Jundishapur J Nat Pharm Prod. 2019 May; 14(2):e63570. doi: 10.5812/jjnpp.63570.

Published online 2019 May 11. **Research Article**

Evaluation of the Anxiolytic Effect of *Vitex agnus-castus* on Female Mice and Possible Role of Estrogen Receptors

Ardeshir Arzi [1, 2], Hoda Mojiri-Forushani [3, *] and Neda Sistani Karampour [1, 2]

[1] Department of Pharmacology and Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2] Physiology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3] Abadan School of Medical Sciences, Abadan, Iran

[*] *Corresponding author*: Abadan School of Medical Sciences, Abadan, Iran. Tel: +98-6153322738, Email: dr.mojiri@yahoo.com

Received 2016 November 01; **Revised** 2017 November 02; **Accepted** 2018 April 22.

Abstract

Background: *Vitex agnus-castus* is a source of phytoestrogens and is used traditionally in the treatment of premenstrual syndrome. The beneficial effects of phytoestrogens on anxiety have been shown in some studies.
Objectives: In this research, we aimed to evaluate the anxiolytic activity of hydroalcoholic *V. agnus-castus* extract in animal models.
Methods: For analyzing the antianxiety effects of the extracts, the elevated plus-maze test was applied. Thirty minutes before the test, different doses of *V. agnus-castus* (25, 50, and 100 mg/kg) were administered to mice. Diazepam and saline were used as the positive and negative controls, respectively. Tamoxifen was used as an antagonist of estrogen receptors to clarify the role of estrogen.
Results: The time spent in the open arms increased at all doses of *V. agnus-castus*, while entries to closed arms reduced than the controls. Groups which received tamoxifen or a combination of tamoxifen with a high dose of *V. angus-castus* did not show any anti-anxiety effects.
Conclusions: *V. agnus-castus* extract exhibited anti-anxiety effects and can be used in the treatment of the anxiety behaviors. Phytoestrogens from *V. agnus-castus* interact with estrogen receptors, which may be the underlying mechanism of its anxiolytic activity.

Keywords: *Vitex agnus-castus*, Elevated Plus-Maze, Anxiety, Tamoxifen, Phytoestrogen

1. Background

Anxiety disorders are a class of mental and behavioral disorders with a high prevalence among the general population. Anxiety disorders markedly impact the function and quality of life (1). More than one-fifth of the population will suffer from anxiety in their lifetime (2). According to the World Health Organization, depression is speculated to be the second most common cause of the premature disability and death by 2020 (3).

Currently, pharmacotherapy is considered a routine and efficacious treatment for anxiety disorders (4). Sedative-hypnotic medicines are usually administered to control these disorders; among them, benzodiazepines are one of the most common types. However, their use has been restricted by the severe adverse effects, including anterograde amnesia (5). Withdrawal symptoms, sedation, and dependence occur with long-term administration of benzodiazepines (6). According to these adverse effects, many pharmaceutical companies and researchers have decided to introduce complementary or herbal medications with specific anxiolytic effects and fewer side effects.

At the present time, herbal therapy is proposed as an effective alternative approach for the treatment of certain neurological disorders. Many studies have been conducted on different animal models to demonstrate the beneficial effects of plant-derived medications (7). Phytoestrogens are plant constituents with a similar structure and function to estrogens, including estradiols, which can bind to estrogen receptors (ERs), selectively (8). Some of the animal studies have indicated the beneficial role of soya as a phytoestrogen in the amelioration of the anxiety symptoms (9, 10).

Vitex agnus-castus, also known as a chaste tree (11), is endemic to Central Asia and Mediterranean Europe and belongs to the family *Verbenaceae* (12). It is a source of phytoestrogens and contains flavonoids and apigenins (13). It has been used traditionally to treat some conditions in females, including premenstrual syndrome, menstrual disorders (dysmenorrheal and amenorrhea), acne, infertility, corpus luteum insufficiency, hyperprolactinemia, lactation problems, and menopause (14-16).

Previous studies have indicated that *V. agnus-castus* has

a modulatory effect on anxiety through interactions with neurotransmitters and neural system, such as dopaminergic and serotonergic neurotransmitters (17,18). In contrast, phytoestrogens from *V. agnus-castus* have a high affinity for ER binding, as well as stimulation of progesterone receptor expression (19).

