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# Does Vitex Agnus-Castus L. Have Deleterious Effect on Fertility and Pregnancy Outcome? An Experimental Study on Rats for Prediction of Its Safety

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**Objectives:** Herbal medicine is a worldwide health topic. Vitex agnus–castus L. (VAC) is a popular plant used for gynecologic problems due to its hormonal effects. The aim of this study is to reveal VAC extract effect on fetus when this herb is used started from antenatal period or during pregnancy.

**Methods:** Performed from starting day of January 2019 till February 2019, 48 rats were assigned in randomly divided eight-member six groups: control (C1), treated group with 365 mg/kg VAC from initiation of insemination (T1) and 30 days prior to pregnancy (T2), control that underwent caesarean section on 15$^{th}$ day of gestational age (C2) and treated group with 365 mg/kg VAC from initiation of insemination (T3) and 30 days prior to pregnancy (T4) that underwent caesarean section. Weight, sex and number of fetuses, abortion and still birth rate and estradiol level were evaluated using t-test by SPSS software.

**Results:** We showed increased weight among T1 group considering totally and sex-dependent which is significant (all p-value < 0.05). We also detected significantly decreased weight in T2 in total (p-value < 0.0001) and when considering female fetuses (0.043) but not males (0.17). Although the results showed slightly non-significant increased weight among fetuses of T3 (totally or based on the fetus sex) compared to the control group (C2), T4 group had statistically decreased weight compared to control group. Pregnancy rate and pregnancy outcome were affected by VAC usage. The time of VAC initiation also affected live birth and abortion rates.

**Conclusion:** VAC extract may affect pregnancy rate, live birth rate, abortion and stillbirth rates. Its effect on the weight and the sex showed dual pattern depends on the time of initiation and pregnancy trimester of evaluation. Prescribing this medicinal plant for patients being prone to pregnancy should be with caution. Further study is recommended.

**Keywords:** abortion, fertility, live birth, still birth, vitex agnus-castus, weight

## INTRODUCTION

Herbal medicine is defined as any form of plant-derived product used for preventive or curative treatment [1]. Its consumption is a global issue, as the World Health Organization estimates 70-80% of individuals worldwide use herbs as medicine [2]. Alternative medicine use during pregnancy varies from 10% to 80% among nationalities, which can be attributed to variations in ethnicity, environment, income, and knowledge status [1, 3, 4]. Also, nearly 30% of breastfeeding mothers use alternative medicines, mostly with the purpose of advancing lactation [1, 5].

Although many publications note the positive effects of herbal medicine, some medicines may cause side effects or

harm to patients, especially pregnant women. Some women experiment with herbal medicine to facilitate labor, improve the course of the pregnancy, or relieve unpleasant complications of pregnancy. However, this may lead to an increased rate of miscarriages, premature labor pain, abnormal uterine bleeding, and physical and mental deficiencies of the fetus. Using herbal medicine can be especially dangerous when the fetus is in the organogenesis period. Complementary medicine may be responsible for an increased rate of cesarean sections, preeclampsia, polyhydramnios, and maternal and neonatal mortality and morbidity. Harmful effects on the fetus may also develop when pregnant women use the topical form of complementary medicines [1, 2, 4-6].

Despite many individuals encouraging women to use herbal medicines during pregnancy, there are no obvious guidelines for use during pregnancy [3, 4]. Some discourage their use during pregnancy and the postnatal period until they are proven to be safe [1]. This animal experimental study was conducted to determine the safety and potential side effects of Vitex agnus-castus L. (VAC), a plant commonly consumed to fix infertility and other gynecologic problems. This study investigated VAC's use prior to and during pregnancy, examining outcomes related to weight, sex, and number of fetuses. This study also explored the abortion and stillbirth rate of fetuses.

