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Original Article

Effect of oral capsule of *Peganum harmala* seeds on bone density in menopausal women prone to osteoporosis

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Abstract

Background and aims: Osteoporosis is one of the most common metabolic bone diseases with systemic involvement of the body skeleton. The *Peganum harmala* seed contains high amounts of carboline alkaloids, which have been shown to have positive effects on bone formation in animal studies. In the present study, the effect of an oral capsule of *P. harmala* seed on bone density was evaluated in menopausal women prone to osteoporosis.

Methods: In this randomized controlled clinical trial, 100 women referring to the orthopedic clinic with a diagnosis of osteoporosis were included and divided into the intervention group treated with calcium D (500 mg) twice a day, Osteofos (70 mg) per week, and *P. harmala* (500 mg) twice-a-day, and the control group treated with calcium D and Osteofos. Before and three months after the intervention, patients were evaluated for osteoporosis using bone densitometry. Finally, independent t-test, paired t-test, and repeated measures ANOVA were used for statistical analysis.

Results: The mean bone mineral density (BMD) of the femur before and after the intervention showed significant improvements in the intervention and control groups ($P<0.001$). The mean differences in BMD before and after the intervention were significant in both control and intervention groups with higher improvements in the intervention group ($P<0.001$). Although the mean BMD of the spine before the intervention was not significantly different between the two groups ($P=0.167$), it was better in the intervention group after the intervention ($P=0.030$).

Conclusion: The findings of the present study confirmed the beneficial effects of *P. harmala* on osteoporosis while the lack of any changes in liver enzymes.

Keywords: *Peganum harmala*, Bone mineral density, Osteoporosis, Liver enzyme, Calcium, Vitamin D

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Introduction

Bone hemostasis in the body is regulated with osteoclasts and osteogenesis by bone absorption and osteoblastic cells, respectively (1). Osteoclasts are produced from blood monocytes and are responsible for decomposing bone matters during osteogenesis (2). Osteoclasts and osteoblasts have exactly reverse roles (3). Excessive bone absorption by osteoclasts plays a role in the pathogenesis of multiple bone diseases such as osteoporosis, hypercalcemia, rheumatoid arthritis, periodontitis, and Paget's disease (4). Osteoporosis, according to the definition of the National Association of Health, is a skeletal disorder that occurs more often in the elderly (5), and reducing bone strength and putting the person at the risk of fracture are among its obvious features (6). Osteoporosis is the most common pathologic cause associated with skeletal weakness and metabolic disease of the bone, which is associated with bone loss and finally

leads to fracture risk (7).

The process is so slow and gradual that the person does not feel any warning signs until the occurrence of the first fracture (8). Therefore, the importance of osteoporosis is associated with the increased femur, pelvic and spinal fractures, which lead to increased mortality and subsequent increases in costs (9). Therefore, it is believed that the complications of this disease are more precarious than the disease, especially fractures, which are the main factor affecting the quality of life (10).

In the United States, there are 1.5 million osteoporotic fractures per year, of which 700 000, 250 000, 250 000, and 300 000 cases are found in the vertebrae, the radius, the pelvis, and other bones, respectively (11). Reduced bone density and the resulting osteoporosis in women are a known phenomenon, and the fractures due to this complication are among the main causes of mortality in older people, especially postmenopausal women. In menopause women, due to estrogen deficiency, the increased activity of osteoclasts and the rate of bone loss, if preventive action is not taken, lead to osteopenia, progressing to osteoporosis (12,13).

Many studies have investigated the beneficial effects of various treatments on this disabling disease, but the lack of acceptance of patients and the side effects of drugs are among the problems that are not ignorable (14). Recently, the therapeutic effects of medicinal plants have been significantly considered, and the positive effects of some medicinal plants on osteoporosis have been proved as well (15). Some plants contain phytoestrogens and have functions similar to estrogen, inhibiting osteoporosis (16). *Peganum harmala* is one of the plants that has been considered in Iranian traditional medicine. Various alkaloids existing in this plant have extensive pharmacological effects such as antispasmodic (17,18), anti-cancer, anti-depressant (19,20), and antibacterial properties (21). They also can inhibit monoamine oxidase (22), bind to different types of receptors such as 5-TH and benzodiazepine receptors (20). They can improve benign prostatic hypertrophy symptoms (23) and the immune system (24). B-carboline alkaloids increase the growth and differentiation of bone and cartilage cells in cells and rats (25,26). Nonetheless, more studies are needed in this regard since no study has so far focused on osteoporosis in menopausal women. Therefore, the present study aimed to evaluate the effect of an oral capsule of *P. harmala* seeds on bone mineral density (BMD) in postmenopausal women with osteoporosis.

