Study of the effect of *Allium porrum* on osteoporosis induced in rats

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ABSTRACT

The present study investigate the effect of alcoholic extract of *Allium porrum* (250 and 500mg/kg) on osteoporosis, which was induced experimentally in male rats by i.p. injection of 20% ethanol (3g/kg/day). Alcoholic extract of *Allium porrum* (250 and 500mg/kg) was given by oral route daily for 11 successive weeks, 8 weeks before the induction of osteoporosis and 3 weeks simultaneously with i.p injection of ethanol 20% for 4 successive days of weekly. Bone mineral density (BMD) was measured by DEXA. Blood samples were collected at the end of the experiments for determination of serum calcium, phosphorus, alkaline phosphatase, malonaldehyde and total antioxidant capacity. Oral administration of alcoholic extract of *Allium porrum* (250 and 500mg/kg) had significant antioxidant activity resulted in a significant elevation in the decreased BMD in osteoporotic rats as compared with control group. It is concluded that alcoholic extract of *Allium porrum* have promising protective effect against osteoporosis induced in rats which may be attributed to its antioxidant capacity.

Keywords: *Allium porrum*, antioxidant, osteoporosis, rats

INTRODUCTION

Osteoporosis is a skeletal condition characterized by decreased bone density (mass/volume) of normally mineralized bone. The reduced bone density leads to decreased mechanical strength, thus making the skeleton more likely to fracture [1]. The prevalence of osteoporosis among the growing population of older people ≥50 years old is almost 20% and have osteoporosis of the hip, spine, or wrist [2]. The diagnosis of osteoporosis is made by measuring the bone mineral density (BMD). The most popular method is dual energy X-ray absorptiometry (DEXA) [3]. Usually, osteoporosis does not cause any symptoms at first; it is often called “silent” disease, because bone loss occurs without symptoms, people often don't know they have the disease until fractures occur. But once bones have been weakened by osteoporosis, symptoms that include back pain, which can be severe if you have a fractured or collapsed vertebra, loss of height over time, with an accompanying stooped posture fracture of the vertebrae, wrists, hips or other bones may appear [4]. Hormonal factors strongly determine the rate of bone resorption; lack of estrogen (e.g. as a result of menopause) increases bone resorption. In addition to estrogen, calcium metabolism plays a significant role in bone turnover, and deficiency of calcium and vitamin D leads to impaired bone deposition; in addition, the parathyroid glands react to low calcium levels by secreting parathyroid hormone (parathormone, PTH), which increases bone resorption to ensure sufficient calcium in the blood [5].
Secondary osteoporosis could occur as a result of nutrition deficiencies low dietary calcium intake, low body mass index, alcohol abuse, tobacco smoking, having certain medical conditions, such as immobilization, endocrine disorders that can induce bone loss including Cushing’s syndrome, hyperparathyroidism, thyrotoxicosis, [6] hypothyroidism, diabetes mellitus type 1 and 2, [7]. Acromegaly and adrenal insufficiency, patients with rheumatologic disorders like rheumatoid arthritis more suspected to get osteoporosis, in addition to hypogonadism, chronic liver disease, leukemia and renal insufficiency can also lead to osteodystrophy [8]. It may also occur as a result of taking medicines known to cause bone breakdown, such as oral or high-dose inhaled corticosteroids (if used for more than 6 months), too high a dose of thyroid replacement, or aromatase inhibitors (used to treat breast cancer). Anti-epileptic drugs, heparin, progesterin without estrogen, hormonal drugs that suppress estrogen, loop diuretics known to increase the risk of bone loss, secondary osteoporosis can occur at any age [9].

Changing of lifestyle for prevention of osteoporosis is in many aspects by potentially avoiding risk factors (as tobacco smoking and alcohol intake) [10]. Achieving a higher peak bone mass through exercise and proper nutrition during adolescence is important for the prevention of osteoporosis. Exercise and nutrition throughout the rest of the life delays bone degeneration and calcium, increased bone density of the lumbar (lower) spine by 5% over nine months [11]. Calcium is required to support bone growth, bone healing and maintain bone strength and is one aspect of osteoporosis treatment. Calcium may cause nausea and vomiting but excessive calcium intake can lead to hypercalcemia and may also lead to renal calculi (kidney stones) and induces symptoms as confusion, delirium, stupor and coma [12].

In recent years there is a growing interest in nutraceuticals which provide health benefits and are alternative to modern medicine. Nutrients, herbals and dietary supplements are major constituents of nutraceuticals which make them instrumental in maintaining health, act against various disease conditions and thus promote the quality of life [13].