## MATERIALS AND METHODS

### 1. Animals

This study was performed on 48 healthy plug-negative female Sprague-Dawley rats within a weight range of 200 g to 250 g (7 weeks old) with the written informed consent from the owner (Animal Laboratory of Shiraz University of Medical Sciences). It was conducted from January 2019 to February 2019 at the animal lab with the guidance of the ethics committee of the Shiraz University of Medical Sciences (ethics number: IR.SUMS.MED.REC.1398.033) that follows the National Institutes of Health guidelines. Experimental rats were kept in standard cages with wire mesh floors, appropriate humidity levels, 12-h light/darkness cycles, a room temperature of (24 ± 2℃), and clean water and proper food ad libitum.

### 2. Study design

The study was designed as a prospective, randomized control study. The study was performed on 48 (plug-negative) fertile female laboratory rats (Sprague-Dawley), with statistical significance considered at the p = 0.05 level and statistical power of 80%. The rats were kept for one week in the animal laboratory for acclimatization. To measure fetal weight, the animals were randomly assigned to six groups, each containing eight rats. Control group 1 (C1) received no medication, while two treated groups (T1, T2) received 365 mg/kg of alcoholic VAC extract derived from the plant's fruit part (Verbenaceae; Chaste tree). The taxonomic validity of the plant name was checked using The Plant List (http://www.theplantlist.org). The drop form was manufactured and quality controlled by the Pursina laboratory (IRC code: 1228091827, Tehran, Iran). Extract components, such as flavonoids, iridoid, glycosides, volatile oils, and palmitic acid, were dissolved in a 96% alcohol solution. The medicine was started in the T1 and T2 groups from the time of insemination and 30 days prior to pregnancy, respectively. This dosage was used because it is considered to interfere with the sexual cycle after 30 days of consumption in rats [7]. Also, in human beings, the effects of the drug can be seen after the first month of usage [8]. For mating, the female rats were caged for 24 h with a fertile matched male rat. Development of vaginal plaque is a confirmation sign of mating. The fact that fertilization led to pregnancy was checked by vaginal smears; the morphology and ratio of leukocytes and epithelial cells in the smear are indicators of pregnancy. The day the insemination sign (vaginal plaque) forms is considered to be the first day of pregnancy [9].

To assess the possible effects of the drug during different trimesters of pregnancy, the other three groups were assigned. Control group 2 (C2) received no medication and underwent a caesarian section on the 15$^{th}$ day of gestational age. Rats are believed to have a 21-day pregnancy cycle. The 15$^{th}$ day is considered equivalent to the end of the second trimester of a human pregnancy. Induction of anesthesia was done by the consumption of 2% sodium pentobarbital at the dose of 50 mg/kg, and the cesarean section delivery was performed in the supine position. An obvious fetus was considered a non-abortion, whereas very small gestational material was labeled as a miscarriage. Treated groups 3 and 4 (T3, T4) received 365 mg/kg of alcoholic VAC extract starting from the time of insemination or 30 days prior to pregnancy, respectively, and they underwent a caesarian section on the 15$^{th}$ day of gestation (Table 1).

To compare the number of pregnant mice, the number of fetuses, and the miscarriage and stillbirth rates, we consider three main groups: C1, T3, and T4. All rats were sampled, recruit-

**Table 1.** Study design of animal randomly divided groups based on receiving Vitex agnus-castus (VAC) extract

| | Control | | Treated | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 3 | 4 |
| 365 mg/kg VAC after insemination | – | – | + | + | + | + |
| 365 mg/kg VAC 30 days prior to pregnancy and continue during pregnancy period | – | – | – | + | – | + |
| Cesarean section on 15th day | – | + | – | – | + | + |

