COMPARISON OF THE EFFECT OF SALVIA OFFICINALIS EXTRACT AND VITEX AGNUS-CASTUS EXTRACT ON ANXIETY IN POSTMENOPAUSAL WOMEN: A RANDOMIZED, TRIPLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Background: This study aimed to determine the effect of Salvia officinalis extract and Vitex agnus-castus extract on anxiety in postmenopausal women. Methods: This study was a triple-blind randomized controlled clinical trial on 99 eligible postmenopausal women in Shiraz, Iran. Sampling was done using at the beginning convenience sampling from among women referring to the clinic of Namazi Hospital in Shiraz, Iran, and then permuted block randomization was used to assign patients into three groups in a 1:1:1 ratio, who were divided into 3 groups (2 intervention groups and 1 control group). Data collection tools were a demographic questionnaire and the Spielberger's State-Trait Anxiety Inventory (STAI) questionnaire. The first intervention group received Agnogel tablets (3.2-1.8 mg) once daily, the second group received Salvigol tablets (100 mg) and the third group received placebo (100 mg starch) 3 times a day for 12 weeks. STAI was again completed by the subjects after 3 months. Data were analyzed by SPSS-18 using ANOVA and paired t-test at a significance level of 0.05. Results: Mean anxiety scores before and after the intervention were 83.3 ± 24.3 and 27.5 ± 12.7 in the Agnogol group; 84.1 ± 27.8 and 26.8 ± 7.4 in the Salvigol group; and 86.3 ± 32.1 and 34.8 ± 14.1 in the control group, respectively. Based on the results of paired t-test, the decrease in mean anxiety scores in the Salvigol and Agnogel groups were significant (P<0.001). Conclusion: The present study showed that Salvia officinalis extract and Vitex agnus-castus extract may have a good effect on relieving anxiety, so they can be used as a low-cost method with no side effects to reduce anxiety and hypochondriasis in postmenopausal women. Keywords: Salvia Officinalis, Vitex Agnus-Castus, Women, Menopause

INTRODUCTION

Menopause is a natural part of a woman's life cycle that occurs in mid or late middle age1. Transition from fertility to infertility (menopause) is associated with several physical and psychological symptoms. Depression and anxiety are two common problems among middle-aged women. It has been reported that the risk of developing these mental illnesses increases in women during premenopause and menopause2-5. The incidence of anxiety symptoms during menopause is approximately twice as high as during premenopausal period5,6. The prevalence of anxiety symptoms among postmenopausal women is 7-25%.6-8 Considering the high prevalence of anxiety (67%) in the study of postmenopausal women

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living in Tehran, finding a way to relieve these symptoms among postmenopausal women is of particular importance. In a study, 51% of women aged 40-55 reported some symptoms of anxiety such as stress, anger or irritability 2 weeks before and during the study, and 25% reported recurrent irritability or nervousness, which is very high.

Compared to premenopausal women, postmenopausal women are at a higher risk for any of the anxiety symptoms. Studies have reported contradicting results on the prevalence of anxiety symptoms at different stages of menopause, with some studies showing no statistically significant differences in menopause while others finding that women with early or late menopause have much higher anxiety symptoms than premenopausal women. Therefore, it is not clear whether menopause is a period of increased risk of anxiety. It is important to better understand the unique impact of menopause on the incidence of anxiety symptoms and syndromes because anxiety is not only common in the general public, but also is a problem often reported by middle-aged women to health care providers. Bahri et al. (2013) showed that the severity of menopausal symptoms has no statistically significant relationship with depression and anxiety in postmenopausal women. Bener et al. (2016) also showed that depression and anxiety in women are more common in the postmenopausal period. These results suggest that depression and anxiety may be associated with an increased risk of metabolic and chronic systemic diseases (e.g., cardiovascular diseases). Considering that relieving the annoying symptoms of menopause is an important care challenge, it is necessary to decide on an appropriate method to reduce them. Hormonal and pharmacological methods have long-term and short-term side effects; hence non-pharmacological methods might be helpful in improving the quality of life of postmenopausal women.

