The Potential Beneficial Role of Parsley (Apigenin Rich Herb) on Prednisolone-Induced Osteoporosis in Rats

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Abstract

This study was carried out to evaluate and compare the effect of parsley (apeginin flavone rich herb), to the effect of apigenin on prednisolone-induced osteoporosis in rats. Four groups were performed in Wistar strain rats, each consisted of ten rats. Group I was considered a control group. In groups 2.3 and 4, prednisolone (10mg/kg), was injected to rats intramuscularly three times a week for 6 weeks to induce osteoporosis. Parsley leaves were prepared by washing, drying then blending with the rats’ diet in a percent of 5gm% from the diet weight. This diet was administered to 3rd group animals. The 4th group was given apigenin orally in a dose of 10mg/kg 3times weekly. Calcium, phosphorus, and alkaline phosphatase serum levels were estimated at the end of experiment and femur specimens were obtained for histological examination. Results showed that prednisolone injections caused significant reductions in calcium, and phosphorus, significant increases in alkaline phosphatase serum levels in the 2nd untreated model group with sings of osteoporosis observed histologically in rats femurs. Oral administration of apigenin or a diet rich in parsley markedly reversed these changes to almost normal values The results of the two treated groups were almost similar. These results suggested that either parsley leaves rich diet or apigenin administration might have a potential prophylactic role against bone loss induced by corticosteroid treatment.

Key Words: Osteoporosis – Calcium – Bone – Alkaline phosphatase – Apigenin – Parsley.

Introduction

OSTEOPOROSIS is a disease of bones that leads to increased risk of fractures. In osteoporosis, the bone mineral density (BMD) is reduced, bone microarchitecture deteriorates, and the amount and variety of proteins in bone are altered. Osteoporosis is defined by the World Health Organization (WHO) as a bone mineral density of 2.5 standard deviations or more below the mean peak bone mass (average of young, healthy adults) as measured by dual-energy X-ray absorptiometry [1]. The disease may be classified as primary type 1, primary type 2, or secondary. The form of osteoporosis most common in women after menopause is referred to as primary type 1 or postmenopausal osteoporosis. Primary type 2 osteoporosis or senile osteoporosis occurs after age 75 and is seen in both females and males at a ratio of 2:1 [2]. Finally, secondary osteoporosis may arise at any age and affect men and women equally. This form results from chronic predisposing medical problems or disease, or prolonged use of medications such as glucocorticoids, when the disease is called steroid-or glucocorticoid-induced osteoporosis [3].

Osteoporosis risks can be reduced with lifestyle changes and sometimes medications. Lifestyle change includes diet and exercise, and preventing falls. Medications includes calcium, vitamin D, bisphosphonates and several others [4].

Recently, approaches for the prevention and management of postmenopausal osteoporosis, such as the use of natural food ingredients rich in flavonoids and calcium, are worth exploring [5].

Many plant-derived substances (e.g. flavonoids and flavones) have estrogenic activities (phytoestrogens) due to their ability to bind the estrogen receptor, these compounds have the potential to counteract the deleterious effects of estrogen deficiency on bone [6].

One of the plant rich sourses of flavonoids, flavones and calcium is parsley. Parsley (Petroselinum crispum) is a species of Petroselinum in the family Apiaceae, native to the central Mediterranean region and widely cultivated as a herb, a spice, and
The Potential Beneficial Role of Parsley (Apigenin Rich Herb)

Parsley leaves contain 138 milligrams of calcium per 100 grams. Moreover, parsley is a rich source of antioxidants (especially luteolin), apigenin, folic acid, vitamin K, vitamin C, and vitamin A. One gram of dried parsley contains about 6.0µg of lycopene and 10.7µg of alpha carotene as well as 82.9µ g of lutein and zeaxanthin and 80.7µ g of beta carotene.

Recently, one of the tested flavones on osteoporosis is apigenin. Many reports have shown that apigenin not only inhibits bone resorption by osteoclasts but also induces osteoclast apoptosis. However, the influence of apigenin on corticosteroid induced osteoporosis in animals is relatively unknown. The purpose of this study was to compare the bone-protective effects of either apigenin or a diet rich in parsley (as an apigenin rich herb) in prednisolone induced osteoporosis in rats.

