COMPARISON OF THE EFFECT OF VITEX AGNUS AND SALVIA OFFICINALIS EXTRACT AT CALCIUM, PHOSPHORUS AND VITAMIN D LEVELS IN POSTMENOPAUSAL WOMEN REFERRING TO BONE MINERAL DENSITOMETRY CENTER: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Osteoporosis is an important problem in women’s health, one of the consequences of which is bone fracture. This clinical trial was performed in 2015 on 99 (33 patients per group) postmenopausal women referred to the bone mineral densitometry center. The first group received one Agnugol tablet (contains 3.2-4.8 mg dry extract of Vitex agnus castus, the second group received three Salvigol tablets (each contains 100 mg Salvia officinalis dry extract daily and the third group three placebo tablets per day for 3 months. Calcium, phosphorus and vitamin D levels were compared before and after the intervention. Results of paired t-test showed that the mean of calcium and phosphorus level increased after intervention in the Vitex agnus and S. officinalis extract groups, but there was no significant difference in the control group. LSD follow-up test was used, showing that the mean difference of calcium and phosphorus score between the Vitex agnus and S. officinalis extract groups, but there was no significant difference in the control group. However, the difference between the score of phosphorus level in Vitex agnus and S. officinalis extract groups was not significant after the intervention. After intervention, the mean of vitamin D increased by 2.4 units in the Vitex agnus group compared to before the intervention. However, there was no statistically significant difference between the Vitex agnus and control groups after the intervention compared to before it. The Vitex agnus was effective in increasing calcium, phosphorus and vitamin D level, while S. officinalis extract was effective in increasing the level of calcium and phosphorus.

INTRODUCTION

Osteoporosis is a systemic skeletal disorder associated with bone loss at bone volume and loss of the microstructure of the bone tissue1. The changes of Osteoporosis increase the fragility of the bone and lead to an increased risk of bone fracture that is the
clinical outcome of osteoporosis\(^2\). Causes of this disorder include a reduction in sex hormones after menopause, insulin-like growth factor deficiency, vitamin D deficiency, a decrease in calcitriol in the kidney and bone, lack of cytokines, increased activity of osteoclasts caused by decreased estrogen levels, disruption in vitamin D activity and disruption in calcium absorption\(^3\).

The role of vitamin D in the bone metabolism, calcium and phosphorus, osteoporosis onset and decreased muscle strength, on the one hand, and the increased risk of falls and fractures, on the other hand, are still the most important issues for specialists in this field\(^4\). In addition, various studies indicate that about one billion people worldwide suffer from deficient levels of vitamin D\(^5\). This issue is not only related to developing countries, and it has been estimated that more than 50% of the world's population has a shortage of vitamin D in a serum level below 30 ng/mL.\(^6,7\)

Increase of the efficiency of calcium absorption (possibly due to increased exposure to vitamin D under the influence of estrogen induction) and the direct role of estrogen receptors in osteoblasts are important potential factors in this regard\(^8\). Estrogen is a golden standard treatment for osteoporosis prevention. On the one hand, study findings suggest that hormone therapy is associated with complications such as cardiovascular disease, stroke, pulmonary embolism, breast cancer, vaginal bleeding, and some serious dangers\(^9,10\).

On the other hand, some researchers have argued that although the use of menopausal hormone therapy has been questioned over recent years in prevention of osteoporosis due to security concerns, this issue needs to be reconsidered according to the latest reports of more agreement on the greater benefits/risks originally expressed in postmenopausal women. This is because the osteoporosis-related fractures can significantly increase the mortality and morbidity of women\(^11\). Different therapies for osteoporosis are available. Most of these drugs are anti-refractory agents that nutritional sources of calcium and vitamin D are ideal\(^12\).

During the past 25 years, amino-bisphosphonates, either orally or intravenously, have remained the dominant and first-line treatment for osteoporosis. These risk factors reduce the risk of spinal fractures by 70%, hip fractures by 40-50%, and non-vertebral fractures by 50-80%\(^13\).

Complementary and Alternative Medicine (CAM) is also one of the treatments that is today popular among menopausal women, particularly in postmenopausal women\(^14\). Among complementary therapies, the change in lifestyle, nutrition, regular exercise, treatment by vitamin E regimen, homeopathy, relaxation techniques, reflexology, yoga, aromatherapy, acupuncture, massage therapy and herbal medicine can be mentioned\(^15,16\).

Conducted studies showed that phytoestrogens played a role in the bone health\(^16\) and contributed to strengthened bone density by preventing bone loss. Phytoestrogens, due to the vasodilator effect, play a role in the bone health by increasing nitric oxide, in addition to vascular expansion, and by inhibiting the activity of osteoclasts and macrophages\(^17\).

