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Original Article

Stachys lavandulifolia Causing Estrogen/Progesterone Imbalance during Pregnancy in Wistar Rat: A Time Course Experimental Study

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Abstract

Stachys lavandulifolia is one of the most abortive herbs used in Iran. The present study aimed to investigate the effects of different doses of hydroalcoholic extract of *Stachys lavandulifolia* (*HAESL*) on serum estrogen and progesterone levels during different stages (implantation, abortion, and parturition) of pregnancy in Wistar rats. After observing vaginal plaque, the pregnant rats were randomly divided into three studied groups. Each group received the *HAESL* for three periods including, implantation (days 3 to 5), abortion (days 10 to 12), and parturition (days 19 to 21) of pregnancy. In the abortion and parturition periods, we observed a significant decrease in the estrogen level following administration of *HAESL* (300 and 900 mg/kg). In addition, we observed a significant decrease in the progesterone level at the highest dose of HAESL in all stages of pregnancy. In conclusion, the present study suggests that *Stachys lavandulifolia* may affect the estrogen/progesterone levels due to the presence of flavonoid compounds that subsequently lead to abortion, especially during the abortion and parturition periods.

Keywords: *Stachys lavandulifolia*, Estrogen, Progesterone, Pregnancy, Abortion, Rat.

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1. Introduction

Natural products have great importance in traditional medicine for many years. The genus *Stachys*, one of the largest genera of the *Lamiaceae* family, contains 300 species [1]. Various biological properties including anti-septic, anti-anoxia, and anti-inflammatory attributed to the genus *Stachys* [2]. In Iran, this

genus is represented by 34 species. *Stachys lavandulifolia* (*SL*) is known under the common names of "Chaye kuhi" and "Wood Betony" in Persian and English, respectively. Several studies have shown that *SL* had significant therapeutic effects on anxiety, infection, hepatitis, asthma, and inflammatory diseases [3, 4]. Recently, the toxicity of a high dose of the extract of *SL* is reported [5]. Besides, some studies have shown that the SL impair fetal growth and caused birth defects in pregnant mice [6]. Apigenin and α-pinene, two main compounds of *SL*, might produce teratogenic effects [7]. The presence of flavonoids in the hydroalcoholic extract of *Stachys lavandulifolia* (*HAESL*) may exert an effect on the pituitary-gonadal axis. Furthermore, the *HAESL* causes a decrease in progesterone concentration and affects fetal life adversely [8].

A large amount of estrogen and progesterone, as two main female sex hormones, are produced at puberty, during sexual activity, and before pregnancy [9]. The estrogen is produced simultaneously by the placenta and corpus luteum at the beginning of pregnancy [10]. The elevated estrogen level was associated with increased follicle maturation, decreased ovarian storage, and recurrent miscarriages [11]. It has a critical effect on many other tissues such as bone, fat, skin, liver, and brain. Although estrogen levels in females are much higher than in males, it also has important roles in males [12]. In general, estrogen implies important roles for dynamic interchange among the placenta and fetus, leading to placental and fetal development during primate pregnancy [13]. Finally, estrogen regulates the functional differentiation of the placental syncy-tiotrophoblast with advancing pregnancy [14].

Progesterone is one of the essential hormones to maintain pregnancy and protects against severe contractions and miscarriage by helping the uterus grow. Progesterone stimulates and maintains endometrial functions necessary for embryonic survival, conceptus growth, implantation, placentation, and development to term [15].

Based on the importance of identifying the effects of *HAESL* on the maintenance of pregnancy, fetal development, and growth during pregnancy, we aimed to investigate the effect of different doses of *HAESL* on the levels of estrogen and progesterone in serum during different stages (implantation, abortion and parturition) of pregnancy in Wistar rat.

2. Materials and Methods

2.1. Plant Collection

Stachys lavandulifolia was collected from the natural habitat in the Hamedan district. This plant with access code 178 is registered in the Herbarium Center in the School of Pharmacy, Hamadan University of Medical Sciences.

2.2. Preparation of Hydroalcoholic Extract

The plant extraction was performed by maceration, using hydroethanolic (80%) as a green solvent. The process was repeated three times with an optimum extraction time of 72 h. The extract was smoothed and concentrated using a rotary evaporator apparatus. The concentrated extract dried by bain-marie and

kept away from light. The dried extract was then stored in a refregrator at 4 ºC for further biochemical examination [16].

2.3. Experimental Animals

A total of 72 adult female rats with an average weight of 200 ± 20 g were obtained from the Animals Lab of Hamadan University of Medical Sciences. The rats were kept in standard plastic laboratory cages at room temperature (24 ± 4 ºC) and humidity (60 ± 5%) with 12 h light/dark cycle. During the experiments study, the rats were fed by standard chow and tap water ad libitum. The rats were housed in the cages after two weeks of adaptation to the laboratory conditions for the 2:1 ratio (two females and one male). After observing vaginal plaque, the pregnant rats were randomly divided into three groups (i.e. implantation, abortion, and parturition periods) [17].