Material and Methods

Animal care and diet:

All animal care and experimental protocols were confirmed to the guidelines for the use of animals in ophthalmic and vision research. Wistar strain rats (90 days old, 200 to 250gm weight) were obtained from the animal house of the Research Institute of Ophthalmology. Animals were housed in environmentally controlled central animal facilities. They were kept at 22ºC, light: Dark (12h: 12h) conditions and fed with a normal calcium level control diet for 2 days before the treatment. The rats were randomly selected and divided into four groups each consisting of ten rats. The 1\textsuperscript{st} group was used as a control normal group; the next 3 groups were injected prednisolone 10mg/kg intramuscularly three times a week for 6 weeks. The 2\textsuperscript{nd} group was considered as a model for osteoporosis. The 3\textsuperscript{rd} group of animals was fed with diet containing parsley 5gm% as illustrated in Table (1). The percentage of parsley was chosen based on a pilot rat study. The 4\textsuperscript{th} group animals were fed the usual diet and apigenin flavone (sigma,Germany) was orally administered in a dose of 10mg/kg intramuscularly three times a week for 6 weeks. Animals were allowed free access to food and water throughout the course of the study. At the end of experiment (6\textsuperscript{th} week), the rats were anesthetized with diethylether. Blood samples were taken from the retro-orbital venous plexus and centrifuged at 3000 rpm for 15 minutes for serum preparation. The rat’s serum samples were stored at −80ºC for later biochemical assay. Then, animals were sacrificed by cervical dislocation, followed by the collection of bone specimens, namely femurs. The specimens were fixed in 10% neutral buffered formalin (nbF) for 12h at 4ºC until histopathological examination.

Preparation of parsley:

Parsley leaves were washed and left to dry in a shaded area at room temperature for 3 to 5 days. Then the leaves were blended thoroughly and mixed with the normal diet in a ratio of 5gm% (5 grams in each 100 gram diet).

Biochemical assays of serum samples:

Calcium, phosphorus and alkaline phosphatase serum concentrations were estimated by the o-cresolphthalein complexing colour development method, p-methylaminophenol method and the hydrolysis of p-nitrophenylphosphate into p-nitrophenol and phosphate method respectively, using commercial kits (Wako Pure Chemical Industries Ltd., Osaka, Japan).

Histopathological examination of the femur neck:

The femur specimens were fixed in 10% neutral buffered formalin (nbF) for 12h at 4ºC, decalcified in 5% ethylenediamine tetraacetic acid (EDTA, pH 7.4) for 7 days. The femurs necks were selected, embedded in paraffin, and cut horizontally into sections of 5µ thickness. The sections were stained with Hemotoxylin and Eosin (H&E). sections were examined by light microscopy to assess the degree of bone loss.

Statistical analysis:

Values were expressed as the mean±sE. Differences between groups were assessed by one-way analysis of variance (ANOVA) and paired sample t-test using the statistical Package for social sciences (sPss) software package for Windows (version 10.0). A value corresponding to \( p<0.05 \) was considered statistically significant.

Table (1): Composition of experimental diets (AIn-93 modified diet for rodents.

<table>
<thead>
<tr>
<th>Ingredients(g)</th>
<th>Normal diet (1)</th>
<th>Normal diet + 5gm% parsley (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, lactic(g)</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Parsley(g)</td>
<td>-</td>
<td>52.65</td>
</tr>
<tr>
<td>L-cystine(g)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch(g)</td>
<td>315</td>
<td>315</td>
</tr>
<tr>
<td>Maltodextrin(g)</td>
<td>35</td>
<td>125</td>
</tr>
<tr>
<td>sucrose(g)</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>Cellulose(g)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>soybean-oil(g)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Lard(g)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mineral mix(g)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Dicalcium phosphate(g)</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Calcium carbonate(g)</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Potassium citrate(g)</td>
<td>16.5</td>
<td>16.5</td>
</tr>
<tr>
<td>Vitamin mix(g)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Kcal/(kg)</td>
<td>4057</td>
<td>4057</td>
</tr>
</tbody>
</table>

AIn: American Institute of nutrition.
1- AIn-93 Modified diet.
2- AIn-93 Modified diet with 5% parsley content by weight.
Results

Biochemical assays:

The untreated model rats of prednisolone-induced osteoporosis (Group II) showed significantly increased levels of serum alkaline phosphatase (465±76)U/l, decreased serum calcium levels (8.2±0.6)mg/dl, and inorganic phosphorus serum levels (4.2±0.15)mg/dl compared to control rats (Group I).

Feeding the animals with a diet rich in parsley (5gm%) in Group-III significantly prevented the elevation of serum alkaline phosphatase levels and restored calcium and inorganic phosphorus back to almost normal ranges (311±55U/l, 10.1±0.47mg/dl, 4.80±.0.55mg/dl respectively).