Incompatible findings have been reported regarding the association of mood changes with the level of hormones. Notable findings with respect to *V. agnus-castus* are described below:

1. Estrogen promotes opiate-containing neuron activities and enhances β-endorphin synthesis and release (20).

2. Estrogen through direct modulation of dopaminergic activity promotes releasing dopamine in the hypothalamus and improves dopamine transmission, as well as D2 receptors (21).

3. Estrogen, and consequently phytoestrogen, may alter GABA receptors; these messengers may affect GABA activity (22-24).

2. Objectives

Since estrogens contribute to anxiety and phytoestrogens imitate estrogens, in this study, the effects of phytoestrogens from *V. agnus-castus* on anxiety were examined in female rats, using the elevated plus-maze (EPM) test; furthermore, we attempted to determine the possible involvement of the estrogenic system.

3. Methods

3.1. Animals

For the experiments, female Swiss mice (body weight: 18 - 22 g) were maintained under standard laboratory conditions in the animal house of School of Pharmacy, Ahvaz University of Medical Sciences (Ahvaz, Iran). Eight rats were kept in cages at 23 $\pm$ 1°C in a 12:12 hours light-dark cycle. The animals had free access to food and water and were given time to adapt to the conditions (1 week) before the test. The animals were euthanized immediately after each experiment. Guidelines for animal care of Ahvaz University of Medical Sciences were respected during the study.

The mice were classified into seven groups (eight per group), and the treatment groups were administered by different doses of *V. agnus-castus* extract (25, 50, and 100 mg/kg). Over four consecutive days, treatments were administered intraperitoneally (i.p.). The EPM test was applied at 30 minutes following the final administration. The positive and negative control groups received diazepam (0.5 mg/kg) and normal saline (1 mL/kg), respectively. The tamoxifen group received 15 mg/kg of tamoxifen. The final group received *V. agnus-castus* extract (100 mg/kg) and tamoxifen.

3.2. Preparation of Methanol Extract

The methanol extract of *V. agnus-castus* fruit was purchased from Pursina Company (Tehran, Iran). Alcohol was evaporated at 35°C and the dry powder of *V. agnus-castus* was obtained.

3.3. EPM Test

The EPM test is a valid method to screen and evaluate antianxiety effects in the rodents (25, 26). The maze includes 2 open and 2 closed arms (30 $\times$ 5 $\times$ 25 cm), which expand from a common platform (5 $\times$ 5 cm). The wooden maze was elevated to 45 cm above the floor, with a 0.25 cm edge on the perimeter of the open arms. The experimental groups were administered 25, 50, and 100 mg/kg of *V. agnus-castus* extract for 4 consecutive days. The positive and negative control groups received diazepam (0.5 mg/kg) and normal saline (1 mL/kg), respectively.

The tamoxifen group received 15 mg/kg of tamoxifen. After 30 minutes, the final group received *V. agnus-castus* extract (100 mg/kg) following tamoxifen injection (15 mg/kg). Each group was placed at the center of the maze at 30 minutes post injection, while facing an open arm. The frequency of entries to the open and closed arms, as well as time spent in the arms, was recorded during the 5-minute test (26).

The anxiolytic effect of various treatments was determined based on the spent time in the open arms. To develop an activity index, entrance into each arm, besides total entry time, was measured. The placement of all 4 paws onto the arm was considered to represent entrance. After each test, ethanol solution was used to clean the maze.

3.4. Statistical Analysis

For data analysis, SPSS version 13 was used, and ANOVA and post-hoc Tukey test were performed. The significance level was set at 0.05. Values are expressed as mean $\pm$ SEM.

4. Results

According to the results, more time was spent in the open arms in mice receiving different doses of *V. agnus-castus* extract and diazepam than the saline group ($P < 0.001$). However, the difference was not significant between the tamoxifen and saline-treated groups (Figure 1). As shown in Figure 2, the groups treated with different doses of *V. agnus-castus* extract, tamoxifen, and diazepam experienced a significant decrease in the frequency of entries to closed arms than the saline group ($P < 0.001$). As

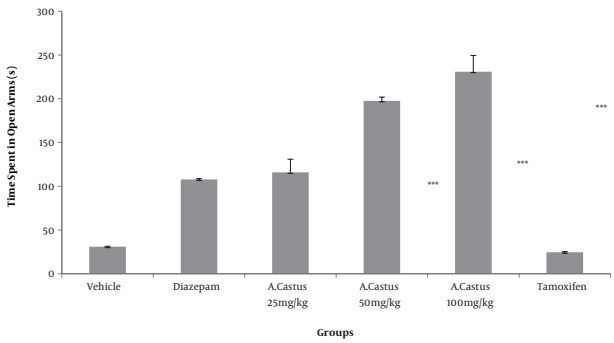

Figure 1. The effects of saline, diazepam, and *V. angus-castus* on the time spent in the open arms in EPM test. Values are presented as mean ± SEM. ***P < 0.001 indicates significant differences in comparison with the vehicle-treated controls.