**Table 2.** Results based on the weight of the experimental studied groups

| Groups | Total number of live births | | | Weight of live births Means ± SD | | |
|---|---|---|---|---|---|---|
| | Male + Female | Male | Female | Male + Female | Male | Female |
| C1 | 61 | 31 | 30 | 5.85 ± 0.75 | 6.04 ± 0.70 | 5.64 ± 0.76 |
| T1 | 28 | 14 | 14 | 6.29 ± 0.47 | 6.43 ± 0.44 | 6.15 ± 0.47 |
| T2 | 11 | 5 | 6 | 3.38 ± 0.59 | 3.27 ± 0.55 | 3.47 ± 0.66 |
| C2 | 67 | 31 | 36 | 4.02 ± 0.66 | 3.95 ± 0.76 | 4.08 ± 0.57 |
| T3 | 76 | 41 | 35 | 4.07 ± 1.10 | 4.12 ± 1.26 | 4.15 ± 0.91 |
| T4 | 38 | 18 | 20 | 2.23 ± 0.82 | 2.57 ± 0.28 | 1.91 ± 1.02 |

C1: Control group that received no medication. T1: Treated groups receiving 365 mg/kg starting from first day of insemination. T2: Treated groups receiving 365 mg/kg starting from 30 days prior to pregnancy. C2: Control group that received no medication and underwent caesarian section on the 15th day of gestational age. T3: Treated groups receiving 365 mg/kg starting from first day of insemination and underwent caesarian section on the 15th day of gestational age. T4: Treated groups receiving 365 mg/kg starting from 30 days prior to pregnancy and underwent caesarian section on the 15th day of gestational age.

ing 2 cc blood by heart puncture, on the 15th day of gestation. Estradiol hormonal level by radioimmunoassay (ELISA) using a rat kit (ab108667-17 beta estradiol ELISA Kit, Abcam, USA) was measured at the Endocrine and Metabolic Research Center affiliated with Shiraz University of Medical Sciences. The detected concentration range for each kit is determined to be 20-2,000 pg/m [10]. The treated group's surviving fetuses were euthanized using 800 mg/kg sodium pentobarbital after being anesthetized by intraperitoneal administration of 100 mg/kg ketamine and 10 mg/kg xylazine [11, 12]. At the end of the survey, the weight, sex, and number of fetuses were evaluated.

### 3. Statistical analysis

Statistical analysis was done using SPSS version 25. Data were analyzed using the Chi-square test after considering the equality of variances on Levene's test. Each analysis was rechecked. $p < 0.05$ was considered statistically significant.

## RESULTS

48 rats were assigned and randomly divided into six groups, including eight rats in each collection, based on the previously stated study design. The weights of the live-delivered fetuses in each group are reported in the form of (mean ± standard deviation) considering the sex of the fetus. T1 and T2 are compared to the control group of C1, and all had natural delivery at the 20th day of pregnancy. Also, T3 and T4 are compared to the control group of C2, all of which underwent a cesarean section on the 15th day of pregnancy (Table 2).

Table 3 shows significantly statistically increased weight among the T1 group, considering totally and sex-dependent variables which are significant (all p-value < 0.05). We also detected significantly decreased weight in T2 in total (p-value < 0.0001) in female fetuses (0.043) but not in males (0.17). Although the results showed slightly non-significant increased weight among fetuses in T3 (totally or based on the fetus sex) compared to the control group (C2), the T4 group had statistically decreased weight compared to the control group. Table 3 shows these details.

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**Table 3.** Group comparison between weight of each experimental groups of alive fetuses using Chi-square test

| Comparison between 2 groups | Male + Female | | Male | | Female | |
|---|---|---|---|---|---|---|
| | 95% CI | p-value | 95% CI | p-value | 95% CI | p-value |
| T1/C1 | (0.71)-(0.82) | 0.02 | (−2.52)-(−1.65) | < 0.0001 | (−1.93)-(−1.17) | < 0.0001 |
| T2/C1 | (−3.00)-(−1.92) | < 0.0001 | (−0.17)-(0.93) | 0.17 | (0.01)-(1.01) | 0.043 |
| T2/T1 | (−3.49)-(−2.32) | < 0.0001 | (1.92)-(3.02) | < 0.0001 | (1.58)-(2.55) | < 0.0001 |
| T3/C2 | (−0.22)-(0.32) | 0.73 | (−0.24)-(0.57) | 0.42 | (−0.43)-(0.29) | 0.71 |
| T4/C2 | (−2.12)-(−1.46) | < 0.0001 | (−1.88)-(−0.86) | < 0.0001 | (−2.59)-(−1.74) | < 0.0001 |
| T4/T3 | (−2.17)-(−1.51) | < 0.0001 | (−2.02)-(−1.05) | < 0.0001 | (−2.53)-(−1.67) | < 0.0001 |