In the last few decades, the use of natural remedies to treat various diseases such as depression and anxiety has increased dramatically. These products are considered as safer alternatives to pharmaceuticals and have a lower risk of side effects or discontinuation. There is evidence of the effectiveness of plants with phytoestrogenic properties in menopausal problems. One of the therapeutic benefits of these plants with phytoestrogenic properties is controlling the symptoms of menopause, so that they affect hot flashes, osteopenia and the mood related to the reduction of estrogen and progesterone hormones. Salvia officinalis and Vitex agnus-castus are two of such plants. The mechanism of action of these plants is through the effect of phytoestrogens on the balance of serotonin receptors and we know that serotonin has a direct effect on the thermoregulatory center in the hypothalamus. Since women are the most important pillars of society and family, and that community health depends on meeting the different needs of this segment of society, trying to eliminate the complications of menopause in this group is one of the duties of the health system of any society. Considering the tendency of postmenopausal women at this age to use natural therapies instead of hormonal drugs and also less side effects of plants compared to hormones and chemical drugs in this field, a comparison of two types of plants was used. Hence, due to the contradictory and limited studies in this field in Iran, the importance of women's mental health during menopause, and the importance of the relationship between anxiety and menopause, the present study was designed to determine the effect of Salvia officinalis and Vitex agnus-castus extracts on anxiety in postmenopausal women.

MATERIALS AND METHODS

Trial design

This study was a triple-blind randomized clinical trial (RCT) based on the effect of Salvia officinalis and Vitex agnus-castus extracts (two intervention groups) and placebo (control group) on anxiety in postmenopausal women in 2015.

Participants

The research environment was Shiraz Namazi Hospital. A total of 99 eligible postmenopausal women were examined. The inclusion criteria were being; 1. Menopausal women, 2. Women who are willing to cooperate and have given their written consent, 3. Do not take any hormonal drugs, 4. Previous lack of sensitivity to herbal medicines, 5. Do not take any medication other than anti-allergies and painkillers (3 months before the study), 6. No other chronic physical and mental illness, 7. They did not use any other medicine that interfered with the study herbal medicine. They
had no medical history of anxiety or depression and did not take any medication. So, sampling of healthy postmenopausal women (based on their health records or self-reported) was performed.

The researcher has reviewed all the above criteria in the initial interview (before completing the questionnaire and taking herbal pills) and if the person has the above conditions, she has entered the study. The exclusion criteria were; 1. allergic reaction to the drug, 2. Occurrence of crisis or stressful event, 3. Emphasis on the correct use of pills: By telephone during the intervention by the researcher (weekly), the need for careful use of pills on time was reminded, but if the research units did not act according to the intervention protocol, the person is out of the study. And someone else would be replaced. 4. Referral to a specialist doctor consulting a research project in cases where certain problems were seen during the study process (no problems occurred in this area).

**Sample size**

According to previous studies and the means comparison formula, the number of samples in each group was estimated at 33 people. According to a statistics expert’s opinion about sampling based on time, all eligible women who have referred to the clinic of Namazi Hospital in 3 consecutive months were examined by census method. According to the referral statistics that the researcher has obtained from the center, at least 99 people (Using the sample size formula to compare the means of the groups) were expected to be included in 3 months. A total of 99 postmenopausal women were divided into the intervention and control groups with randomized block design.

\[
n_1 = \frac{\left( z_{1-\alpha} + z_{1-\beta} \right)^2 \left( \sigma_1^2 + \sigma_2^2 \right)}{d^2}
\]

\[
\sigma_1 = 30, \sigma_2 = 29.7
\]

\[
\alpha = 0.05, \beta = 0.2
\]

Finally, 89 patients completed the study. During the study, 5 participants in the intervention groups (3 participants in Vitex agnus-castus group and 2 in Salvia groups) and 5 patients in the placebo group were excluded from the study due to incomplete consumption of tablets and individual or family participation problems. The remaining 89 people stayed until the end of the project according to the design (Figure 1).