The leek, Allium ampeloprasum var. porrum (L.), also known as Allium porrum. It which is a plant belonging to family Alliacea (Liliaceae), commonly named leek, is a biennial herb, closely related to garlic and onion [14]. Allium genus such as garlic (A. sativum), onion (A. cepa) and leek (A. porrum) are widely known vegetables, cultivated and consumed as flavors and foods throughout the world. In fact, garlic, onions and leek were likely cultivated in ancient Egyptian times; they are the oldest cultivated plants and are still used both as a food and for medical purposes. They are a rich source of a number of phytonutrients which make them important elements of the diet. They are also useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, type II diabetes, hypertension, cataract, osteoporosis and disturbances in the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia) [15].

MATERIALS AND METHODS

Animal
Adult albino male rats Wister strain, weighing 200-250 g, were used in the experiment of this study. They were obtained from the animal house colony of the National Research Center (Dokki, Giza, Egypt) and were housed under conventional laboratory conditions throughout the period of experiments. The animal were fed a standard rat pellet diet and allowed free access to water. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals [16].

Drugs and herbs
Calcium acetate (Marcyrl Company, Egypt) and alcoholic extract of Allium porrum bulbs, and leaves (cultivated in Egypt) were used in this study. All drugs were freshly dissolved in distilled water and were given po. The concentration was adjusted so that each 100 g animal body weight received 0.5 ml, containing the required dose of each drug.

Chemicals and test reagent kits
Chemical :
- Diethyl ether from Chemicals from El Nasr Pharmaceutical.
- Thiopental sodium 0.5 g from Sandoz Co., Austria.
Reagent kits:-

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- Calcium according to Gindler and King, [17].
- Phosphorus according to El-Merzabni et al., [18].
- Alkaline phosphatase according to Belfield and Goldberg, [19].
- Lipid Peroxide according to Satoh, [20].
- Total antioxidant according to Koracevic et al., [21].

All kits are colorimetric method and were obtained from Biodiagnostic Co., Egypt.

Identification of the Allium porrum

Allium porrum were collected from Banha city, Egypt during the months of May and June; 2006. The plant was botanically identified and authenticated by Dr. Ahmed Abd Al-Aziz Abd Al-Fattah, Ass. Prof. of aromatic and medicinal plant department, agricultural research centre. Then leaves and bulbs were dried and ground to powder using clean mill and mortar then soaked in 1L of 70% ethanol and left to stand 24 hours with shaking, the solution was filtered and alcohol removed from the filtrates using rotary evaporator under 60-70°C [22].

Experimental design

Osteoporosis inducted in male rats by i.p. injection of 20% (vol/vol) alcohol/saline solution (3g/kg/day) [where each 1ml of absolute alcohol is equivalent to 0.793gm of absolute alcohol] for four consecutive days for 3 weeks [23], then examined the effects of alcohol treatment on bone resorption by measuring bone mineral density (BMD) of treated rats and compared them with negative control group using DEXA.

Bone mineral content (BMC) and bone mineral density (BMD) of femur were examined in each rat using Dual Energy X-ray Absorptiometry (DEXA). Rats were anaesthetized with i.p. injection of thiopental sodium (40mg/Kg) 10 min before exposing to DEXA [24].

Rats were divided to five groups, of 8 rats/each as follows:

Group 1: rats given p.o. distilled water (0.5ml/100g) throughout the experiment and served as negative control group,

Group 2: rats injected i.p. only by 20% ethanol (3g/ kg/day) for 4 successive days for 3 weeks) and served as positive control,

Group 3,4: rats were given p.o. the extract of Allium porrum (250 & 250 mg/kg) respectively for 8 successive weeks before induction of osteoporosis and simultaneous with i.p. ethanol 20% for 4 successive days of each week for 3 successive weeks, Group 5: rats were given calcium acetate powder (53 mg/kg) p.o for 8 successive weeks before induction of osteoporosis and simultaneous with i.p. ethanol 20% injection for 4 successive days of each week for 3 successive weeks.

At the end of the experiment blood samples were withdrawn from retro orbital venous plexus of rats after light ether anesthesia and the serum was separated by centrifugation at 3000 rpm for 10 min.

Statistical analysis

Data was expressed as mean ± SE where (n=8). Statistical comparisons between the control group and the treated groups were carried out using one-way ANOVA, followed by Duncan’s multiple range tests. Significance difference between groups were determined at the corresponding time at p<0.05.