The rats in each pregnancy period were randomly divided into four groups (i.e., control and three treatment groups). Each group contained six rats. The three treatment groups were treated with 100, 300, and 900 mg/kg *HAESL* on implantation (for three consecutive days starting from days 3 to 5 of gestation), abortion (for three consecutive days starting from days 10 to 12 of gestation), and parturition periods (for three consecutive days starting from days 19 to 21 of gestation). At the end of the experiments, rats were laparotomied under Ketamine/ Xylazine (50:10 mg/kg) anesthesia [18] to minimize the painful effect of sacrificing steps on days of 9, 13, and 22 of pregnancy. The animal's peripheral blood from vena cava veins was collected. The serum separation from blood samples was done using a centrifuge at 3500 g for 10 min and then frozen at -20 ºC until processing. Additionally, the number of embryos adsorbed in uterine tubes, which represented the number of aborted fetuses, was counted. All procedures were approved in advance by the Ethical Committee for Hamadan University of Medical Sciences (IR.UMSHA.REC.1397.373).

2.4. Estrogen Measurement

In this study, 17β estradiol as an essential estrogen was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (DiaMetra, Spello, Perugia, Italy) and taking absorbance at 450 nm, according to instructions of the manufacture. The lowest detectable concentration of this estrogen that can be distinguished from the calibrator zero is 8.68 pg/ml at the 95% confidence limit.

2.5. Progesterone Measurement

Progesterone commercial ELISA kit was purchase from DiaMetra (Spello, Perugia, Italy). The enzyme-substrate reaction was measured spectrophotometrically at 450 nm. The lowest detectable concentration of progesterone that can be distinguished from the calibrator zero is 0.05 ng/ml at the 95% confidence limit.

2.6. Statistical Analysis

Statistical analysis was performed with SPSS 15.0 (SPSS Inc., Chicago, Ill., USA) statistical program. The results were presented as the mean ± SD. Statistical significance was

examined by one-way ANOVA followed by Tukey's post hoc test. A difference of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

Herbal medicine consumption is a worldwide public health concern, especially in women during pregnancy. Herbal medicines contain active ingredients that are perceived to have therapeutic benefits [19]. About 80% of the world's population uses a variety of traditional and herbal medicine, for the diagnosis, prevention, and treatment of illnesses, and the improvement of general well-being [20]. Even though the usage of herbal medicine is becoming more common recently, their unidentified side effects are a serious threat to human health, especially during pregnancy. In this regard, it is crucial to identify herbs with side effects that can affect pregnancy through estrogen and progesterone secretion. Therefore, this study evaluates the *HAESL* effect on estrogen and progesterone levels during pregnancy in the Wistar rats.

The rats were treated with 100, 300, and 900 mg/kg of the *HAESL* at different stages of pregnancy (i.e. implantation, abortion, and parturition). We observed the *HAESL* reduced the number of fetuses in all of the groups that received 100 mg/kg, 300 mg/kg, and 900 mg/kg compared to the control groups in all tested days of pregnancy (Table. 1).

Table 1. Effect of hydroalcoholic extract of *Stachys lavandulifolia* on viable fetuses, and aborted fetuses in three periods of pregnancy.

Groups	Fetuses	Abortion
Day 9 (Implantation period)		
Control	45	0
100 mg/kg	30	0
300 mg/kg	34	2 (5.9%)
900 mg/kg	33	8 (24.2%)
Day 13 (Abortion period)		
Control	69	0
100 mg/kg	30	0
300 mg/kg	32	3 (9.4%)
900 mg/kg	28	8 (28.6%)
Day 22 (Parturition period)		
Control	60	0
100 mg/kg	56	4 (7.1%)
300 mg/kg	48	6 (12.5%)
900 mg/kg	42	8 (19%)

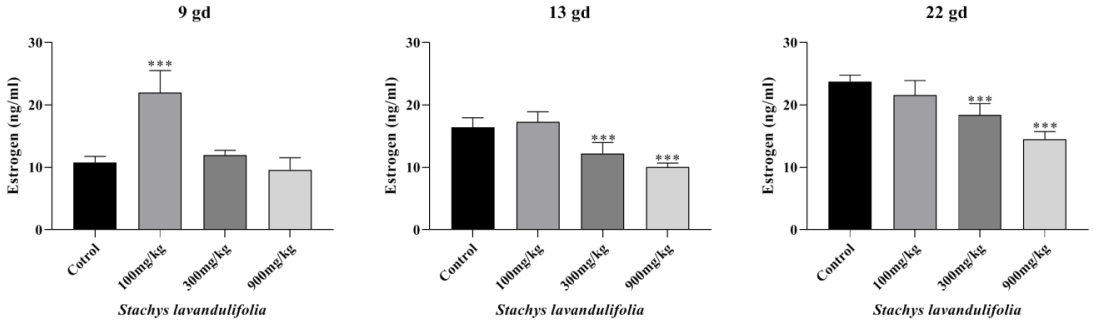

Figure 1. The effects of different concentrations of *S. lavandulifolia* extract on the estrogen levels on days 9, 13, and 22 of pregnancy. Data represent means ± SD (n = 6 in each group). (*** p<0.001). gd, gestation day.