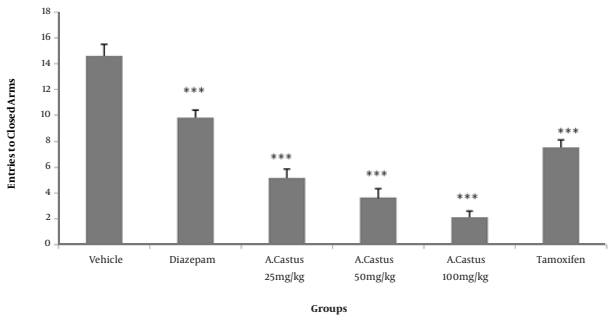

Figure 2. The effects of saline, diazepam, and *V. agnus-castus* extract on the number of entries to the closed arms during the 5-minute test. Values are expressed as mean ± SEM. ***P < 0.001 indicates a significant difference versus the controls.

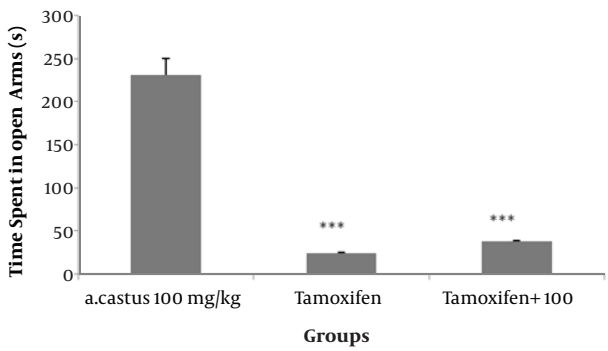

Figure 3. The effects of *V. agnus-castus* (100 mg/kg) extract, tamoxifen, and a combination of tamoxifen and *V. agnus-castus* extract (100 mg/kg) on the spent time in the open arms during the 5-minute test. Data are presented as mean ± SEM.***P < 0.001 indicates significant differences compared with the high-dose *V. agnus-castus* extract group (100 mg/kg).

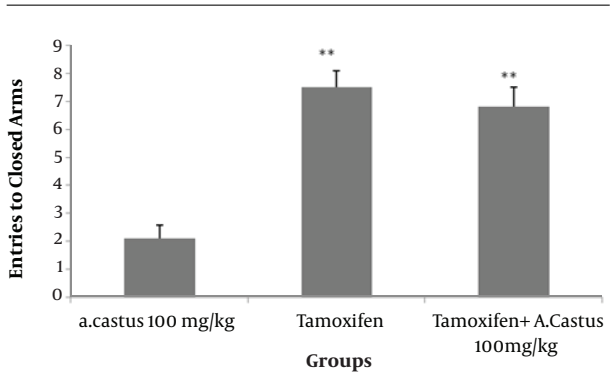

Figure 4. Effects of *V. agnus-castus* extract (100 mg/kg), tamoxifen, and combination of tamoxifen and *V. agnus-castus* extract (100 mg/kg) on the frequency of entries to the closed arms on the 5-minute test. Values are presented as mean ± SEM. **P < 0.01 in comparison with the high-dose *V. agnus-castus* extract (100 mg/kg).

shown in Figure 3, the spent time in the open arms significantly reduced in groups receiving tamoxifen and tamoxifen plus 100 mg/kg *V. agnus-castus*, compared with the group treated with 100 mg/kg of *V. agnus-castus* alone.

The results also showed that the number of entries to the closed arms significantly increased ($P < 0.001$) in tamoxifen and tamoxifen plus 100 mg/kg *V. agnus-castus* groups, compared with the group treated with 100 mg/kg extract (Figure 4).