CI, confidence interval. C1: Control group that received no medication. T1: Treated groups receiving 365 mg/kg starting from first day of insemination. T2: Treated groups receiving 365 mg/kg starting from 30 days prior to pregnancy. C2: Control group that received no medication and underwent caesarian section on the 15th day of gestational age. T3: Treated groups receiving 365 mg/kg starting from first day of insemination and underwent caesarian section on the 15th day of gestational age. T4: Treated groups receiving 365 mg/kg starting from 30 days prior to pregnancy and underwent caesarian section on the 15th day of gestational age.

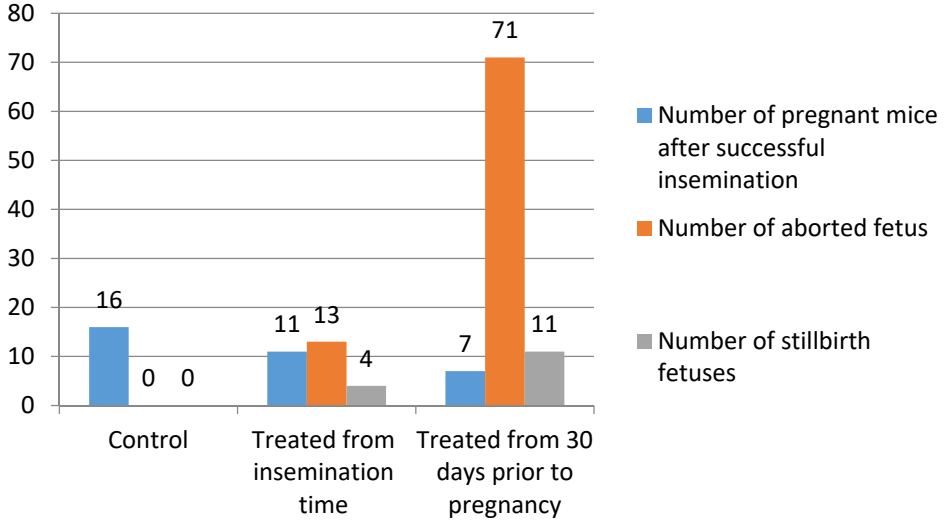


**Figure 1.** Diagram comparing number of pregnant mice, abortion and still birth in each group.

**Table 4.** Prevalence of pregnancy and pregnancy outcome among patients receiving VAC 30 days prior or simultaneously with insemination

| Groups | Pregnant mice (%) | Live birth (%) | Abortion (%) | Still birth (%) |
|---|---|---|---|---|
| Control | 16/16 (100) | 128/128 (100) | 0/128 (0) | 0/128 (0) |
| Receiving 365 mg/kg starting from first day of insemination | 11/16 (68.75) | 104/121 (85.95) | 13/121 (10.74) | 4/121 (3.30) |
| Receiving 365 mg/kg starting from 30 days prior to pregnancy | 7/16 (43.75) | 49/131 (37.40) | 71/131 (54.10) | 11/131 (8.39) |

To compare the number of pregnant mice, the number of fetuses, and the abortion and stillbirth rates, we considered three main groups: the control (C1 + C2), the group receiving 365 mg/kg from initiation of insemination (T1 + T3), and the group receiving the herb 30 days prior to pregnancy (T2 + T4) (Fig. 1, Table 4).