RESULTS

Effect of oral administration of Allium porrum alcoholic extract (250 and 500 mg/kg) and calcium (54mg/kg) daily for 11 successive weeks, 8 weeks before induction of osteoporosis and 3 weeks simultaneous with 20% ethanol i.p (3gm/kg/day for 4 days) is resulted in the following:

Bone mineral density

The mean value of BMD of the normal control group (given distilled water) was 0.1003± 0.0041unit. Administration of 20% ethanol (3g/kg) leads to a significant decrease in BMD by 40%. administration of Allium porrum alcoholic extract (250, 500mg/kg) and calcium (54mg/kg) p.o. lead to restoration of BMD by 31, 46 and 32% respectively compared with negative control group (Fig. 1).

Bone mineral content:

The mean value of BMC of the negative control group was 0.03524±0.0024 unit. There weren’t any significant change in BMC of all other groups.
Table (1): Effect of oral administration of *Allium porrum* alcoholic extract (A.p.ext.) (250, 500 mg/kg/day) on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in rats.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Time</th>
<th>Groups</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Normal control</td>
</tr>
<tr>
<td>ALT (unit/ml)</td>
<td>Basal</td>
<td>14.3±0.5</td>
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<td></td>
<td>4 weeks</td>
<td>13.8±1.2</td>
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<td></td>
<td>8 weeks</td>
<td>15.7±1.4</td>
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<tr>
<td>AST (unit/ml)</td>
<td>Basal</td>
<td>108.5±1.56</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>112.6±1.7</td>
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<tr>
<td></td>
<td>8 weeks</td>
<td>105.7±3.2</td>
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<tr>
<td>ALP (IU/L)</td>
<td>Basal</td>
<td>187.4±5.4</td>
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<tr>
<td></td>
<td>4 weeks</td>
<td>199.6±18.7</td>
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<td></td>
<td>8 weeks</td>
<td>211.8±89.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using two-way ANOVA followed by Duncan’s multiple range tests.

No significant difference between groups of all treated rats at the corresponding time at p<0.05.

Table (2): Effect of oral administration of *Allium porrum* alcoholic extract (A.p.ext.) (250, 500 mg/kg/day) on blood urea nitrogen (BUN) and serum creatinine in rats.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Time</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal control</td>
</tr>
<tr>
<td>BUN (mmol/l)</td>
<td>Basal</td>
<td>7.45±0.3</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>7.1±0.4</td>
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<tr>
<td></td>
<td>8 weeks</td>
<td>6.7±0.25</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>Basal</td>
<td>0.7±0.021</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>0.7±0.0089</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>0.68±0.023</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using two-way ANOVA followed by Duncan’s multiple range tests.

No significant difference between groups of all treated rats at the corresponding time at p<0.05.

Fig.1. Effect of oral administration of alcoholic extract of *Allium porrum* (A.p.ext.) (250, 500mg/kg) and calcium (54mg/kg) on bone mineral density (BMD) in 20% ethanol-treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range test.

@ Statistically significant from 20% ethanol-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.

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Fig. 2. Effect of oral administration of alcoholic extract of *Allium porrum* (A.p.ext.) (250, 500mg/kg) and calcium (54mg/kg) on bone mineral content (BMC) in 20% ethanol-treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range test.

No significant difference between groups of all treated rats at the corresponding time at p<0.05.

Fig. 3. Effect of oral administration of alcoholic extract of *Allium porrum* (A.p.ext.) (250, 500mg/kg) and calcium (54mg/kg) on serum calcium (Ca++) level in 20% ethanol-treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range test.

@ Statistically significant from 20% ethanol-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.
Fig.4. Effect of oral administration of alcoholic extract of *Allium porrum* (A.p.ext.) (250, 500mg/kg) and calcium (54mg/kg) on serum phosphorus (P++) level in 20% ethanol-treated rats .

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range

No significant difference between groups of all treated rats at the corresponding time at p<0.05.

Serum biochemical tests:

**Serum calcium (Ca++) level:**
The mean serum Ca++ of the negative control group was 9.93±0.154 mg/dl. There weren’t any significant change in any group except for calcium (54mg/kg) treated group which exhibited increase in serum Ca++ by 22.4% as compared with negative control group (Fig. 3).

**Serum phosphorus (P++) level**
The mean serum P++ of the negative control was 7.29±0.197mg/dl. There wasn’t any significant change caused by any of other group as compared with negative control of rats (Fig. 4).

**Serum alkaline phosphatase (ALP) level**
The mean serum ALP of the negative control was 194.34±9.16 IU/L. Ethanol and calcium treated group of rats showed a significant increase in serum ALP by 35.9%, 40.2% respectively compared with negative control group. Administration of alcoholic extract of *Allium porrum* (250 and 500mg/kg) showed significant reverse to the elevation in serum ALP levels to reach approximately negative control values (Fig. 5).