**Fig1:** consort diagram for sampling
Method of randomization

After sample size calculation, permuted block randomization was used to assign patients into three groups in a 1:1:1 ratio. Then, the subjects were selected through simple random sampling and were divided into Salvia officinalis extract, Vitex agnus-castus extract, and control groups using stratified block randomization. In doing so, a number was randomly selected from the table of random numbers and the researcher moved toward the right or left column or row and wrote the 5 digit numbers down. Since the participants were divided into 3 groups in this study, 3-therapy method was used and classification was performed as follows: A: Salvia officinalis extract group, B: Vitex agnus-castus extract group, and C: control group. Accordingly, ABC: 1, ACB: 2, BAC: 3, BCA: 4, CAB: 5, and CBA: 6. Then, 17 blocks of 6 were prepared and the patients were consecutively assigned to the blocks until the sample size in each group was completed.

Blinding

Placebo, Salvigol, and Agnugol tablets looked the same in size and shape, but slightly differed in color. Therefore, they were placed in black envelopes and coded by the researcher assistant. The researcher was blind to the codes until the end of the study, and patients did not meet to share information. Besides, Statistical consultant analysis also did not know the intervention and control groups.

Intervention methods

The first intervention group received one 3.2-4.8 mg tablet of Salvia officinalis per day for 3 months (In each coated tablet of Salvigel 100 mg dry extract of young shoots of Salvia (Salvia officinalis), Standardized in terms of 19-25 mg tannins (in terms of tannic acid) per tablet). The second intervention group received three 100 mg tablets of Vitex agnus-castus per day for 3 months (Each coated tablet contains the dry extract of the fruit of the five-fingered plant (Vitex - agnus castus) at a rate of 3.2-4.8 mg, Standardized In terms of 0.58 - 0.42 mg of acobine), which were made by Goldaru Pharmaceutical Company (Isfahan, Iran). The control group received 3 placebo tablets per day for 3 months (containing 100 mg of starch produced by Shiraz School of Pharmacy).

The results of several clinical trials have confirmed the effectiveness of daily consumption of 20 to 40 mg of plant fruit extract in relieving menopausal symptoms. Therefore, according to previous studies, the dose of drugs has been selected18-22.

At first, the women who were willing to participate completed the informed consent form, demographic questionnaire, and STAI. Three months later, they again completed STAI, and then data were entered into SPSS-18 for analysis.

Research tools

STAI is used to measure anxiety. STAI has 40 items that measure state and trait anxiety. The trait anxiety section has 20 items scored based on a Likert scale from 1 (almost never) to 4 (almost always). The total score ranged from 40 to 160 for each person. Cronbach's alpha coefficient of the state and trait anxiety scales were 0.89 and 0.94, respectively 23. The reliability of this scale in Iran was obtained as 0.97. The reliability and validity indexes reported by Aghamohammadi et al. (2007) are the basis of the present study24.

Statistical analysis

ANOVA was used to compare the mean score of STI between the 3 groups before the intervention, and paired t-test was used to compare the mean scores of STAI in the three groups between before and after the intervention. Also ANOVA test to compare clinical features and Chi-square test was performed for demographic characteristics in three groups The significance level was considered at 0.05.