Methods

Participants

The trial was a randomized clinical trial study with 100 osteoporotic patients diagnosed with BMD. The criteria for including the trial were no digestive disturbance disorders, no thyroid, renal and hepatic diseases, and no psychiatric disorders. On the other hand, the exclusion criteria included drug allergy, restlessness, convulsion, and mental disorders. Each group of 50 patients was determined using the following formula:

$$n = \frac{z_{(1-\frac{\alpha}{2})} + z_{(1-\beta)}}{d})^2$$ n=50, d=0.4 1.96=$z_{(1-\alpha/2)}$, 0.84=$z_{(1-\beta)}$

Study design, intervention, and randomization

Patients were selected by convenience sampling and randomly assigned to the two groups using Random Allocation Software. Random allocation was performed by a person who had no role in any procedures of the study (Figure 1). The first group (intervention group) received capsules containing *P. harmala* (500 mg) twice a day for 3 months in addition to routine drugs (calcium 500 mg twice a day was produced by Tehran *Chemie* Pharmaceutical *Company*, alendronate sodium 70 mg or Osteofos once per week produced by Alborzdarou Pharmaceutical Company). The second group (control group) received routine drugs with the same dose. Participants were requested not to change their food intake and physical activity during the intervention.

The inclusion criteria were postmenopausal women who, according to the World Health Organization (WHO), had at least 12 months of menstruation and osteoporosis with a T-score of ≤ -2.5 in bone densitometry. According to the WHO definition, osteoporosis in postmenopausal women based on densitometry is a BMD reduction of 2.5 or greater than the standard deviation under the mean value for young adults (T-score ≤ -2.5). In this definition, -2.5 ≤ T-score < -1 is considered osteopenia while T-score ≥ -1 is considered normal (27).

Exclusion criteria included pregnancy, breastfeeding, smoking, drinking alcohol, other hormonal disorders, and use of hormones or drugs with a negative or positive effect on bone metabolism (e.g., estrogens and bisphosphates) in the past year. Other criteria were the lack of volunteering to participate in the study, allergy to drugs, certain complications such as hallucinations, confusion, and numbness, nausea, vomiting, restlessness, seizure, disorders of intestinal absorption, and thyroid, kidney, liver disorders.

After the botanist approval of the Medical Plants Research Center in Shahrekord University of Medical Science, *P. harmala* seed (500 mg) was prepared in the form of capsules. Study objectives and data confidentiality were explained to the participants, and then informed consent was obtained from them for participation in the study.

However, due to the possible hepatotoxic effects of *P. harmala*, liver factors such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were checked in all patients before treatment, as well as in the first, second, and third month after the treatment.

Bone Densitometry Assessment

The dual X-ray absorption was used to perform bone densitometry reported as standardized values called T- and Z-scores (28,29). In this procedure, two X-ray sources

