Evaluation of possible preventive activity of *Elaeagnus angustifolia* L. against osteoporosis, an *in vivo* study

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\textbf{Abstract}

Osteoporosis is generally associated with decrease and degeneration of bone tissue which may lead to fragileness and fracture. A part from conventional interventions, application of natural medicine in prevention and management of osteoporosis is also given consideration. Accordingly, current study aimed to assess the possible effects of *Elaeagnus angustifolia* L. (Elaeagnaceae) fruits hydroalcoholic extract on some biochemical parameters related to bone density. In an 8-week intervention, seventy virgin rats were distributed in seven study groups and sixty of those were bilaterally ovariectomized. Groups were as; first or control with no surgery or intervention, second as ovariectomized control group with no intervention, third ovariectomized with estradiol (2 mg/kg)-injected, fourth as ovariectomized with *E. angustifolia* extract (oral, 500 mg/kg), fifth as ovariectomized *E. angustifolia* extract (oral, 1000 mg/kg), sixth as ovariectomized rats with *E. angustifolia* extract (oral, 1500 mg/kg), and seventh as ovariectomized and received both *E. angustifolia* extract (oral, 1000 mg/kg) and estradiol (IM, 2 mg/kg). At the end, mean serum calcium, phosphorus, alkaline phosphatase and prostatic acid levels in each group were evaluated. Neither estradiol, nor extract could elevate the serum calcium and phosphorus levels. Also the level of alkaline phosphatase remained stable. Prostatic acid phosphatase-usually increases during osteoporosis- significantly declined in intervention groups (extracts), dose dependently. To assess the preventive effects of *E. angustifolia*, the intervention was performed once after ovariectomy. The negative results for some parameters may be related to poor osteoporosis intensity. Although impacts of the extract on serum calcium, phosphorus and alkaline phosphatase was not satisfactory, significant decline in tartrate-resistant (Prostatic) acid phosphatase was found. Based on the results, *E. angustifolia* may have positive preventive effects on osteoporosis. However, complementary studied are to be added to this study to conclusively proved the results.

\textbf{Keywords:} Osteoporosis, *Elaeagnus angustifolia*, Traditional Medicine, Animal study.

1. Introduction

Osteoporosis is generally associated with decrease and degeneration of bone tissue which may lead to fragileness and fracture (1, 2). This disorder is spoken as a main cause for mortality in old subjects (3). Epidemiologically, it is estimated that the prevalence of this complication will reach...
to around 45% of all fractures up to 2050 (4).

Femur and hip fractures are of most prevalent but life threatening complications especially in geriatrics. Based on etiological factors, osteoporosis is raised by aging and also is more observed in females and low weight subjects (5). However, secondary parameters such as vitamin D deficiency, endocrine or genetic disorders, and pharmacotherapy with corticosteroids and anti-convulsants, as well as gastrointestinal complications have considerable impact on the occurrence of osteoporosis (6, 7).

Management approaches to this disorder is involved preventive strategies and treatment lines (8). Life style modification and vitamin D supplementation are of most preventive approaches (9). Treatment lines are often based on inhibition of mineral reuptake and substitution or development of bone mass and density (10). Mostly, bisphosphonates, Calcium, vitamin D, estrogen and progesterone and also human recombinant parathormone hormone and calcitonin are extensively administered in the management of osteoporosis (11-13).

Other than the conventional remedies, traditional and complementary medical systems have reported numerous natural medicaments for the management of osteoporosis and bone complications (14). Traditional Persian Medicine (TPM) which is a complex of ancient and medieval medical knowledge and experiences of indigenous scholars during the golden Islamic era (15) have presented various medicinal herbs for the management of such disorders (16). One of these natural remedies which also deeply believed to be effective by natural healers and traditional practitioners in Iran is Elaeagnus angustifolia L. (Elaeagnaceae) (17). This medicinal plant is a nitrogen-fixing shrub which is being traditionally used as an analgesic, and to treat rheumatoid arthritis, osteoarthritis, respiratory and gastrointestinal problems (18, 19). Despite the continuous administration and relevant ethnopharmacology, no decisive studies have been performed on the effectiveness of this natural medicine on osteoporosis. In this regard, current study aimed to assess the possible impact of E. angustifolia fruits extract on some biochemical parameters related to bone density in an animal study.

2. Materials and methods

2.1. Plant preparation and extract

Totally ten samples of E. angustifolia fruits were purchased from different medicinal plants markets in Shiraz (South of Iran), out of those four samples were selected based on botanical freshness. One hundred grams of each selected fruit samples were individually subjected to hydroalcoholic extraction via a Soxhlet extractor for almost 6 h. Among those extract, a sample with highest yield of extraction %W/W was selected for animal study.