Ethical consideration

This study was approved by the ethics committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1394.185) and registered with the code IRCT2015111713940N2 in the clinical trial center. Written consent was taken from all women to enter the study and the method and objectives of the study were explained. It was also stated that not participating in the study or canceling the study will not have any negative effect on providing services to them.
Table 1: Comparison of mean age, menopausal age, duration of menopause, in the three groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention group 2 Salvia</th>
<th>Intervention group 1 Vitex</th>
<th>Control Group</th>
<th>p-value*</th>
<th>F</th>
<th>df (between)</th>
<th>df (within)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>3.7 ± 55.6</td>
<td>4.2 ± 55.6</td>
<td>3 ± 56.3</td>
<td>0.747</td>
<td>0.292</td>
<td>2</td>
<td>86</td>
</tr>
<tr>
<td>Menopausal age</td>
<td>5.9 ± 48.8</td>
<td>7.3 ± 46.6</td>
<td>6 ± 47.8</td>
<td>0.409</td>
<td>0.904</td>
<td>2</td>
<td>86</td>
</tr>
<tr>
<td>Duration of menopause</td>
<td>7.1 ± 6.6</td>
<td>7.9 ± 8.8</td>
<td>6/3 ± 8.5</td>
<td>0.612</td>
<td>0.494</td>
<td>2</td>
<td>86</td>
</tr>
</tbody>
</table>

Table 2: Comparison of mean anxiety score before and after intervention in intervention and control groups

<table>
<thead>
<tr>
<th>Anxiety</th>
<th>Intervention group 2 Salvia</th>
<th>Intervention group 1 Vitex</th>
<th>Control Group</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD ± mean</td>
<td>SD ± mean</td>
<td>SD ± mean</td>
<td></td>
</tr>
<tr>
<td>Before intervention</td>
<td>84.1 ± 27.8</td>
<td>83.3 ± 24.3</td>
<td>86.3 ± 32.1</td>
<td>0.914</td>
</tr>
<tr>
<td>After intervention</td>
<td>26.8 ± 7.4</td>
<td>27.5 ± 12.7</td>
<td>34.8 ± 14.1</td>
<td>0.021</td>
</tr>
<tr>
<td>Change</td>
<td>57.3</td>
<td>55.8</td>
<td>51.5</td>
<td>0.555</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

RESULT AND DISCUSSION

Result
The study results of one-way ANOVA showed no significant difference between the study groups regarding the clinical characteristics including mean age, menopausal age, duration of menopause and had a similar status (p > 0.05). In addition, the results of Chi-square test in terms of education (p= 0.704) and job (p= 0.604) showed no significant difference between the three groups and were identical in terms of education and occupation (Table 1). Kolmogorov-Smirnov test was used to investigate the normality of the quantitative variables distribution. Based on the results, all quantitative variables were normal and thus parametric tests were used for analysis. The independent t-test indicated that the two groups were homogeneous in terms of education, occupation and housing. Mean anxiety scores before and after the intervention were 83.3 ± 24.3 and 27.5 ± 12.7 in the Vitex agnus-castus group; 84.1 ± 27.8 and 26.8 ± 7.4 in the Salvigel group; and 86.3 ± 32.1 and 34.8 ± 14.1 in the control group, respectively. The paired t-test showed that the decrease in mean anxiety scores in the Salvigel and Agnogel groups were significant (P< 0.001) (Table 2).

Discussion
This study examined the effect of Salvia officinalis extract and Vitex agnus-castus extract on anxiety in postmenopausal women. The results showed that anxiety significantly reduced after the intervention in the Salvia officinalis and Vitex agnus-castus groups. It was noteworthy that along with the intervention group, the mean anxiety scores decreased in the placebo group, which can be attributed to the effect of the suggestion in this group, although the trend of improving anxiety in the intervention groups was significantly more prominent. These are consistent with the results obtained by Lipovac (2010). Lipovac showed that isoflavones derived from red clover are effective in reducing depression and anxiety symptoms in postmenopausal women25. Cognitive and psychological functions change during menopause. The study by Naseri et al showed that after the intervention, the mean scores of complete menopause, anxiety and vasomotor dysfunction in the Vitex group were significantly lower than the placebo group26. Anxiety decreased in all three groups however, the decreasing trend was more in the intervention groups. In control group which could be attributed to the suggestibility effect. To justify this, previous studies have shown that V. agnus-castus has a modulatory effect on anxiety through interaction with
neurotransmitters and the nervous system, such as dopaminergic and serotonergic neurotransmitters\(^28\text{-}34\). In contrast, V. agnus-castus phytoestrogens have a high affinity for binding to estrogen receptors, as well as stimulation of progesterone receptor expression\(^29\). Other studies have shown that Vitex agnus castus showed similar effects to estrogen in reducing anxiety-like behaviors in ovarian rats. ERs may also be responsible for their anti-anxiety effects\(^30\text{-}31\).