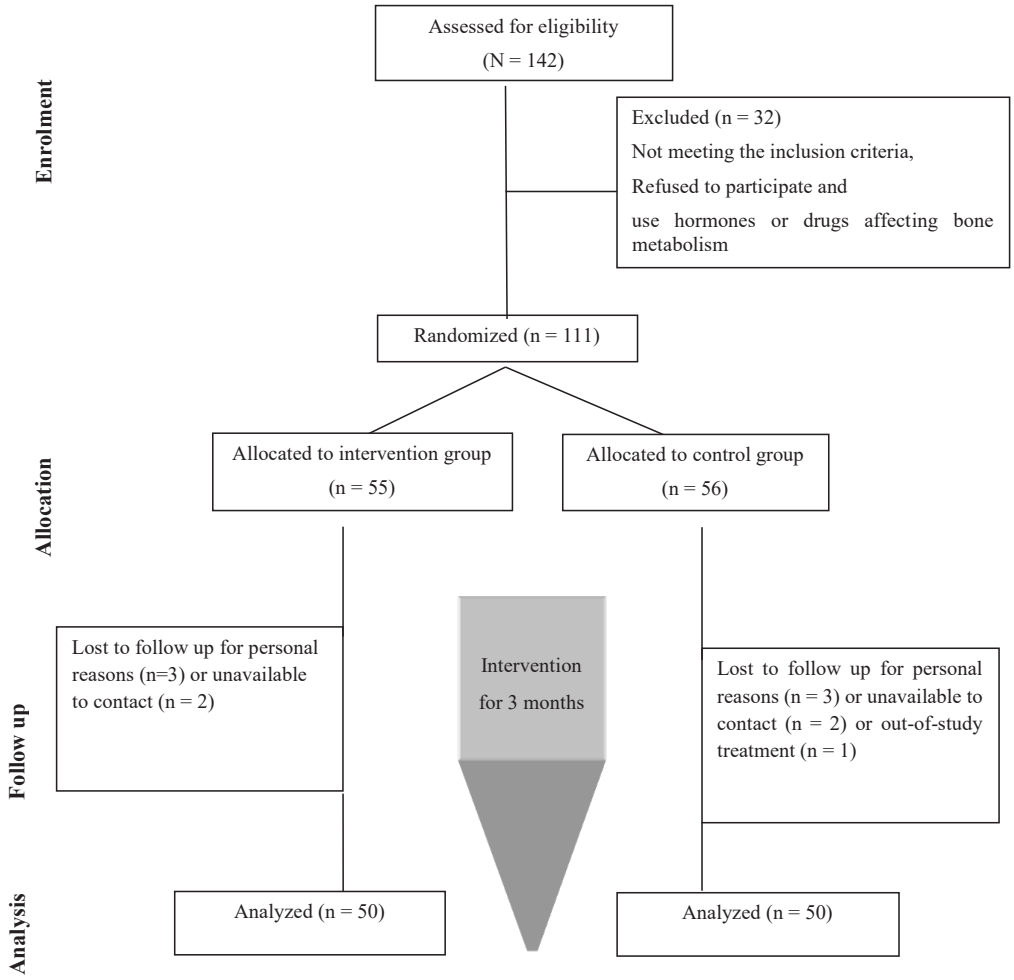

Figure 1. Algorithm of study participants in the two groups.

are sent to the bone to measure the density of the spine, hip, or forearm. Two sources of X-rays are applied to assure accurate measurement. The femur and spine bones were examined in this study. The WHO classification of BMD according to the t-score (30) was normal (-1, 0, or greater in the t-score), osteopenia (-1, 0, or greater in the t-score), osteoporosis (-2.5 or less in the t-score), and severe osteoporosis (-2.5 or less in the t-score with fracture). BMD was measured at the beginning and end of the study (30).

Statistical Analysis

Data were entered into SPSS software (version 18) and analyzed by descriptive (Mean and standard deviation) and analytical (independent *t* test, paired *t* test, and repeated measures ANOVA with post hoc Tukey's test) statistics, and $P<0.05$ was considered as the significance level.

Results

The mean age was not significantly different between the intervention (59.02±10.49) and control (59.06±9.71) groups ($P=0.984$). The mean BMD of the femur before treatment were not significantly different in the intervention and control group ($P=0.927$) and the mean BMD of the femur after treatment showed no significant difference in the control group ($P=0.181$), but a significant decrease was observed in the mean BMD of the femur in each of the intervention and control groups ($P<0.001$). In general, the mean of changes in the BMD of the femur was significantly higher in the intervention group (-0.37) compared to the control group (-0.24) before and after the treatment ($P<0.001$, Table 1).

Conversely, the mean BMD of the spinal cord before the treatment were not significantly different in the intervention and control groups ($P=0.167$) while the BMD of the spinal cord was significantly lower in the intervention group after the treatment in comparison with the control group ($P=0.030$). Based on the results, a significant decrease was found in the mean BMD of the spinal cord in each of the intervention and control groups ($P<0.001$). In general, the mean of changes in BMD of the spinal cord before and after treatment in the intervention group (-0.44) is significantly higher than the control group (-0.22) ($P<0.001$, Table 1).

The results of the repeated measures analysis test demonstrated that the mean ALT in the intervention and control groups was not significantly different ($P=0.592$).

However, the mean ALT in the times of the first, second, and third month significantly differed from the time before the intervention ($P=0.001$). The mean ALT in each of the intervention and control groups in the studied times of treatment represented no significant difference ($P=0.996$, Table 2 and Figure 2A).