**Serum malondialdehyde (MDA) level:**
The mean serum MDA of the negative control group was 2.56±0.31 nmol/ml, this result was increased in significant way in calcium (54mg/kg) and 20%ethanol (3g/kg) treated groups of rats by 148, 123% respectively in comparison
with negative control. Administration of alcoholic extract of *Allium porrum* (250 and 500mg/kg) significantly reduced the elevation in serum MDA levels to reach approximately the normal control values (Fig. 6).

![Graph of ALP levels](image)

**Fig.5.** Effect of oral administration of alcoholic extract of *Allium porrum* (A.p.ext.) (250, 500mg/kg) and calcium (54mg/kg) on serum alkaline phosphatase (ALP) level in 20% ethanol-treated rats. Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range test.

@ Statistically significant from 20% ethanol-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.

**Serum total antioxidant capacity (TAC) level:**

The mean serum TAC of the negative control group of rats (given distilled water) was 1.834±0.17 Mm/L. This result was decreased in significant way in ethanol (3g/kg) and calcium (5mg/kg) treated groups by 59.2, 54.7% respectively in comparison with negative control rats. Administration of alcoholic extract of *Allium porrum* (250 and 500mg/kg) significantly reduced the elevation in serum TAC levels to reach approximately the normal control values (Fig. 7)
Fig. 6. Effect of oral administration of alcoholic extract of *Allium porrum* (A.p.ext.) (250, 500 mg/kg) and calcium (54 mg/kg) on serum malondialdehyde (MDA) level in 20% ethanol-treated rats. Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range test.

@ Statistically significant from 20% ethanol-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.

Fig. 7. Effect of oral administration of alcoholic extract of *Allium porrum* (A.p.ext.) (250, 500 mg/kg) and calcium (54 mg/kg) on serum total antioxidant capacity (TAC) level in 20% ethanol-treated rats.

Data are expressed as mean ± SE (n=8).

Statistical comparison of difference between the control group and the treated groups was carried out using one-way ANOVA followed by Duncan’s multiple range test.

@ Statistically significant from 20% ethanol-treated group at the corresponding time at p<0.05.

* Statistically significant from the normal control group at the corresponding time at p<0.05.
DISCUSSION

In this study the i.p. injection of 20% ethanol for 4 consecutive days for 3 weeks was used to induce osteoporosis. BMD measured in rats proximal femur by DEXA scan as indicative parameter of osteoporosis. Ethanol administration resulted in a significant decrease in BMD compared with negative control group rats. This finding agreed with Callaci et al., [23]. There is evidence that ethanol either directly inhibit osteoblast function or other ethanol-related complications may aggravate bone loss, these complications could be liver disease, under nutrition, hypercalcuiuria, changes in parathyroid hormone (PTH) secretion or function and abnormal vitamin D metabolism [25]. Diamond et al., [26] stated that ethanol may be responsible for osteoblastic dysfunction resulting in diminished bone formation and reduced bone mineralization. Keller et al., [27] reported that ethanol may contribute to osteoporosis by direct action. There is evidence that ethanol may increase bone resorption and inhibit bone production, resulting in osteopenia. The proinflammatory cytokine, interleukin-6 (IL-6), is an important mediator of osteoclast activity on bone, the investigators provide evidence that ethanol induces IL-6 expression and IL-6 promoter activity in a human bone marrow stromal cell line and increases serum IL-6 levels in mice [28].

Oral administration of Allium porrum (250 and 500 mg/kg) keep BMD in the same range as in negative control group in the same time while daily oral administration of calcium (53mg/kg) restore BMD partially but remained less significantly than negative control group.