Moreover, treatment of animals in Group VI with oral apigenin 10mg/kg three times weekly resulted also in a significant decrease in serum alkaline phosphatase levels paralleled with increases in serum inorganic phosphorus and calcium levels reaching values of (291±75.5U/l, 5.10±0.35mg/dl, 10.33±0.67mg/dl) respectively.

Histopathological results:

Fig. (1) shows the normal architecture of a cross section of the femur neck. Notice the thickness of the cortex and its ratio to the medullary cavity, the little number of osteoclasts and the prevalence of osteoblasts. Whereas, Fig. (2-A,B) shows a cross section of a femur neck in group 2 which were used as a model of prednisolone induced osteoporosis. Fig. (2) shows a marked thinning of the cortex with widening of the medullary cavity, the number of osteoclasts is notably increased with increased resorption areas noted at the subperiosteal and endosteal surfaces of the cortex.

Figs. (3,4-A,B) show the treated groups with either parsley rich diet (Group III) or apigenin administration (Group IV). There is a marked preservation of the cortical thickness and medullary area width. Also the number of osteoclasts is minimal as compared to the model untreated rats (Group II).

Table (2): Levels of calcium, inorganic phosphorus and alkaline phosphatase in rat serum of studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (I)</th>
<th>Prednisolone induced osteoporosis + normal diet (II)</th>
<th>Prednisolone induced osteoporosis + normal diet + 5% parsley (III)</th>
<th>Prednisolone induced osteoporosis + normal diet + oral apigenin 10mg/kg (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>Range 11.61±0.26</td>
<td>8.2±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.1±0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.33±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Mean±sE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inorganic phosphorus (mg/dl)</td>
<td>Range 5.3±0.71</td>
<td>4.2±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.10±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Mean±sE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>Range 240±67</td>
<td>465±76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>311±55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>291±75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Mean±sE</td>
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</table>

Values are expressed as mean±sD of 10 rats per group.
<sup>a</sup>: P<0.05 vs. control group (I).
<sup>b</sup>: P<0.05 vs. group (II).

Fig. (1-A,B): Histophotomicrographs showing the normal architecture of a cross section of the femur neck. Notice the thickness of the cortex and its ratio to the medullary cavity, the decreased activity of osteoclasts and the prevalence of osteoblasts (x40, x100, H&E).
Fig. (2-A,B): Histophotomicrographs showing cross sections of a femur neck in group 2 which were used as a model of prednisolone induced osteoporosis. There is a marked thinning of the cortex with widening of the medullary cavity, the number of osteoclasts is notably increased with increased resorption areas (haustrations) noted at the subperiosteal and endosteal surfaces of the cortex (x40, x100, H&E).

Fig. (3,4-A,B): Histophotomicrographs showing cross sections of a femur neck in the treated groups with either parsley rich diet (3-A,B) (Group III) or apigenin administration (4-A,B) (Group IV). The cortical thickness and medullary area width are markedly preserved. Also the number of osteoclasts is minimal as compared to the model untreated (Group II) (x40, x100, H&E).
Discussion

Osteoporosis is now widely recognized as a public health problem since this disease, which increases bone fragility and thereby the risk of fractures, is associated with high mortality, morbidity and medical expenses throughout the world [10].

Corticosteroids have several adverse effects on bone metabolism. They cause direct inhibition of osteoblast function, enhancement of bone resorption, inhibition of gastrointestinal calcium absorption, increases in urine calcium loss and inhibition of gonadal hormones [11].

It has been demonstrated that high concentrations of glucocorticoids delay maturation and formation of osteogenic cell colonies, inhibit the synthesis of collagen (stimulate the synthesis of collagenase III) and osteocalcin (which is a noncollagenous protein found in bones and plays a role in bone mineralization and calcium homeostasis) and interfere with bone matrix mineralization [12]. In addition, they lead to apoptosis of osteoblasts through disruption of their cytoskeleton [13].

Glucocorticoids modulate the Wnt signaling pathway. The Wnt signaling pathway plays a role in differentiation and function of osteoblasts. They also inhibit the synthesis of osteoprotegerin (Osteoprotegerin is a glycoprotein receptor that can reduce the production of osteoclasts by inhibiting the differentiation of osteoclast precursors), while the production of RANKL (Receptor activator of nuclear factor kappa B ligand) is stimulated, which, in turn, increases the activity, proliferation and maturation of osteoclasts [12].

Moreover, steroids reduce synthesis of estrogen, testosterone, adrenal androgen and calcitonin by C thyroid cells [13].