To explain the effect of VAC on the pregnancy rate, we examined the rate of abortion, the stillbirth and alive fetuses, and the number of pregnant mice affected by the extract. The same relationship is present for pregnancy outcomes. Table 5 presents the statistical status of all groups. Except for the pregnancy rate and stillbirth rate among mice given the VAC extract at insemi-

**Table 5. Statistical evaluation of pregnancy rate and outcome among different groups**

| Comparison between proportions | p-value | | | |
|---|---|---|---|---|
| | Pregnant mice | Live birth | Abortion | Still birth |
| Group receiving from first day of insemination/control | 0.02 | < 0.0001 | 0.0001 | 0.04 |
| Receiving 365 mg/kg starting from 30 days prior to pregnancy/control | 0.0005 | < 0.0001 | < 0.0001 | 0.0008 |
| Receiving 365 mg/kg starting from 30 days prior to pregnancy/group receiving from first day of insemination | 0.16 | < 0.0001 | < 0.0001 | 0.09 |

nation or 30 days prior to pregnancy, others were affected statistically. Table 5 shows additional details.

## DISCUSSION

Nowadays, alternative medicine usage is becoming more popular, with rising concerns for possible side effects. VAC, an herbal medicine, is popular for treating infertility and gynecological diseases. The herb has long leaves and tender branches, along with flowers and ripening berries. It contains major components of vitexin, casticin, agnuside, p hydroxybenzoicacid, alkaloids, and diterpenoids. The fruit of the plant also has herbal effects due to flavonoids, terpenoids, neolignans, phenolic, and glyceride constituents [13, 14]. This study was performed to evaluate the effects of VAC on pregnancy rates and outcomes when started prior to pregnancy or simultaneously with insemination.

Menstrual effects of VAC use have been described in the literature. Some clinicians believe that this herb can affect the volume of menstrual bleeding. In a systematic review, Mollazadeh et al. revealed no significant effects of VAC on the volume of bleeding compared to the placebo [13]. However, VAC use has been found to be helpful for managing oligomenorrhea and amenorrhea problems [15]. Several clinical studies have found this herb beneficial in treating polycystic ovary syndrome and premenstrual syndrome in women [14, 16], but a meta-analysis by Csupor et al. found no definite benefit [17].

In addition, the dopaminergic effects of VAC are supposed to be beneficial in treating cyclic mastalgia [8]. In 2019, Niazi et al. related this effect to antioxidant compounds with anti-inflammatory and analgesic effects [18]. Due to VAC's phytoestrogen effect, it can alleviate menopausal symptoms and improve the quality of life of women [19]. Molaie et al. also demonstrated that VAC is beneficial, in combination with conventional drugs, in relieving hot flushes of menopause patients [20]. It also can improve sleep disturbances during menopause [21]. Also, the effects of VAC on the endocrine system have been demonstrated. The terpenoid component of the herb is responsible for lowering prolactin levels, due to dopaminergic effects [13, 22]. In addition, the pituitary–thyroid and pituitary–adrenocortical axes have been found to be affected by VAC extract if used for long period.

VAC is traditionally administered to improved fertility [23]. Its hormonal effects play a role in the treatment of both males and females [14]. In 2019, Antoine et al. found a positive relationship between VAC use and regulated menstrual cycles and ovulation, leading to an increase in the likelihood of fertility [24, 25]. In contrast to previous studies, we found a decreased fertility rate and pregnancy rate when the treated groups were compared to control groups. However this decrease was not statistically significant among patients who received VAC 30 days prior to pregnancy, compared to the mice that received VAC starting from the first day of insemination (Tables 4, 5). This may be due to an impaired estrogen to progesterone ratio and the estradiol hormone leading to a disturbance of implantation. Other possible effects of the herb can be attributed to pH change of the uterine environment, affecting the thickness of the endometrium, developing myometrium contractions or cytokine release. Also, this can be attributed to decreased rate of ovulation with VAC usage, especially when prescribed for a longer period. A logical justification of this result can be in relation to Luteinizing hormone (LH) levels. As the LH is decreased with the VAC extract prescription, LH surge is impaired. Decreased ovulation is the final consequence of this process.