On the other hand, some studies on the effects of phytoestrogens on the bone mass density (BMD) or bone turnover are largely contradictory. Currently, in some studies, the use of phytoestrogens is not recommended for menopausal osteoporosis. Therefore, hormone replacement therapy (HRT) is recommended to be considered for the prevention of osteoporosis\(^11\).

There is no study on the effect of *Vitex agnus* and *S. officinalis* extract on the prevention of osteoporosis in postmenopausal women in Iran and there few studies conducted on the effect of these two plants on physical and psychological symptoms. The present study was carried out to evaluate the effect of *Vitex agnus* and *S. officinalis* extract on some osteogenic factors (calcium, phosphorus, vitamin D serum) in postmenopausal women referred to bone mineral densitometry center of Nemazee Hospital in Shiraz.

**MATERIAL AND METHODS**

This **A randomized, double-blind, placebo-controlled trial was conducted in 2015 in Shiraz. The study population consisted of postmenopausal women referred to bone mineral densitometry center of Namazi Hospital.**
The sample size for each group was estimated to be 31 individuals by using Philip et al.’s (2015) study and its generalization into three groups with confidence level of 95% and the powering test of 0.8. Finally, with a 5% probability of drop-out, 99 people were enrolled in this study and with Lost of 10 people in follow in three groups during the study, 89 of them completed the study (Figure 1). A simple purposive sampling method was used in this study. Samples were randomly assigned to three groups of A, B and C, using random permuted blocks. The groups were included in the study after obtaining the written consent.

The inclusion criteria in this study were as follows: postmenopausal women who gave their written consent, no use of certain drugs that affect bone density and any hormonal medication, postmenopausal women who had received supplements of calcium and vitamin D, women who were osteopenic but were not treated with osteogenic drugs, and no sign of sensitivity to herbal medicine. Exclusion criteria in this study included allergic reaction to a drug and diagnosis of a disease during the study that required the use of drugs that affect bone density.

**Intervention methods**
We selected the study samples from patients who referred to bone mineral densitometry center. Then, the eligible women were randomly divided into three groups of Agnugol tablets-, Salvigol tablets-, and placebo-taking groups. Tablets used for the intervention group were made by the Goldaru Pharmaceutical Company in Iran and the placebo tablets were made at Shiraz Faculty of Pharmacy. Then, they completed the written consent form and personal information form for the intervention. Then, they were introduced to the laboratory for calcium, phosphorus and vitamin D level tests. In the next step, the first intervention group received one 3.2-4.8 mg Agnugol tablet as a daily dose, the second intervention group received three 100 mg Salvigol tablets and the third group received three placebo tablets per day for three months.

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**Fig1: CONSORT Diagram**
After three months, the tests were repeated and the results were analyzed by a statistician. Double-blindness in this study was in the pharmaceutical form of the tablets in three groups and their size was slightly different, but the subjects could not see each other. Moreover, the researcher put the tablets in black packs so that the contents of the tablets in each pack was not clear.

**Statistical analysis**

Data analysis was performed using SPSS16 software. For data analysis, Chi-square test, analysis of variance (ANOVA) and paired t-test were used. Least Significant Difference (LSD) follow-up test was used if there was a significant analysis of variance.

**RESULTS AND DISCUSSION**

**Results**

According to the paired t-test, there was an increase in the mean calcium (0.5 and 0.6 units) in the *Vitex agnus* and *S. officinalis* extract groups, respectively, after the intervention compared to before it, which showed a significant increase in calcium (p< 0.001). However, there was no significant difference in the control group (p= 0.5).

Also, the mean of calcium analysis before and after the intervention was the same in the three groups using ANOVA. The three groups had the same level of mean calcium before and after the intervention (p= 0.259) (Table 1).

According to the paired t-test results, there were significant phosphorus level increases of 0.6 and 0.5 units (p< 0.001) in the *Vitex agnus* and *S. officinalis* recipients, respectively, after the intervention compared to before it. While, there was no such significant difference in the control group (p= 0.083).

Also, the results of ANOVA test showed that the mean phosphorus level was the same in the three groups before the intervention (p= 0.221), while after the intervention, there was a significant difference (p< 0.001). Using the test, we found that the difference between the mean score of phosphorus in the *S. officinalis* group and control group after the intervention was 0.55 while it was 0.78 in *Vitex agnus* group and control group after intervention, which was statistically significant in both plants (p< 0.001). However, this difference between the *Vitex agnus* and *S. officinalis* group after the intervention was 0.23, which was not statistically significant (p= 0.068) (Table 2).

According to paired t-test results, there was an increase in the mean of vitamin D (4.2 units) in the *Vitex agnus* groups after the intervention compared to before it (p< 0.001). However, there was no significant difference in the control group (p= 0.377). Also, the result of the ANOVA test showed that the mean of vitamin D was the same in the three groups before and after the intervention (p= 0.476) (Table 3).