Additionally, glucocorticoids in high doses interfere with active transmembrane calcium transport. They also inhibit the expression of genes, dependent on vitamin D3. Thus, calcium absorption in the gastrointestinal tract is significantly decreased, while calciuria is increased, which causes a negative calcium balance. This, in turn, leads to hyperstimulation of the parathyroid glands and secondary hyperparathyroidism. As a result of negative calcium balance and inhibited osteoblast activity, bone tissue demineralization foci occur [14].

In the present study osteoporosis was induced in rats by intramuscular injection of prednisolone 3 times a week for 6 weeks. The untreated model animals showed a significant reduction in serum calcium and phosphorus levels, also a significant elevation in alkalinephosphatase (AIP) serum levels was noticed. These finding where in concordance with those reported by Rianthavorn et al., 2012 [14]. Histological findings of the femur neck confirmed the occurrence of bone loss in the form of marked thinning of the cortex and widening of the medulla.

Treatment of rats in Group III with a diet rich in parsley showed significant improvement in serum calcium, phosphorus, and AIP levels, with a noticeable improvement in the histological picture.

similarly, a marked improvement was also noticed in rats of Group IV treated with oral apigenin in all serum tested parameters as well as the histological picture findings.

To explain the beneficial effects of apigenin on bones, recent studies have proven that apigenin could attenuate osteoclastogenesis and osteoclast function by inhibition of tumor necrosis factor alpha (TNFalpha) and interferon gamma (IFNgamma). Ligands of the TNF receptor family constitute the most potent osteoclastic cytokines [15].

Moreover, apigenin was found to inhibit osteoclast differentiation by reducing receptor activator of nuclear factor kappa ligand (RANKL), RANK, and calcitonin receptor, resulting in the inhibition of multinucleated osteoclast formation [16].

In addition, apigenin was reported to inhibit expression of the osteoclast differentiation markers TRAP, and RAnK1, in osteoclast precursor cells obtained from mouse bone marrow following treatment with RANKL and macrophage colony stimulating factor (MCsF). Furthermore, apigenin induced apoptosis of mature osteoclasts obtained from rabbit long bone and inhibited bone resorption [17].

These data suggested that apigenin has multiple effects on the bone cells that could prevent bone loss in vivo.

To explain the beneficial results of parsley enriched diet observed in the present study. It has been reported that apigenin and its glycosides are the main flavonoids in parsley, it can be found in relatively large amounts in the leaves [18]. However, the presence of apigenin is only one factor in the beneficial role of parsley against osteoporosis. Parsley leaves are considered a rich source of a broad range of flavonoids, flavones, vitamins and minerals important to maintain healthy bones including calcium, vitamin K1 and boron [19].
Vitamin K1 is the form of vitamin K that is found in plants. A function of vitamin K1 that is often overlooked is its role in converting inactive osteocalcin to its active form. Osteocalcin, the major non-collagen protein in bone, anchors calcium molecules to the protein matrix [20].

A deficiency of vitamin K leads to impaired mineralization of bone due to inadequate osteocalcin levels. Other studies have shown that the lower the level of circulating vitamin K, the lower the bone density [21,22]. This evidence clearly indicates the importance of vitamin K.

In addition to vitamin K1, the high levels of minerals especially calcium and boron in green leafy vegetables may also be responsible for this protective effect. Boron is a trace mineral gaining recent attention as a protective factor against osteoporosis. Supplementing the diet of postmenopausal women with 3mg of boron per day reduced urinary calcium excretion by 44% and dramatically increased the levels of 17 beta-estradiol, the most biologically active estrogen [23]. It appears that boron is required to activate certain hormones including estrogen and vitamin D. Boron is apparently required for the conversion of vitamin D to its most active form (1,25-(OH)2D3) within the kidney. A boron deficiency may contribute greatly to osteoporosis as well as menopausal symptoms. Boron has been shown to mimic some of the effects of estrogen therapy in postmenopausal women [21].

Furthermore, an interesting report had mentioned that some of the flavones in parsley (namely: Apigenin, diosmetin. And kaempferol) showed a potent estrogenic activity resembling phytoestrogens. Phytoestrogens had a beneficial role against osteoporosis in females [24].

In conclusion, the results of the present study suggest that either parsley leaves rich diet or apigenin administration may have a potential prophylactic role against bone loss induced by corticosteroid treatment. The results of both of them were almost similar. However, parsley has the favor of being an easy, cheap, and economic nutritional advice to people susceptible to osteoporosis. Future studies are required to uncover more nutritional treasures for prophylaxis against osteoporosis.

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References