According to our study, birth weight showed a dual pattern that was affected by the herb usage initiation time. In T1, both male and female fetuses experienced a significant increase in weight. This increase is also seen in T3, but it was not statistically significant in either sex or the total group. The VAC extract was given at the insemination time for both T1 and T3 and at different point at the termination of pregnancy. T1 had natural deliveries at the end of the study (equivalent to the end of

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the third trimester in humans), while the T3 pregnancies were terminated on the 15th day of the rat's pregnancy cycle (equivalent to the end of the second trimester in humans) (Tables 2, 3). Based on these results, when VAC is started simultaneously with insemination, it has a positive effect on the fetus weight. Also, most of the weight gain occurs in the late pregnancy period (equivalent to the third trimester of human pregnancies). For T2 and T4 groups, when VAC was started 30 days prior to pregnancy, a weight decrease was observed in both genders, except for the T2 male fetuses, while both genders showed a decrease in weight in T4. There may be two explanations for the dual pattern of the VAC on fetal weight when prescribed before pregnancy. When it is started prior to pregnancy, it may decrease the weight of male fetuses mostly in the first and second trimesters, rather than in the third trimester. As a result, there could be weight gain in the third trimester of pregnancy, leading to the result that T2 male fetuses had no statistically decreased weight in comparison to the control group. Another aspect of this result is that the male fetuses gained more weight in the third trimester of pregnancy in comparison to female fetuses, and that only male fetuses were not statistically affected by VAC usage. Different effects of the herb may be attributed to the duration of usage, the altered hormonal properties of the extract by individuals, or the primary hormonal environment in which the herb is started. More studies are recommended to find the possible consequences and related pathways contributing to this effect.

VAC usage affects pregnancy rates, live birth rates, abortion rates, and stillbirth rates. The live birth and abortion rates are influenced by the time at which VAC use begins, as found when comparing the results of use 30 days prior to pregnancy and use at the time of insemination (Tables 4, 5). It can be developed secondarily to decreased blood circulation, which is in line with the work of Louei Monfared and Hamoun Navard [26]. They present a decreased placental trophoblastic cell after VAC extract consumption in rat models that leads to decreased blood circulation. Lin et al. revealed that low levels of estradiol during pregnancy can lead to inflammation and vascular endothelial dysfunction [9]. Increased rates of abortion and stillbirth after VAC extract consumption showed in our study can also be attributed to hormonal alteration. Also, VAC can reach the fetus through the placenta barrier and cause malformations on mice fetuses [27]. Direct effects of the herb on the conception material are another mechanism to explain this result.

This study had several important findings, including the assessment of VAC extract effects on different aspects of fetuses. Also, we studied the time of initiation of the extract and the potentially harmful effects during different stages of the pregnancy period. Studying the effects on fetal sexes offers a guide to studying the effects of VAC on fetal chromosomes and different physiological patterns related to each sex. To our knowledge, there has been no such study to date. There are several limitations, such as some confusing factors affecting the results. Due to the physiological differences between rats and humans, the results may not be completely generalizable to humans. Also, the method and dosage of the consumption may vary at home and may not be based on the recommendation of other individuals, which may be different from a scientific studies' recommendations [4]. To overcome this gap, patient and healthcare education should be facilitated to provide safe recommendations for VAC use [6]. Besides, the effects may be influenced by the cultivation region differences in soil, weather, and altitude, the quality of the active prepared herb, and the incorrect or poor quality manufacturing process [28]. In addition, the type of the nanobarrier used in the VAC extract could potentially impact the oral bioavailability of the drug [29].

## CONCLUSION

Our study revealed that VAC extract may affect pregnancy rates, live birth rates, and abortion and stillbirth rates. Its effects on the weight and the sex showed dual patterns depending on the time of initiation and the pregnancy trimester of evaluation. It is important to educate individuals about the possible harmful consequences of herbal medicines, especially among pregnant women. Physicians should keep in mind the potential for unwanted pregnancy in women of child-bearing ages. Prescribing this herb for patients who may be or become pregnant is not recommended.