**Table 1:** The comparison of serum calcium levels before and after in the two intervention groups and control group

| Ca (mg/dl) | Salvia Group | Vitex Group | Control Group | p-valueb | f | Df (between) | Df (within) |
|------------|--------------|-------------|---------------|----------|---|--------------|-------------|
|            | M± SD        | M± SD       | M± SD         | p-valuea |   |              |             |
| Before     | 9.5±0.6      | 9.5±0.5     | 9.8±0.5       | 0.061    | 2.897 | 2            | 86          |
| After      | 10.1±0.5     | 10±0.5      | 9.9±0.5       | 0.259    | 1.372 | 2            | 86          |
| CV         | 0.6          | 0.5         | 0.1           | <0.001   |     |              |             |

a. paired sample t-test
b. One-way ANOVA
Table 2: The comparison of serum Phosphorus levels before and after the two intervention groups and control group

|         | Salvia Group | Vitex Group | Control Group | p-value b | f    | Df (between) | Df (within) |
|---------|--------------|-------------|---------------|-----------|------|--------------|-------------|
|         | M± SD        | M± SD       | M± SD         |           |      |              |             |
| Before  | 3.8±0.5      | 3.9±0.4     | 3.8 ± 0.5     | 0.221     | 1.53 | 2            | 86          |
| After   | 4.3 ± 0.5    | 4.5±0.5     | 3.7 ± 0.5     | <0.001    | 19.18| 2            | 86          |
| CV      | 0.5          | 0.6         | 0.1           | <0.001    |      |              |             |
| p-value a| <0.001       | <0.001      | 0.083         |           |      |              |             |

a. Paired sample t-test
b. One-way ANOVA

Table 3: The comparison of serum Vitamin D levels before and after the two intervention groups and control group

| Vit-D(mg/dl) | Salvia Group | Vitex Group | Control Group | p-value b | f    | Df (between) | Df (within) |
|--------------|--------------|-------------|---------------|-----------|------|--------------|-------------|
|              | M± SD        | M± SD       | M± SD         |           |      |              |             |
| Before       | 33.3±20.1    | 26.9±14.4   | 27.9±16.5     | 0.303     | 1.212| 2            | 86          |
| After        | 35.4±19.4    | 31.1±16.9   | 29.7±19.8     | 0.476     | 0.749| 2            | 86          |
| CV           | 2.1          | 4.2         | 1.8           | 0.484     |      |              |             |
| p-value a    | 0.078        | <0.001      | 0.377         |           |      |              |             |

a. Paired sample t-test
b. One-way ANOVA

Discussion

In this study, although the mean of calcium increased significantly after the intervention compared with before it, this increase was not significant in comparison to the control group (P= 0.259). This insignificant effect of the Vitex agnus and S. officinalis extract on the control group in our study may be due to the use of calcium supplementation by all postmenopausal women who were included in the study.

In a study carried out by Hoylian et al. (2014), the effect of olive on mice was investigated. In this study, 120 mice were divided into 4 groups (30 mice in each group). The first group was the control group, the second one was ovarian removed group, the third group was ovarian removed + olive group, and the fourth one was ovarian removed + estradiol group. The amount of calcium in the olive treatment group did not change significantly compared to the control group, which is consistent with our study. The reason for this inconsistency in terms of significance may be the oscillations of calcium in the blood during osteoporosis. In another study conducted by Noorafshan et al. (2015), the effect of black and white olive extract (containing phytoestrogen flavonoids) on 90 mice was studied. The obtained result of no change in the calcium level in this study was consistent with the finding of our study. However, in a study by Qiong et al. (2012), the effect of S. officinalis extract (Danshen) on osteoporosis was studied. In this study, 4.5 mg/kg prednisone was given to 4-month-old mice every day to cause osteoporosis in them. In these mice, calcium, phosphorus, magnesium and hydroxyproline levels decreased. Next, the blue extract of the root of S. officinalis was applied for 12 weeks. Finally, calcium and BMD levels increased in mice, but the level of phosphorus, magnesium and PH did not change. Their results, have not been consistent with those of our study on calcium.

Although the two intervention groups are very similar in the effectiveness of serum calcium score, Salvia Officinalis Extract has performed better than vitex in improving serum calcium score because the changes after the intervention are more than vitex compared to before the intervention in the S. officinalis group. Although this amount is not significant.