5. Discussion

To examine the anti-anxiety effects of hydroalcoholic *V. agnus-castus* extract, an EPM model of anxiety was applied. The model focuses on the rats' natural reticence to avoid open and elevated places (27). First, we examined the antianxiety activity of different doses of *V. agnus-castus* extract, as well as the effect of tamoxifen on anxiety. Finally, we administered tamoxifen to block ERs and then applied an effective dose of *V. agnus-castus* extract to assess the possible role of ERs. According to the findings, different doses of the extract (including 25, 50, and 100 mg/kg) were effective.

In this study, tamoxifen, which is an effective nonselective ER antagonist, was used (28). Administration of tamoxifen to mice blocks ERs. Therefore, tamoxifen induces anxiety-like behaviors in animals. A high dose of *V. agnus-castus* extract (100 mg/kg) reduced the anxiety symptoms in the animals, whereas coadministration of tamoxifen and high-dose *V. agnus-castus* extract reversed the anti-anxiety effects of *V. agnus-castus* extract and caused the mice to exhibit anxiety. Inhibition of the anti-anxiety effects of *V. agnus-castus* extract may be related to the blockade of ERs induced by tamoxifen.

In the different pharmacological studies, diazepam has been used as a reference sedative-hypnotic drug. Our findings revealed the substantial anti-anxiety effects in animals receiving diazepam. Loch et al. indicated that *V.*

agnus-castus caused a decrease in depression and anxiety disorders in females with premenstrual syndrome (PMS) (29). Schellenberg et al. evaluated the impact of *V. agnus-castus* on other PMS symptoms, such as anxiety, headache, mood swings, agitation, and breast pain. Their findings showed its beneficial effects on the alleviation of symptoms (30).

The fruits of *V. agnus-castus* contain iridoids and flavonoids; in addition, the leaves and flowers contain compounds that are structurally similar to sex hormones (31). The available knowledge suggests that phytoestrogens are suitable for the prevention and treatment of several diseases (32). Phytoestrogens are the analogs of estrogens and have estrogenomimetic properties with a high affinity for binding to ERs; however, their ability is weaker than endogenous estrogens (33). They originate from vegetables and have a high therapeutic potential (32).

In the present study, *V. agnus-castus* was used for its phytoestrogen content. Studies have previously indicated the anxiolytic effects of phytoestrogens, such as, soya and fennel (9, 34). Two types of ERs are present, ER-α and ER-β. According to studies on rats and mice, ER-β is responsible for the anxiolytic effects of estrogens (35, 36). To elucidate the role of ER-α and ER-β in anxiety, it has been demonstrated that the anxiety behavior increased in female ER-β knockout mice, whereas no differences were seen in ER-α knockout mice (37).

ER-β is more expressed in the amygdala and paraventricular nucleus of the hypothalamus, which are related to fear and the anxiety responses (38, 39). Hence, the inability of estradiol to act on these brain areas may explain the anxiety symptoms in ER-β knockout mice (37). However, since phytoestrogens possess estrogenic actions (40) and have a greater affinity to ER-β (8), these receptors may contribute to the anxiolytic-like effects of *V. agnus-castus*.

Dietary phytoestrogens, such as, genistein have a high affinity and activity for ER-β and have been shown to decrease anxiety in both female and male rats in EPM test (39). *V. agnus-castus* extract exerted anti-anxiety effects through interference in the dopaminergic and serotonergic systems (17, 18). In this study, the importance of ERs in anxiety was investigated, and the anti-anxiety potential of *V. agnus-castus* may be associated with estrogen system modulation.

These findings were partly consistent with previous research, demonstrating the anxiolytic effects of selective ER-β modulators in the open field (17 β-estradiol, diarylpropionitrile, and 7, 12-dihydrocoumes), EPM test, light-dark transition, emergence, Vogel punished drinking tasks, and defensive freezing, inhibited via tamoxifen coadministration (41).

Consumption of phytoestrogen influences the fertility and morphogenesis of ovaries in animals (42). As phytoestrogens may change the level of sex hormones in consumers, the treatment doses of phytoestrogen should be precisely determined (43). Eventually, the efficiency of isoflavones in animal and in vitro studies and clinical trials should be evaluated to confirm their beneficial effects on humans.

5.1. Conclusions

Our study results indicated that *V. agnus-castus* extract exerted anxiolytic effects in mice subjected to the EPM test. When ERs were blocked by the administration of tamoxifen, the inhibitory effects of the extract on anxiety diminished. The present study supports previous research, reporting the effectiveness of phytoestrogens in the reduction of anxiety. Also, ERs may be responsible for the antianxiety effects.