Wetli et al., [29] study demonstrates that structural analysis of ethanolic extract of Allium cepa produce nonprotein sulfur amino acids derived from cysteine which is γ-L-glutamyl-trans-S-1-propenyl-L-cysteine sulfoxide (γ-GPeCSO) compound and inhibiting significantly the osteoclast activity and there is significant (p < 0.05) correlation between the amounts of γ-GPeCSO and the osteoclast activity inhibition. According to Dugravot et al., [30] analysis of Allium porrum produced nonprotein sulfur amino acids derived from cysteine such as propylcysteine sulfoxide this may describe this preventive effect of Allium porrum. The green leaves of Allium porrum mainly contain kaempferol glycoside and traces of quercetin-3-glucoside were identified by TLC [31]. The bulbs of Allium porrum contain only few milligrams of glycosides of kaempferol and quercetin per kg fresh weight. A phytochemical investigation of the extracts obtained from bulbs of leek. Allium porrum has led to the isolation of five flavonoid glycosides based on the kaempferol aglycone [32]. According to Wattel et al., [33] flavonoids widely distributed such as quercetin and kaempferol, exert a potent inhibitory effect on in vitro bone resorption. Flavonoids derivatives as quercetin and kaempferol can stimulate osteoblastic activity and such compounds may represent new pharmacological tools for the treatment of osteoporosis [34]. Also Trivedi et al., [35] reported that kaempferol has no-estrogenic effect in vivo but exerts bone anabolic activity at a dose 5 mg/kg of kaempferol completely prevented bone loss in the femur caused by ovariectomy, these results obtained by DEXA analysis and thus may be due to inhibition of osteoclast differentiation.

In present study the serum calcium level were elevated significantly in calcium (53mg/kg) treated rats. This result is in agreement with Polley et al., [12] results who reported that one of the side effects of calcium treatment is hypercalcemia.

As regard the level of alkaline phosphatase in current study was significantly increased as compared with negative control rats after ethanol administration. This was agreed with Yamada et al., [36] who stated that the increased serum alkaline phosphatase levels observed in response to chronic ethanol feeding may be due, at least in part, to increased lability of this plasma membrane enzyme [37]. Oral administration of Allium porrum (250 and 500 mg/kg) restore the elevated serum alkaline phosphatase in positive control group. This finding was agreed with El-Meihry, [38] who reported that ethanolic Allium porrum cause significant decrease in alkaline phosphatase.

The significant increase MDA and decrease in total anti oxidant capacity compared with negative control group rats could be explained by the high oxidative stress of ethanol. The involvement of free radical mechanisms in the pathogenesis of alcoholic liver disease (ALD) is demonstrated by the detection of lipid peroxidation markers in the liver and the serum of patients with alcoholism, as well as by experiments in alcohol-feed rodents that show a relationship between alcohol-induced oxidative stress and the development of liver pathology. Ethanol-induced oxidative stress is the result of the combined impairment of antioxidant defenses and the production of reactive oxygen species by the mitochondrial electron transport chain, the alcohol-inducible cytochrome P450 (CYP) 2E1 and activated phagocytes. Furthermore, hydroxyethyl free radicals (HER) are also generated during ethanol metabolism [39].
Bozhko et al., [40] stated that under conditions of acute ethanol intoxication an increase of the MDA concentration is caused by the acetaldehyde (ethanol metabolite) action. The long-term ethanol intoxication induces a significantly increase the MDA concentration (if compared with acute intoxication). Acetaldehyde is an important factor among those determining disturbances of cell biogenesis in the animal organism, causing an increase of the blood serum MDA. The oxidative stress is an important mediator of bone loss since deficiency of antioxidant vitamins has been found to be more common in the elderly osteoporotic patients. Increased free radicals production overwhelms the natural antioxidants defense mechanisms, subjecting individuals to hyperoxidant stress and thus leading to osteoporosis [41].

Oral administration of *Allium porrum* daily with the same dose and duration mentioned before reverse the oxidative effect of 20% ethanol and this reducing capacity can be described by the presence of its phenolic content, this result is agreed with Tsai et al., [42] who reported that aqueous extracts of *Allium porrum* appeared to contain more phenolic compounds than those of garlic and green onion and thus the antioxidant activities of *Allium porrum* is bigger than green onion and garlic. Also Rose et al., [43] demonstrated that several organosulfur compounds identified in *Allium* species have antioxidant properties, the organosulfur compounds found in *Allium porrum* extract, can reduce lipid peroxidation and hydrogen peroxide formation.

Borek, [44] reported that organosulfur compounds, such as S-allylcysteine and S-allylmercaptocysteine which extracted from *Allium porrum* exert an antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells. They also inhibit lipid peroxidation. Yin et al., [45] demonstrated that the antioxidant protection of organosulfur agents was concentration dependent.

It is concluded that oral administration of *Allium porrum* (250 and 500 mg/kg) has significant protective effect against osteoporosis induced by ethanol. This effect may be achieved due to the presence of sulfur amino acid compounds which have antioxidant effect in *Allium porrum* [46].

In addition to sulfur amino acid, there is also flavonols such as kampferol which inhibit significantly osteoclasts activity and bone resorption [30, 31].

Thus safety and efficacy of *Allium porrum* make the isolation of its active constituents and testify their therapeutic effect on different biological diseases is recommended.

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