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## AUTHORS' CONTRIBUTIONS

N.F, T.P, M.S, N.N, and A.O conducted the research. Designing of study was by N.F, M.S, and A.O. to contribute essential agents preparation. Z.SH and N.N were involved. Z.SH and M.S entered the data, N.N rechecked and SH and T.P analyzed the data. All authors were engaged in drafting the paper and have read and confirmed the final edited manuscript. They also are accountable for all aspects of the work in accuracy or reliability of any part of the work.

## CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

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## REFERENCES

1. Munoz Balbontin Y, Stewart D, Shetty A, Fitton CA, McLay JS. Herbal medicinal product use during pregnancy and the postnatal period: a systematic review. Obstet Gynecol. 2019;133(5):920-32.
2. Ossei PPS, Appiah-Kubi A, Ankobea-Kokroe F, Owusu-Asubonteng G, Ayibor WG, Aninkora OK, et al. The culture of herbal preparations among pregnant women: a remedy or a suicide potion? A case report and mini review. Case Rep Pathol. 2020; 2020:6186147.
3. Onakpoya I. Use of herbal products in pregnancy: harms are reported but the benefit to harm balance is uncertain. BMJ Evid Based Med. 2020;25(2):1-2.
4. Eid AM, Jaradat N. Public knowledge, attitude, and practice on herbal remedies used during pregnancy and lactation in West Bank Palestine. Front Pharmacol. 2020;11:46.
5. Aljofan M, Alkhamaiseh S. Prevalence and factors influencing use of herbal medicines during pregnancy in Hail, Saudi Arabia: a cross-sectional study. Sultan Qaboos Univ Med J. 2020;20(1): e71-6.
6. Adane F, Seyoum G, Alamneh YM, Abie W, Desta M, Sisay B. Herbal medicine use and predictors among pregnant women attending antenatal care in Ethiopia: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2020;20(1):157.
7. Jelodar G, Askari K. Effect of Vitex agnus-castus fruits hydroalcoholic extract on sex hormones in rat with induced polycystic ovary syndrome (PCOS). Physiol Pharmacol. 2012;16(1):62-9.
8. Ooi SL, Watts S, McClean R, Pak SC. Vitex agnus-castus for the treatment of cyclic mastalgia: a systematic review and meta-analysis. J Womens Health (Larchmt). 2020;29(2):262-78.
9. Lin ZH, Jin J, Shan XY. The effects of estradiol on inflammatory and endothelial dysfunction in rats with preeclampsia. Int J Mol Med. 2020;45(3):825-35.
10. Ben Hameid AS, Al-Sindi TA, Allow AK, Nafie EM, Alahmad BE, Faisal GG. Substantial effect of fenugreek seeds aqueous extract on serum Estradiol level in ovarian hyperstimulation syndrome rat model. Oman Med J. 2019;34(3):238-43.
11. Moorthy M, Khoo JJ, Palanisamy UD. Acute oral toxicity of the ellagitannin geraniin and a geraniin-enriched extract from *Nephelium lappaceum* L rind in Sprague Dawley rats. Heliyon. 2019;5(8):e02333.
12. Zatroch KK, Knight CG, Reimer JN, Pang DS. Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats (Rattus norvegicus). BMC Vet Res. 2017;13(1): 60.
13. Mollazadeh S, Mirghafourvand M, Abdollahi NG. The effects of Vitex agnus-castus on menstrual bleeding: a systematic review and meta-analysis. J Complement Integr Med. 2019;17(1): 20180053.
14. Rafieian-Kopaei M, Movahedi M. Systematic review of premenstrual, postmenstrual and infertility disorders of Vitex agnus Castus. Electron Physician. 2017;9(1):3685-9.
15. Moini Jazani A, Hamdi K, Tansaz M, Nazemiyeh H, Sadeghi Bazargani H, Fazljou SMB, et al. Herbal medicine for oligomenorrhea and amenorrhea: a systematic review of ancient and conventional medicine. Biomed Res Int. 2018;2018:3052768.
16. Moini Jazani A, Nasimi Doost Azgomi H, Nasimi Doost Azgomi A, Nasimi Doost Azgomi R. A comprehensive review of clinical studies with herbal medicine on polycystic ovary syndrome (PCOS). Daru. 2019;27(2):863-77.
17. Csupor D, Lantos T, Hegyi P, Benko R, Viola R, Gyongyi Z, et al. Vitex agnus-castus in premenstrual syndrome: a meta-analysis of double-blind randomised controlled trials. Complement Ther Med. 2019;47:102190.