Acknowledgments

This work was supported by the Department of Pharmacology and Toxicology, Ahvaz University of Medical Sciences, Ahvaz, Iran. The code number is APRC-9302.

Footnotes

Authors' Contribution: Study concept and design: Hoda Mojiri Forushani and Ardeshir Arzi; acquisition of data: Hoda Mojiri Forushani; analysis and interpretation of data: Hoda Mojiri Forushani and Ardeshir Arzi; drafting of the manuscript: Hoda Mojiri Forushani; critical revision of the manuscript for important intellectual content: Hoda Mojiri Forushani; statistical analysis: Hoda Mojiri Forushani; administrative, technical, and material support: Hoda Mojiri Forushani and Neda Sistani Karampour; study supervision: Hoda Mojiri Forushani.

Conflict of Interests: Authors have no financial interests related to the material in the manuscript.

Funding/Support: It is not declared by the authors.

References

1. Somers JM, Goldner EM, Waraich P, Hsu L. Prevalence and incidence studies of anxiety disorders: A systematic review of the literature. *Can J Psychiatry*. 2006;**51**(2):100–13. doi: 10.1177/070674370605100206. [PubMed: 16989109].
2. Dang H, Sun L, Liu X, Peng B, Wang Q, Jia W, et al. Preventive action of Kai Xin San aqueous extract on depressive-like symptoms and cognition deficit induced by chronic mild stress. *Exp Biol Med (Maywood)*. 2009;**234**(7):785–93. doi: 10.3181/0812-RM-354. [PubMed: 19429857].
3. Onasanwo SA, Chatterjee M, Palit G. Antidepressant and anxiolytic potentials of dichloromethane fraction from Hedranthera barteri. *Afr J Biomed Res*. 2010;**13**(1):76–81.