2.2. Animals, study groups and interventions

Seventy virgin rats with normal weight of 150-200 g were randomly and equally distributed in seven study groups. Prior to distribution, sixty of those were subjected to bilateral ovariectomy under aseptic condition and ether anesthesia in order to induce the osteoporosis. Groups of investigation were as; the first or control with no surgery or intervention, the second as the control group which was ovariectomized (OVX), but received no intervention, the third group is the ovariectomized rats with estradiol (2 mg/kg)-injected intramuscularly, the fourth group is the ovariectomized rats with E. angustifolia extract (oral, 500 mg/kg daily), the fifth group is the ovariectomized rats with E. angustifolia extract (oral, 1000 mg/kg), the sixth group is group is the ovariectomized rats with E. angustifolia extract (oral, 1500 mg/kg), and finally the seventh group is group is the ovariectomized rats which have been received both E. angustifolia extract (oral, 100 mg/kg) and estradiol (IM, 2 mg/kg). The study was conducted for 8 weeks and all animals were received the respective interventions during the study. All animals in groups were fed with usual daily diet during the study. At the end of the protocol, animals were anesthetized ethically with diethyl ether and exsanguinated by drawing blood from the aorta. Blood samples were kept for biochemical analysis. In this study, Serum Calcium, Phosphorus, Alkaline phosphatase and Tartrate resistant acid phosphatase were evaluated.
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2.3. Statistical analysis

Biochemical data and comparison between study groups were analyzed using an ANOVA and LSD tests. Prior analysis, normality with mean and variance (Asymp. Sig.>0.05) among resulted data was performed via Kolmogorov-Smirnov Test.

3. Results and discussion

In this study, the preventive effects of *E. angustifolia* hydroalcoholic extract against osteoporosis in ovariectomized rats were evaluated. Following 8 weeks of intervention, mean serum calcium, phosphorus, alkaline phosphatase and prostatic acid phosphatase (tartrate resistant acid phosphatase) levels in each group were evaluated. Subsequently, differences between each groups were assessed in regard of each of the aforementioned parameters.

Figures 1 to 4 respectively represented the comparison among all study groups for the serum level of aforementioned parameters. According to figure 1, no significant differences have been observed between intervention groups of the study. Neither estradiol, nor extract in different concentrations could elevate the serum calcium level. However, despite ovariectomy, the level of calcium remained stable and no decline was observed. Therefore, it can imply that the effectiveness of extract in stabilization of calcium level is the same as estradiol, the conventional medicine. On the other hand, the level of calcium in control group was also stable. However, compensatory mechanisms such as impacts of parathormone hormone or kidney function may remain the calcium level at a constant condition. Similarly, the levels of phosphorus and alkaline phosphatase were remained stable at the end of the study (Figure 2, 3).

![Figure 1. Levels of calcium in study groups.](image1.png)

![Figure 2. Levels of phosphorus in study groups.](image2.png)
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Phytochemically, presence of different types of flavonoids and phenols in the extract possesses therapeutic and also preventive effects against osteoporosis. The underlying mechanisms of these classes of metabolites are included the inhibitory effects on the release of minerals from bone tissue, improving the osteoblast function, and increasing in the resorption of calcium and phosphorus in bone tissue (20-22). Based on these aspects, *E. angustifolia* with various types of flavonoids, phenols and related medicament (23-25) should exert positive effects on bone tissue and osteoporosis.

Studying on natural medicaments, previous investigation resulted in different outcomes in regard of the aforementioned osteoporosis-related parameters. An investigation has evaluated the preventive effects of dietary soybean protein on bone loss in ovariectomized osteoporosis-rat model. In that study, following 30 days of intervention, both soybean and estradiol ovariectomized groups showed significant increase in bone density as compared to positive control. However, both alkaline phosphatase and tartrate-resistant acid phosphatase showed higher serum activities, indicating the controversies with the bone density (26).

A similar study revealed that standard diet contained soybean extract could improve serum calcium level in ovariectomized rat model. In that study, rat which were ovariectomized three months before were fed with the above diet for three months (27).

A recent investigation simultaneously evaluated the impact of *Litsea glutinosa*, *Curcuma aromatica* and *Terminalia arjuna* aqueous extracts on serum level of related parameters in ovariectomized rats. In that assessment, both extracts could reduce the levels of alkaline phosphatase and tartrate-resistant acid phosphatase in ovariectomized rats (28).

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Figure 3. Levels of alkaline phosphatase in study groups.

Figure 4. Levels of tartrate-resistant acid phosphatase in study groups.
Also hydroalcoholic extract of *Urtica dioica* (20 mg/kg) was checked for possible anti-osteoporotic activity in an ovariectomized rat model in comparison with raloxifene. According to the results of that investigation, no significant differences in calcium level were observed among all groups of intervention. However, the levels of alkaline phosphatase and phosphorus significantly changed in intervention groups compared to those of the control (29). One of the main differences of this current study with the previous items was that here the preventive effects of *E. angustifolia* were evaluated. Whereas, many of those studies have focused on therapeutic activities.

To assess the preventive effects of *E. angustifolia*, the intervention in this study was performed once after ovariectomy. The negative results for some parameters may be related to poor osteoporosis intensity.

4. Conclusion
Among many of the studies focusing on the anti-osteoprotic activity of natural medicines, current investigation was performed on an herb, ethnopharmacologically used to prevent osteoporosis. Accordingly the intervention was started once the ovariectomy performed in rat model. Although the impact of the extract on serum calcium, phosphorus and alkaline phosphatase was not satisfactory, significant decline in tartrate-resistant acid phosphatase was found dose dependently. Based on the results, *E. angustifolia* hydroalcoholic extract may have positive preventive effects on reducing osteoporosis. However, complementary studies are to be added to this study to conclusively proved the results.

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Conflict of Interest
None declared.

5. References

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