18. Niazi A, Rahimi VB, Hatami H, Shirazinia R, Esmailzadeh-Dizaji R, Askari N, et al. Effective medicinal plants in the treatment of the cyclic mastalgia (breast pain): a review. J Pharmacopuncture. 2019;22(3):131-9.
19. Naseri R, Farnia V, Yazdchi K, Alikhani M, Basanj B, Salemi S. Comparison of Vitex agnus-castus extracts with placebo in reducing menopausal symptoms: a randomized double-blind study. Korean J Fam Med. 2019;40(6):362-7.
20. Molaie M, Darvishi B, Jafari Azar Z, Shirazi M, Amin G, Afshar S. Effects of a combination of Nigella sativa and Vitex agnus-castus with citalopram on healthy menopausal women with hot flashes: results from a subpopulation analysis. Gynecol Endocrinol. 2019;35(1):58-61.
21. De Franciscis P, Grauso F, Luisi A, Schettino MT, Torella M, Colacurci N. Adding agnus castus and magnolia to soy isoflavones relieves sleep disturbances besides postmenopausal vasomotor symptoms-long term safety and effectiveness. Nutrients. 2017;9(2):129.
22. Heskes AM, Sundram TCM, Boughton BA, Jensen NB, Hansen NL, Crocoll C, et al. Biosynthesis of bioactive diterpenoids in the medicinal plant Vitex agnus-castus. Plant J. 2018;93(5):943-58.
23. Veal L. Complementary therapy and infertility: an Icelandic perspective. Complement Ther Nurs Midwifery. 1998;4(1):3-6.
24. Antoine E, Chirila S, Teodorescu C. A patented blend consisting of a combination of Vitex agnus-castus extract, Lepidium meyenii (Maca) extract and active folate, a nutritional supplement for improving fertility in women. Maedica (Bucur). 2019;14(3):274-9.
25. Feyzollahi Z, Mohseni Kouchesfehani H, Jalali H, Eslimi-Esfahani D, Sheikh Hosseini A. Effect of *Vitex agnus-castus* ethanolic extract on hypothalamic *KISS-1* gene expression in a rat model of polycystic ovary syndrome. Avicenna J Phytomed. 2021;11(3):292-301.
26. Louei Monfared A, Hamoun Navard S. The effect of ethanol extracts of Vitex on histomorphological placenta in the rat model. Armaghan Danesh. 2014;19(7):614-24.
27. Azarnia M, Ejtemaee-Mehr S, Shakoor A, Ansari A. Effects of Vitex agnus castus on mice fetus development. Acta Med Iran. 2007;45(4):263-70.
28. Sogame M, Naraki Y, Sasaki T, Seki M, Yokota K, Masada S, et al. Quality assessment of medicinal product and dietary supplements containing Vitex agnus-castus by HPLC fingerprint and quantitative analyses. Chem Pharm Bull (Tokyo). 2019;67(6):527-33.
29. Piazzini V, Monteforte E, Luceri C, Bigagli E, Bilia AR, Bergonzi MC. Nanoemulsion for improving solubility and permeability of Vitex agnus-castus extract: formulation and in vitro evaluation using PAMPA and Caco-2 approaches. Drug Deliv. 2017;24(1):380-90.