were lower in OVX control vs. sham control (p<0.05 for each). The OVX aniseed supplemented group has higher BMD and BMC values vs. OVX control group (p<0.05) (Table 4). There was no statistical difference between the sham control and sham aniseed groups in the absolute and ratio values per weight for BMD and BMC (p>0.05 for each) regardless of the type of diet (Table 4).
Table 4. The effect of diet on femur BMD and BMC in different experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Sham Control</th>
<th>Aniseed Control</th>
<th>Sham OVX Aniseed Control</th>
<th>Aniseed</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD (g/cm²)</td>
<td>0.156 ± 0.007</td>
<td>0.151 ± 0.006</td>
<td>0.137 ± 0.007 *</td>
<td>0.152 ± 0.008 **</td>
</tr>
<tr>
<td>BMD/wt (g/cm²)/kg</td>
<td>0.64 ± 0.03</td>
<td>0.61 ± 0.02</td>
<td>0.52 ± 0.03 *</td>
<td>0.62 ± 0.01 **</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>0.366 ± 0.021</td>
<td>0.367 ± 0.019</td>
<td>0.312 ± 0.016 *</td>
<td>0.359 ± 0.015 **</td>
</tr>
<tr>
<td>BMC/wt (g/kg)</td>
<td>1.46 ± 0.07</td>
<td>1.47 ± 0.03</td>
<td>1.25 ± 0.06 *</td>
<td>1.43 ± 0.05 **</td>
</tr>
</tbody>
</table>

The data presented as mean ± SD. n = 15 for each group. * Significantly different at p<0.05 vs. Sham control and ** significantly different at p<0.05 vs. OVX control values within a raw. BMD = bone mineral density, wt = weight, BMC = bone mineral concentration.

4. Discussion

Osteoporosis is a metabolic bone disease characterized by a defect in bone mineralization. After age 40, a slow process of bone loss begins in both sexes and continues until late in life. In women after menopause, there is an accelerating rate of bone loss because of the decreasing estrogen secretion associated with aging [29]. Bone metabolism is affected by genetic, endocrine, mechanical, and nutritional factors. Calcium has been reported as the most important nutrient associated with bone mass [30]. Dietary calcium moderately reduces the rate of cortical bone loss in late menopause [31]. Low calcium intake is particularly common in many developing countries.

The results of this study indicate that body weight gain was higher in both OVX groups than in SHAM groups (p<0.05 for each) and the weight gain for the OVX rats was not different between aniseed supplemented and control diet. A positive association between bone density and body weight has been well documented in many large epidemiologic studies [32]. The weight gain might have bone-protective effects which can be explained by the increase in aromatization of androgen to estrogen in adipose tissue [33], or increase bone formation induced by high circulating concentrations of insulin [31] and other hormones secreted by the cells of the pancreatic islets [34]. The exact mechanisms by which OVX induces increase in body weight are not clear [35]. Aniseed did not prevent the increase in body weight induced by OVX in rats. It also was devoid of any uterotrophic activity because uterine weight was not different in OVX and anise group (data not shown). The results suggest that aniseed at the given dose did not behave like estrogen in the regulation of body weight and uterine tissue growth in the OVX rats.

As expected, OVX resulted in significant decrease in the femur BMD after 20 weeks. This loss of bone mass was accompanied by a significant increase in bone remodeling, as was evidenced by the increased levels of the bone turnover markers (serum OC, ALP, and urinary DPD). Aniseed prevented the decreases in BMD, which were reflected by the decreases in serum OC and ALP levels, and the urinary DPD/Cr ratio, indicating a reduction in bone turnover. A decrease in urinary calcium excretion and increase in calcium absorption might contribute to the increase in BMD [36]. Aniseed prevented the OVX-induced increase in urinary Ca and P excretion. These effects of aniseed are consistent with the maintenance of bone mass by inhibiting bone resorption.
It has been demonstrated that reactive oxygen species intervene in bone resorption, promoting osteoclastic differentiation in such a manner that bone resorption is increased with oxidative stress [37-39]. The mechanism of the effects of aniseed on bone appeared to be related to its high content of antioxidant polyphenolic compounds such as lignans and flavonoids, which would influence oxidative stress [23,24]. Another possibility would be that the polyphenolic compounds such as flavonoids in aniseed affect bone, at least in part, through estrogen receptors (ER) as phytoestrogens do [40]. The rat and human ER exists as two subtypes ERα and ERβ. ERβ is more abundant than ERα in bone tissue while ERα is mainly distributed in reproductive system, especially the breast and uterus. Moreover, the earlier study has showed the presence of ERβ-like immunoreactivity in the nuclei of human and murine osteoblast and osteocytes and in the cytoplasm of osteoclasts [41]. ERβ messenger RNA (mRNA) is present in rat osteoblasts [42]. Thus, aniseed flavonoids, as naturally occurring SERMs, might show higher affinity for ERβ than for ERα that produces optimal action in preventing bone loss without stimulating an unwanted proliferation of the uterine tissues.

5. Conclusion

The present study clearly demonstrates that daily oral administration of aniseed over a 20-week period in the adult OVX rat can prevent the estrogen deficiency-induced bone loss, thereby maintaining biomechanical competence of bone. Consequently, aniseed might be a potential alternative medicine for prevention of postmenopausal osteoporosis.

References


[37] Sontakke AN, Tare RS: A duality in the roles of reactive oxygen species with respect to