Salvia officinalis extract contains bitter substances and flavonoid diterpene, phenolic acids and tannins and a substance called tojan. It also has antiseptic and antioxidant properties rich in calcium, potassium, magnesium and zinc, and they dilate blood vessels, thus applying anti-anxiety properties\(^32\).

In addition, several studies\(^33\text{-}34\) have multi-plant formulas to improve anxiety symptoms, many of which are phytoestrogenic and consistent with the present study. Although such compounds are thought to have more therapeutic value than medicinal plants, it is difficult to discern what effect each plant has on overall effects.

Researchers have shown that the compounds in Foeniculum vulgare and Melissa can be effective in reducing depression and anxiety in postmenopausal women\(^35\text{-}36\). In addition, Forouzanmehr et al. reported a reduction in the symptoms of anxiety, depression, and improved sleep in postmenopausal women using an herbal formula that also contained valerian\(^37\). However, the findings of Atayi, who studied oral administration of curcumin and vitamin E during menopause\(^38\), are inconsistent because of the shorter intervention time in their study.

Thus, it appears that the effects of Salvia officinalis and Vitex agnus-castus extracts on the treatment of anxiety problems in postmenopausal women on the one hand are related to their effect on GABA receptor and on the other hand are due to their phytoestrogenic and antioxidant properties. Their effect on other menopausal symptoms such as hot flashes and sleep problems indicate that they are well tolerated by women and have no side effects\(^39\).

Therefore, Vitex contains phytoestrogens, which are weak estrogen agonists. Therefore, it can be imagined that in low estrogen environments, similar to the environments of postmenopausal women, this plant can have more radical estrogenic effects\(^40\). It is important to note the effect of herbal supplements on the human body, including postmenopausal women. In addition to physiological factors, they are affected by individual, social and psychological factors. Some postmenopausal women are less concerned about their symptoms, which can affect quality of life and the outcome of herbal remedies\(^41\). The efficacy of Salvia officinalis extract on menopausal symptoms has been reported in several studies but has been limited to menopausal anxiety studies\(^42\text{-}44\). The effectiveness of Salvia officinalis extract on menopausal symptoms has been reported in several studies but has been limited to menopausal anxiety studies. However, some studies have not shown the superiority of sage over placebo in reducing menopausal symptoms\(^45\text{-}46\). Studies have shown that sage's effectiveness is due to its phytoestrogens, which include a mixture of ten compounds including monoterpenes, sesquiterpenes, diterpenes and alpha and tojan. Caffeic acid derivatives such as rosmarinic acid, chlorogenic acid, flavonoids and tannins\(^47\).

Due to the multiple effect of plants on various menopausal symptoms, the willingness of women at this age to use natural remedies instead of hormonal drugs, the less side effects of plants than hormones and chemical drugs in this regard, and with the recommendations to do more complete research in this field, these plants can be considered as an alternative and appropriate option in reducing anxiety. In our study, no significant harms or unintended effects in each group were reported. However, in a study of the fruit of the five fingers, mild and reversible side effects occurred, including: nausea, mild gastrointestinal complaints, fatigue, menstrual disorders, dry mouth, acne, pruritus, rash and erythematosus\(^48\).