Moreover, repeated measures analysis findings revealed no significant difference in the mean AST in the intervention and control groups ($P=0.942$). The mean AST in the times of the first, second, and third month significantly varied from the time before the intervention ($P<0.001$). Contrarily, the mean AST in each of the intervention and control groups in the studied times of treatment was not significantly different ($P=0.213$, Table 2 and Figure 2B).

Based on repeated measures ANOVA results and the post hoc test , there was a significant decrease in the mean in the first, second, and third month, which was significantly different from the time before the treatment ($P<0.001$).

Discussion

This study aimed to investigate the effect of *P. harmala* seeds on osteoporosis. Traditional treatments are the commonly used methods for diseases in many parts of the world (26,31). According to the results of bone densitometry, the mineral density of the bone in the spine and hip in the intervention and control groups had a significant improvement after the intervention, and their improvements in the intervention group were significantly higher compared to the control group, reflecting the significant effects of *P. harmala* seeds on bone density in patients prone to osteoporosis.

The positive effect of *P. harmala* can be due to the existence of secondary metabolites, especially alkaloids. Previous research showed that *P. harmala* contains high amounts of beta-carboline alkaloids such as harmalol, harmaline, and harmine (33).

The findings of an animal study demonstrated that the β-carboline alkaloids present in *P. harmala* increase the activity of alkaline phosphatase (*ALP*) in MC3T3-E1 cells and expand the expression of mRNA, which increases osteoblast markers, including *ALP* and osteocalcin, and accelerates the mineralization of MC3T3-E1 cells. Additionally, harmine increases the differentiation in primary osteoblasts and stem cells by inducing the expression of the bone morphogenetic protein (BMP) and Runx2 pathways (33-35). Yonezawa et al reported that harmine, which is a compound found in *P. harmala*, has anabolic effects on osteoblast (34).

Osteocalcin and ALP are two of the most important markers of bone formation, reflecting various aspects of osteoblast function and bone formation. A change in the ALP gene and osteocalcin is an important determinant of bone loss due to age (36). The ALP gene plays an important role in the process of mineralization and regulates the concentration of the extracellular pyrophosphate inhibitor (PPI) via the hydrolysis of PPI, which can inhibit the formation of hydroxyapatite crystals and is required in the process of bone mineralization. Since osteoblasts are a major source of ALP, increasing the expression of the ALP gene and its serum level is an indication of the stimulation of osteoblast cells, and then cellular signaling increases with increased BMD (37). Furthermore, the ALP gene has an essential role in the active metabolism of inorganic phosphate release via the hydrolysis of phosphorus

Table 1. Comparison of bone densitometry results in intervention and control groups

Variables		Intervention group (n=50) Mean ± SD	Control group (n=50) Mean ± SD	*P* value[a]
BMD of the femur	Before	-2.32±0.86	-2.32±1.89	0.927
	Sfter	-1.88±0.81	-2.11±0.89	0.181
	P value[b]	0.000**	0.000**	
	Difference between before and after treatment[a]	-0.37±0.09	-0.24±0.17	0.000**
BMD of the spinal cord	Before	-2.46±0.82	-2.67±0.66	0.167
	After	-2.10±0.82	-2.43±0.68	0.030*
	P value[b]	0.000**	0.000**	
	Difference between before and after treatment[a]	-0.44±0.12	-0.22±0.07	0.000**

Note. SD: Standard deviation; BMI: Body mass index.
** Significant at $P<0.001$; * Significant at $P<0.05$; [a] Independent t-test; [b] Paired t-test.

Table 2. Effects of group (control and intervention), time (before, first month, second month, and third month after treatment), and their interaction group*time

Scale	Group			Time			Group * Time		
	F	df	*P*	F	df	*P*	F	df	*P*
ALT	0.289	1.98	0.592	6.291	3.294	0.001[a]	0.016	3.294	0.996
AST	0.005	1.98	0.942	8.750	3.294	<0.001[a]	1.510	3.294	0.213

Note. ALT: Alkaline aminotransferase; AST: Aspartate aminotransferase.