As shown in figure 1, in the implantation period (gestation day 9th), a group of rats that received 100 mg/kg of the extract, the highest amount of estrogen was observed compared to the control group ($p < 0.001$). However, there were no significant differences between the estrogen levels of groups that received 300 and 900 mg/kg of the *HAESL* compared to control. In the abortion and parturition periods (gestation day 13th and 22nd), we observed a decrease in the estrogen level in the animals that received the doses of 300 and 900 mg/kg of the *HAESL* compared to the control group ($p < 0.001$). In agreement with our findings, previous studies have reported that the SL modulates the intensity and duration of pain caused by primary dysmenorrhea due to estrogen secretion reduction [16, 21]. In addition, the *HAESL* phytochemical evaluation has shown that the presence of apigenin flavonoids might be related to its abortive effect [15, 22]. Moreover, apigenin was shown to lower the estrogen receptor alpha concentration in the uterus, which leads to a decrease in estrogen response [23]. Therefore, it seems that a decrease in estrogen levels in abortion and parturition periods of pregnancy can cause pregnancy problems and increase the risk of miscarriage.

As shown in figure 2, on the day 9th and 13th of pregnancy (implantation and abortion periods), a significant decrease was observed in the progesterone level in the treated animals with 900 mg/kg of *HAESL* ($P < 0.01$).

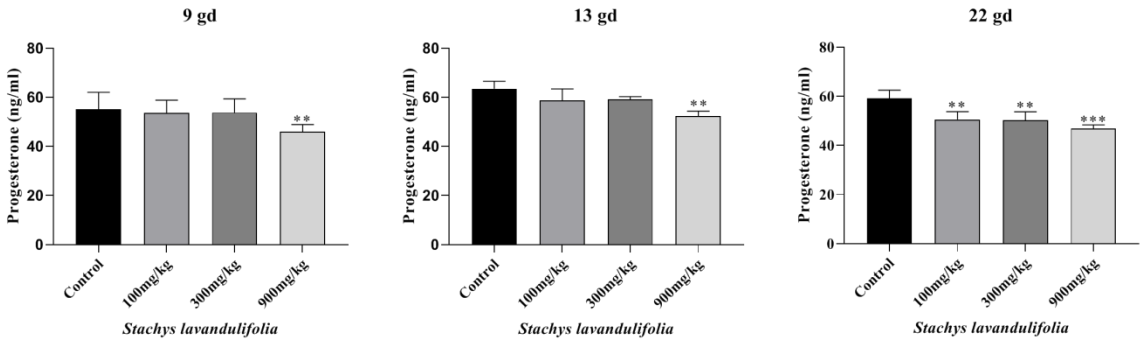

Figure 2. The effects of different concentrations of S. lavandulifolia extract on the progesterone levels on days 9, 13, and 22 of pregnancy. Data represent means ± SD (n = 6 in each group). (** p < 0.01, *** p<0.001). gd, gestation day.

In the parturition period (gestation day 22nd), we found a decreased level of progesterone in the groups that received 100 and 300 mg/kg ($P < 0.01$) and 900 mg/kg ($P < 0.001$) of *HAESL*. In agreement with our findings, Jafarzadeh *et al*. showed that administration of *HAESL* could cause a decrease in progesterone. They also showed that *HAESL* decreases the number of fetuses and increases abortion [16]. Previously, the inhibitory effects of apigenin on progesterone activity and uterine endometrial cell proliferation have been confirmed [24]. In addition, previous studies showed that flavonoids can significantly decrease progesterone levels [25, 26]. Therefore, it seems that a decrease in progesterone levels is associated with *SL* flavonoids, specially apigenin, that may lead to miscarriage and even premature labor.

4. Conclusion

Taken together, the present study suggests that *SL* may affect the estrogen/progesterone levels due to the presence of flavonoid compounds that subsequently lead to abortion, especially during the abortion and parturition periods. Thus, the use of Stachys lavandulifolia could interfere with the pregnancy process and cause premature labor and even miscarriage. However, further studies need to evaluate the exact molecular mechanism involved in these processes in the future.

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