4. Sandford JJ, Argyropoulos SV, Nutt DJ. The psychobiology of anxiolytic drugs. Part 1: Basic neurobiology. *Pharmacol Ther*. 2000;**88**(3):197–212. doi: 10.1016/S0163-7258(00)00082-6. [PubMed: 11337025].
5. Beracochea D. Anterograde and retrograde effects of benzodiazepines on memory. *ScientificWorldJournal*. 2006;**6**:1460–5. doi: 10.1100/tsw.2006.243. [PubMed: 17115086]. [PubMed Central: PMC5917174].
6. Lader M, Tylee A, Donoghue J. Withdrawing benzodiazepines in primary care. *CNS Drugs*. 2009;**23**(1):19–34. doi: 10.2165/0023210-200923010-00002. [PubMed: 19062773].
7. Galdino PM, Nascimento MV, Sampaio BL, Ferreira RN, Paula JR, Costa EA. Antidepressant-like effect of Lafoensia pacari A. St.-Hil. ethanolic extract and fractions in mice. *J Ethnopharmacol*. 2009;**124**(3):581–5. doi: 10.1016/j.jep.2009.05.001. [PubMed: 19439172].
8. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*. 1998;**139**(10):4252–63. doi: 10.1210/endo.139.10.6216. [PubMed: 9751507].
9. Lund TD, Lephart ED. Dietary soy phytoestrogens produce anxiolytic effects in the elevated plus-maze. *Brain Res*. 2001;**913**(2):180–4. doi: 10.1016/S0006-8993(01)02793-7. [PubMed: 11549384].
10. Lephart ED, Setchell KD, Handa RJ, Lund TD. Behavioral effects of endocrine-disrupting substances: Phytoestrogens. *ILAR J*. 2004;**45**(4):443–54. doi: 10.1093/ilar.45.4.443. [PubMed: 15454683].
11. Mills S, Bone K. *Principles and practice of phytotherapy. Modern herbal medicine*. Edinburgh: Churchill Livingstone; 2000.
12. Hankey A. CAM and the phenomenology of pain. *Evid Based Complement Alternat Med*. 2006;**3**(1):139–41. doi: 10.1093/ecam/nek002. [PubMed: 16550235]. [PubMed Central: PMC1375225].
13. Jarry H, Spengler B, Porzel A, Schmidt J, Wuttke W, Christoffel V. Evidence for estrogen receptor beta-selective activity of Vitex agnus-castus and isolated flavones. *Planta Med*. 2003;**69**(10):945–7. doi: 10.1055/s-2003-45105. [PubMed: 14648399].
14. Chopin Lucks B. Vitex agnus castus essential oil and menopausal balance: A research update [complementary therapies in nursing and midwifery 8 (2003) 148-154]. *Complement Ther Nurs Midwifery*. 2003;**9**(3):157–60. doi: 10.1016/S1353-6117(03)00020-9. [PubMed: 12852933].
15. Bergmann J, Luft B, Boehmann S, Runnebaum B, Gerhard I. [The efficacy of the complex medication Phyto-Hypophyson L in female, hormone-related sterility. A randomized, placebo-controlled clinical double-blind study]. *Forsch Komplementarmed Klass Naturheilkd*. 2000;**7**(4):190–9. German. doi: 10.1159/000021343. [PubMed: 11025394].
16. Adams JD Jr, Garcia C. Women's health among the Chumash. *Evid Based Complement Alternat Med*. 2006;**3**(1):125–31. doi: 10.1093/ecam/nek021. [PubMed: 16550233]. [PubMed Central: PMC1375244].
17. Yaghmaei P, Oryan S, Fatehi Gharehlar L, Salari AA, Solati J. Possible modulation of the anexiogenic effects of vitex agnus-castus by the serotonergic system. *Iran J Basic Med Sci*. 2012;**15**(2):768–76. [PubMed: 23493923]. [PubMed Central: PMC3586884].
18. Zarrindast MR, Babapoor-Farrokhran S, Babapoor-Farrokhran S, Rezayof A. Involvement of opioidergic system of the ventral hippocampus, the nucleus accumbens or the central amygdala in anxiety-related behavior. *Life Sci*. 2008;**82**(23-24):1175–81. doi: 10.1016/j.lfs.2008.03.020. [PubMed: 18456284].
19. Liu J, Burdette JE, Xu H, Gu C, van Breemen RB, Bhat KP, et al. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J Agric Food Chem*. 2001;**49**(5):2472–9. doi: 10.1021/jf0014157. [PubMed: 11368622].
20. Genazzani AR, Petraglia F, Mercuri N, Brilli G, Genazzani AD, Bergamaschi M, et al. Effect of steroid hormones and antihormones on hypothalamic beta-endorphin concentrations in intact and castrated female rats. *J Endocrinol Invest*. 1990;**13**(2):91–6. doi: 10.1007/BF03349515. [PubMed: 2139451].
21. Jacobs PA, Hyland ME. An evaluation of the benefits of taking hormone replacement therapy with other prescription drugs. *Maturitas*. 2003;**46**(4):273–81. doi: 10.1016/S0378-5122(03)00198-1. [PubMed: 14625124].
22. Blasi C. Influence of benzodiazepines on body weight and food intake in obese and lean Zucker rats. *Prog Neuropsychopharmacol Biol Psychiatry*. 2000;**24**(4):561–77. doi: 10.1016/S0278-5846(00)00093-2. [PubMed: 10958151].
23. Lucion AB, Charchat H, Pereira GA, Rasia-Filho AA. Influence of early postnatal gonadal hormones on anxiety in adult male rats. *Physiol Behav*. 1996;**60**(6):1419–23. doi: 10.1016/S0031-9384(96)00246-6. [PubMed: 8946485].
24. Mora S, Dussaubat N, Diaz-Veliz G. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. *Psychoneuroendocrinology*. 1996;**21**(7):609–20. doi: 10.1016/S0306-4530(96)00015-7. [PubMed: 9044444].
25. File SE, Pellow S. The effects of PK 11195, a ligand for benzodiazepine binding sites, in animal tests of anxiety and stress. *Pharmacol Biochem Behav*. 1985;**23**(5):737–41. doi: 10.1016/0091-3057(85)90064-4. [PubMed: 3001779].
26. Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. *Pharmacol Biochem Behav*. 1986;**24**(3):525–9. doi: 10.1016/0091-3057(86)90552-6. [PubMed: 2871560].
27. Weiss SM, Wadsworth G, Fletcher A, Dourish CT. Utility of ethological analysis to overcome locomotor confounds in elevated maze models of anxiety. *Neurosci Biobehav Rev*. 1998;**23**(2):265–71. doi: 10.1016/S0149-7634(98)00027-X. [PubMed: 9884119].
28. Etgen AM, Shamamian P. Regulation of estrogen-stimulated lordosis behavior and hypothalamic progestin receptor induction by antiestrogens in female rats. *Horm Behav*. 1986;**20**(2):166–80. doi: 10.1016/0018-506X(86)90015-2. [PubMed: 3522395].
29. Loch EG, Selle H, Boblitz N. Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing Vitex agnus castus. *J Womens Health Gend Based Med*. 2000;**9**(3):315–20. doi: 10.1089/152460900318515. [PubMed: 10787228].
30. Schellenberg R. Treatment for the premenstrual syndrome with agnus castus fruit extract: Prospective, randomised, placebo controlled study. *BMJ*. 2001;**322**(7279):134–7. doi: 10.1136/bmj.322.7279.134. [PubMed: 11159568]. [PubMed Central: PMC26589].
31. Newall CA, Anderson LA, Phillipson JD. *Herbal medicines. A guide for health-care professionals*. London: The Pharmaceutical Press; 1996.
32. Schulz V, Hänsel R, Tyler VE. *Fitoterapia razionale: Scienza e piante medicinali*. Italy: Libreria Scientifica Ghedimedia; 2003.
33. Russo R, Corosu R. The clinical use of a preparation based on phytooestrogens in the treatment of menopausal disorders. *Acta Biomed*. 2003;**74**(3):137–43. [PubMed: 15055018].
34. Pourabbas S, Kesmati M, Rasekh A. Study of the the anxiolytic effects of fennel and possible roles of both gabaergic system and estrogen receptors in these effects in adult female rat. *Physiol Pharmacol*. 2011;**15**(1):134–43.
35. Lund TD, Rovis T, Chung WC, Handa RJ. Novel actions of estrogen receptor-beta on anxiety-related behaviors. *Endocrinology*. 2005;**146**(2):797–807. doi: 10.1210/en.2004-1158. [PubMed: 15514081].
36. Krezel W, Dupont S, Krust A, Chambon P, Chapman PF. Increased anxiety and synaptic plasticity in estrogen receptor beta -deficient mice. *Proc Natl Acad Sci U S A*. 2001;**98**(21):12278–82. doi: 10.1073/pnas.221451898. [PubMed: 11593044]. [PubMed Central: PMC59805].
37. McEwen BS, Alves SE. Estrogen actions in the central nervous system. *Endocr Rev*. 1999;**20**(3):279–307. doi: 10.1210/edrv.20.3.0365. [PubMed: 10368772].
38. Couse JF, Korach KS. Estrogen receptor null mice: What have we learned and where will they lead us? *Endocr Rev*. 1999;**20**(3):358–417. doi: 10.1210/edrv.20.3.0370. [PubMed: 10368776].