In our study, the amount of phosphorus in the two intervention groups was significantly different from that of the control group (p=
changes and the release of calcium from the bone and its loss. The mean of vitamin D, before and after the intervention, was significant different in the Vitex agnus group, but the mean of vitamin D in the S. officinalis group (p= 0.078) and control group was not significantly different (p= 0.377). In this regard, Marini et al. (2007) investigated the effect of genistein (phytoestrogens) in postmenopausal women. They divided 389 postmenopausal women for 24 months into two groups of treatment with genistein (54 mg per day) and treatment with placebo, while both groups received calcium and vitamin D supplements. Finally, it was found that calcium and vitamin D levels in both groups increased significantly, but no significant difference was observed in the treatment group compared to the placebo. In a clinical trial carried out by Filip et al. (2015), the effect of polyphenolic compounds on olive extracts in postmenopausal women have been investigated.

The study of Filip et al. have shown that, alkaline phosphatase level decreased and calcium, phosphorus and vitamin D level increased in the olive treated group compared to the control group. These results are not consistent with our study. The level of vitamin D in postmenopausal women with osteoporosis is low. In other studies, vitamin D levels increased after estrogen therapy in postmenopausal women. The importance of this vitamin is very important and should be taken care of in order to balance it in the body. This vitamin increases the absorption of calcium and phosphorus from the lumen into the plasma by acting on the enterocytes of the small intestine, and also plays an essential role in the absorption of calcium from bone in the presence of parathyroid hormone. This vitamin, along with parathyroid hormone, improves the reabsorption of calcium from the distal tubules, which ultimately increases calcium and phosphorus levels and prevent muscle contractions following hypocalcemia. Calcium and phosphorus requirements are regulated by the production of vitamin D.

Although the two intervention groups were very similar in their effects on calcium and phosphorus levels, S. officinalis group performance was better than the Vitex agnus group in terms of the improved scores of
calcium and phosphorus serum. It was due to more changes in the *S. officinalis* group compared to the *Vitex agnus* group before and after the intervention.

**Conclusion**

After the intervention, calcium and phosphorus serum levels in the two *Salvia officinalis* and *Vitex agnus* groups were significantly higher than those before the intervention, but calcium and phosphorus levels increased significantly compared to the control group. The level of vitamin D serum in the *Vitex agnus* group after the intervention was significantly higher than before it, but it did not significantly increase compared to the control group. Moreover, there was no significant increase in the *S. officinalis* group after the intervention compared to the control group before the intervention.

**Ethics Approval and Consent to Participate**

This research was carried out in Nemazee Hospital after obtaining approval from the Ethics Committee of Shiraz University of Medical Sciences with the license number of IR.SUMS.REC.1394.185 dated in 2015 and also after registration on Iranian Clinical Trial website with the code number of 2015111713940N2 in Nemazee hospital. The postmenopausal women were enrolled in the study after signing an informed consent forms, if they wished to cooperate and if they observed the entry requirements. They were excluded from the study if they did not agree to continue the intervention.

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مقارنة بين تأثير مستخلص كل من فيتكس أجنس و سالفيا أوفيشيناليس على مستويات الكالسيوم والفسفر فيتامين د لدى النساء بعد سن الایاس بالإشارة إلى مركز قياس كثافة المعدن بالظام: تجربة عشوائية مزدوجة التعمية خاضعة للتحكم بالغقل

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هشاشة العظام مشكلة مهمة في صحة المرأة، ومن عواقبها كسور العظام. أجريت هذه التجربة السريرية في عام 2015 على 99 مريضة (33 مريضة لكل مجموعة) من النساء بعد سن الایاس تحت إختبار مستخلص Agnugol والمجموعة الأولى قياماً على (تحتوي على 2- 3-4 مجم مستخلص جاف من فيتكس أجنس) ثم المجموعة الثانية ثلاثة أفراد (تحتوي كل منها على 100 مجم سالفيا أوفيشيناليس عبارة عن مستخلص جاف يوميًا Salvigol والمجموعة الثالثة ثلاثة أفراد من العلاج الوعائي يوميًا لمدة 12 شهرًا. تم تدشين مجموعة مستويات الكالسيوم والفسفر فيتامين D قبل الدخول وبعد. أظهرت نتائج اختبار t المزدوج أن متوسط مستوى الكالسيوم والفسفر زاد بعد الدخول في مجموعة مستخلص فيتكس أجنس و سالفيا أوفيشيناليس وسلفيا أوفيشيناليس ليكي فرق معنوي. في المجموعة الضابطة تم استخدام اختبار متابعة LSD أظهر أن متوسط التحكم كان معنوي في المقارنات الزوجية. لم يكن المستوى في مجموعة مستخلص فيتكس أجنس و سالفيا أوفيشيناليس معنوي، بعد الدخول، زاد متوسط فيتامين D ب. 20 ووحدة في مجموعات فيتكس أجنس مقارنة بما قبل الدخول. ومع ذلك، لم يكن هناك فرق معنوي به إحصائيا بين
مجموعة فيتامين D والفوفرور والفوسفور والكالسيوم، بينما كان مستخلص سالفيا أوفيتياليس فعالاً في زيادة مستوى الكالسيوم والفوسفور."

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