The strength of this study was the use of two natural plants to improve anxiety and characteristics of women during menopause, and the results showed their effectiveness. One of the limitations of this study was the exclusion criteria that excluded a number of women from the study. Another limitation of the study was the problem of follow-up of samples, which reduced the sample size. Also, the presence of other symptoms with different intensities in postmenopausal women can affect the severity of anxiety. However, more studies are needed to prove the effect of these plants on
anxiety. It is recommended to use these plants in further studies in combination with cognitive-behavioral group therapy and group memory to reduce anxiety in postmenopausal women.

Generalizability of project findings, considering that sampling in a general center that comes from all parts of the city to perform bone density tests is important, but to prove the effect of these herbs on menopausal symptoms, including anxiety, there is a need for further research in other areas.

Conclusion
Evidence suggests that Salvia officinalis and Vitex agnus-castus extracts are helpful in reducing anxiety. Considering the significant difference in the level of trait anxiety observed after receiving placebo and then after receiving medication, it appears that the duration of treatment has a decisive role in the effectiveness of Salvia officinalis and Vitex agnus-castus extracts in reducing anxiety in postmenopausal women. Therefore, it is suggested that further studies pay more attention to increasing the length of treatment and receiving medication. Besides, considering that the quality of life of postmenopausal women is an important health issue in different societies and the basic goals of health care in this period, if it is determined that menopausal symptoms can lead to psychological disorders such as anxiety and depression, by treating its symptoms, it can prevent depression and anxiety and improve the quality of life of postmenopausal women.

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ممتازه بین تأثیر مستخلص سالفیا اوفیسینالیس ومستخلص فیتکس أجنوس-کاستوس على القلق لدى النساء بعد سن الیاس: تجربة عشوائية ثلاثية التعمیم مضبوطة بالغفل

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الکلیفیه: هدف از این پژوهش آن است تأثیر مستخلص سالفیا اوفیسینالیس ومستخلص فیتکس أجنوس-کاستوس على القلق لدى النساء بعد سن الیاس

الطريقة: کانوی از تعداد سربیع معشیة ذات شاوه تثلیثیة التعمیم على 99 امرأة مؤهلة بعد سن الیاس في شیراز، ایران. تم أخذ العینات استعمال عینات ملأت به البیناء من بين النساء اللائي يشترتن إلى عینة مستشنفی نمایی في شیراز، ایران، ثم تم استخدام التوزیع العشوائي للکلیة العشوائیة لتعیین المرضى إلى ثلاث مجموعات بنسبة 1: 1: 1، والذین تم تقسمهم إلى 3 مجموعات (مجموعات تدخل ومجموعة مراقبة واحدة). كاینت أدوات جمع البيانات علیة عن استبان

دیمجرافی واستبان STAI. تلتقت المجموعة الأولى أقرuras أجنوس (3.5-18 مجم) مرة واحدة يومیًا. تلتقت المجموعة الثانیة أقرuras سالفیجول (100 مجم) والمجموعة الثالثة تلتقت العلاج الیومی (100 مجم) مرة أخرى من قبل الموضوعات بعد 3 أشهر. تم تحلیل البيانات بواسطة 18-ANOVA باستخدام SPSS

النتائج: متوسط درجات القلق قبل وبعد التدخل كانت 32±6 و 27±5 و 15±4.2 في مجموعات اجنوس، 84±1، 27±8 و 16±4.2 و 34.8±27.1 و 36±4.2 في مجموعات سالفیجول، و 32.1±32.1 و 36.3±34.8 و 14.1±14.1 في المجموعة الضابطة، على التوالي. بناءً على نتائج اختبار المقترن، كان الانخفاض في

الخلاصة: أظهرت الدراسة الحالية أن مستخلصات نبات سالفیا اوفیسینالیس ومستخلص فیتکس أجنوس-کاستوس قد يكون لها تأثیر جذب في تخفیف القلق، لذلك يمكن استخدامهما كطريقة منخفضة التكلفة مع عدم وجود آثار جانبیة لتقلیل القلق والتورم المرضی عند النساء بعد سن الیاس.