39. Lephart ED, West TW, Weber KS, Rhees RW, Setchell KD, Adlercreutz H, et al. Neurobehavioral effects of dietary soy phytoestrogens. *Neurotoxicol Teratol*. 2002;**24**(1):5–16. doi: 10.1016/S0892-0362(01)00197-0. [PubMed: 11836067].
40. Almstrup K, Fernandez MF, Petersen JH, Olea N, Skakkebaek NE, Leffers H. Dual effects of phytoestrogens result in u-shaped dose-response curves. *Environ Health Perspect*. 2002;**110**(8):743–8. doi: 10.1289/ehp.02110743. [PubMed: 12153753]. [PubMed Central: PMC1240943].
41. Walf AA, Frye CA. ERbeta-selective estrogen receptor modulators produce antianxiety behavior when administered systemically to ovariectomized rats. *Neuropsychopharmacology*. 2005;**30**(9):1598–609. doi: 10.1038/sj.npp.1300713. [PubMed: 15798780].
42. Poluzzi E, Piccinni C, Raschi E, Rampa A, Recanatini M, De Ponti F. Phytoestrogens in postmenopause: The state of the art from a chemical, pharmacological and regulatory perspective. *Curr Med Chem*. 2014;**21**(4):417–36. doi: 10.2174/09298673113206660297. [PubMed: 24164197]. [PubMed Central: PMC3963458].
43. Martinez J, Lewi JE. An unusual case of gynecomastia associated with soy product consumption. *Endocr Pract*. 2008;**14**(4):415–8. doi: 10.4158/EP.14.4.415. [PubMed: 18558591].