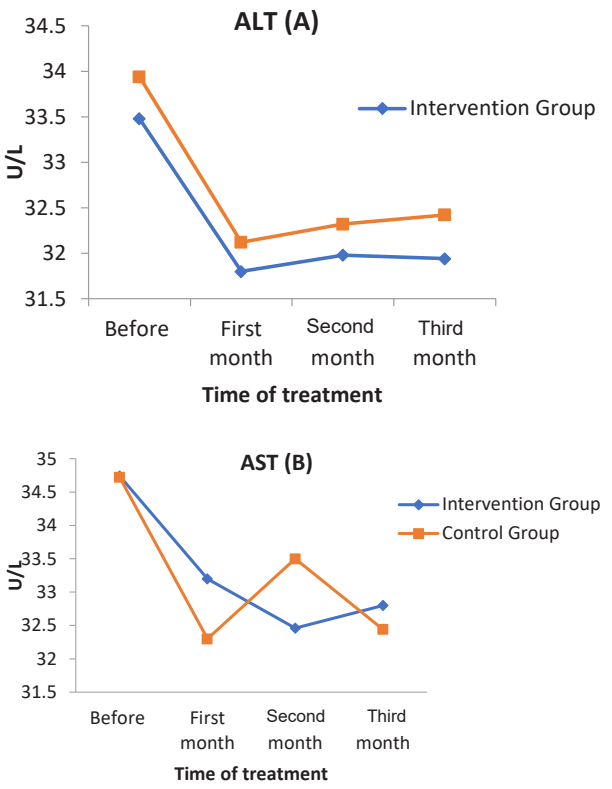

Figure 2. Mean of liver enzymes at the time of interventions (before, first, second, and third month after the treatment) between control and intervention groups. *Note*. ALT: Alkaline aminotransferase; AST: Aspartate aminotransferase.

components (38).

In a study focusing on determining the mechanism of hormone estrogenic effects in the cell culture, it was reported that the use of the BMP antagonist and its receptor inhibition reduced harmine effects in increasing ALP activity and the growth and differentiation of bone cells (39). Harmine also increases the expression of Runx2 and Osterix mRNA, which are the main transcription factors in differentiating osteoblasts (40). Harmine treatment activates the receptors of BMP and Runx2 (41). Similarly, it was found that harmonium (10 mg/kg) prevented bone loss in ovariectomized mice with osteoporosis (42). In addition, another study reported that harmine increased the expression of cartilage markers including agricon and COL2α1, and led to an increase in the primary regulator of chondrogenesis, SOX-9, and chondrogenesis in ATDC5 chondrogenesis in ATDC5 cells (41). Considering that the material of the bone fields contains collagen and cartilage, harmine may increase bone formation and bone density by increasing the production of the cartilage (43).

The study showed that the levels of liver enzymes such as AST and ALT did not changed, Previous histological studies as well indicates liver degeneration does not occur in the rats treated with a dose of 1 g/kg (44). Moreover, animal studies confirmed that *P. harmala* do not cause hepatocytotoxicity while the extracts of *P. harmala* are efficient for decreasing AST and ALT activities and bilirubin levels in male rats (45,46) Based on the evidence, harmaline was the most potent compound against hepatocarcinoma and fibrosarcoma (47,48).

The limitations of this study were the lack of the measurement of serum vitamin D levels and exposure to sunlight at the beginning and end of the study. Another limitation was the lack of using a placebo for the control group due to financial restrictions. However, the strength of this clinical trial study was the continuous follow-up of the intervention.

Conclusion

Overall, the results of this study approved that the use of *P. harmala*, along with a conventional drug improves the bone density of the thigh and spine in postmenopausal women with osteoporosis, which may reduce osteoporosis in postmenopausal women while indicating no hepatic or renal complications. The effects of *P. harmala* on BMD are probably due to its alkaloids of β-carboline, especially the harmine. These compounds have been shown to improve bone formation in cell culture media and animal models. To the best of our knowledge, the present study was the first one to demonstrate the beneficial effects of spin, including high levels of these compounds. Further studies are needed for more complete results in effect of compounds of *Peganum harmala* seeds.

Conflict of Interests

There is no conflict of interests.

Ethical Approval

This study was performed according to the guidelines presented in the Declaration of Helsinki. In addition, the study was approved by the Medical Ethics Committee of Shahrekord University of Medical Sciences (code: IR.SKUMS.REC.1395.33), and its protocol was confirmed by the Iranian *Registry of Clinical Trials* (identifier: IRCT2017091910222N122). The recommended dosage was regarded safe, namely, it does not harm the liver and kidney function (49,59).

Authors' Contributions

MD, ZKH, and FD jointly conceived and designed the study. ZLG and FF supervised the clinical trial, and MD, FD, and SF supervised orthopedics data analysis. Further, NA and ZKH conducted diagnostic tests, and SF and FM drafted the manuscript. ZLG and SF obtained funding. All authors read and approved the final manuscript.

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