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Original article:

THE EFFECT OF HYDROALCOHOLIC EXTRACT OF STACHYS LAVANDULIFOLIA VAHL ON PREGNANT MICE

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ABSTRACT

Objectives: *Stachys lavandulifolia* is commonly used for many health problems including anxiety. A couple of reports indicate that this plant might have an abortifacient effect on pregnant women. Here we examined this effect on pregnant mice.
Materials and methods: Incremental doses of 0, 50, 100, 150 and 200 mg/kg of the extracts or normal saline (control group) were injected intraperitoneally to the pregnant mice between 7^{th} to 12^{th} days of pregnancy. On day 16, uterine tubes were resected and absorbed fetuses were counted.
Results: Our study showed that the different average of absorbed fetuses between treated and control groups is significant ($P < 0.05$). *S. lavandulifolia* changes the activity level of hypothalamus-pituitary gland-gonad axis due to flavonoid compounds. Also, *S. lavandulifol*ia decreases progesterone concentration resulting in a significant difference between treated and control groups ($P < 0.05$) and gives rise to failure in fetus survival and consequently, abortion. The length and weight of fetuses decreased in treated groups and there was a significant difference between treated and control groups ($P < 0.05$).
Conclusion: Owing to the possible abortive effect of *Stachys lavandulifolia*, it is highly recommended to use it cautiously during pregnancy.

Keywords: *Stachys lavandulifolia*, medicinal plants, abortifacient agents, pregnant mice

INTRODUCTION

For thousands of years people used plants and herbs as healing compounds (Kazi et al., 2003). The importance of some plants as a source of antifertility drugs has been emphasized by many researchers (Farnsworth et al., 1975; Yakubu et al., 2007). Antifertility agents obtained from indigenous medicinal plants would be immense benefit especially to inhabitants of developing countries, since the cost of these drugs would be within their means (Goonasekera et al., 1995). Naturally occurring compounds exhibiting estrogenic activity are widely distributed in the plant kingdom (Breinholt et al., 2000).

The antifertility plants with estrogenic effect can directly influence the pituitary action through peripheral modulation of luteinizing (LH) and follicle-stimulating hormones (FSH) by decreasing the secretion of these hormones and blocking ovulation (Brinker, 1997). In addition, the plant may also intercept the synchronized development of the ovum and endometrium while others may have abortifacient or antiprogestational effects (Gark et al., 1978; Prakash et al., 1985).

The climate of Iran and the favored geographical situation have contributed to the biodiversity of medicinal plants. Among the species of Iranian plants, which are known as the source of folk medicine, *Stachys la-*

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vandulifolia has been commonly used for its useful effects on insomnia and anxiety. There are about 300 species of the genus Stachys widespread throughout the world (Evans, 1996). In Iran, 34 species of this genus exist, among which, 13 are endemic (Mozaffarian, 1996). Flavonoids, quinines, iridoids, phenolic acids and diterpenoids are reported as secondary metabolites of different species of this genus (Duru et al., 1999). Germacrene-D, beta pinene, alpha pinene, myrcene and beta phellandrene have been reported to be the main compounds of the essential oil *S. lavandulifolia* (Javidnia et al., 2004). In Iranian folklore medicine, *S. lavandulifolia* is claimed to be effective for the treatment of infection, asthma, inflammatory diseases, especially rheumatism (Sajjadi and Amiri, 2007). However, despite an obvious abortifacient effect of *S. lavandulifolia* extract utilized in Iranian folklore medicine, there is no published scientific evidence to approve or refuse this claim. Therefore, we have decided to provide scientific evidence to show abortifacient potential of the hydroalcoholic extract of *S. lavandulifolia* (different doses) in pregnant mice.

MATERIALS AND METHODS

Plant material

Aerial parts of *S. lavandulifolia* were collected from Chaharmahal and Bakhtiyari Province. The plant was identified at the Medical Plants Research Center, Shahrekord University of Medical Sciences. A voucher specimen of the plant has been deposited in herbarium unit in Shahrekord University of Medical Sciences, Iran (voucher specimen no. 204).

Hydroalcoholic extract

Dried and powdered aerial parts of the plant (100 g) were macerated at room temperature with 1 L of ethanol: water (75:25). The extraction continued two times and then was concentrated in a rotary evaporator under low pressure to give one third of the primary substance. The net extract weighted (dried mass) 7 g.

Laboratory animals

Thirty six female Syrian mice (Pasture, Tehran, Iran) weighting between 35 to 40 g (8-12 weeks old) were housed in cages with controlled room temperature 22-25 °C.

Determination of Abortifacient Activity

The mice were mated (ratio 2:1) in cages with food and water ad libitum. Vaginal plug and smear were checked by means of 5 % eosine. The day on which a vaginal plug was observable was considered day 0 of pregnancy. Then the pregnant mice were randomized into six groups, each consisting of six animals. Between 7^{th} to 12^{th} day of pregnancy, groups 1 to 4 were intraperitoneally injected with extract doses of 50, 100, 150 and 200 mg/Kg, respectively,. Two control groups were administrated as following: group 6 received nothing and group 5 received sterile distilled water. On the 16^{th} day of pregnancy, the animals were sacrificed by cervical dislocation and were also laparotomized. The ovaries and uterine horns were exteriorized, and the uteri were opened for counting of live and degenerated/dead fetuses and late reabsorptions. All procedures were performed in accordance with the local principles for laboratory animals caring in Shahrekord University of Medical Sciences.

The amount of flavonoid components was measured colorimetrically by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The total amount of flavonol was measured by colorimetry of by the Rutin method with aluminum chloride (Loziene et al., 2007). A commercial kit (Mouse estradiol and Mouse sterogene elisa 96 tests catalog no. csb-eo51o4m, cusabio.co) was used for progesterone measurements.

Statistical analysis

Kruskal-Wallis test was done by using SPSS software v11.5. Results are expressed as the mean ± SD and p-value of less than 0.05 was considered statistically significant.

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RESULTS

The reproductive parameters determined after exposure to doses of 50, 100, 150 and 200 mg/Kg body weight of the extract and of two control groups are exhibited in Table 1.

In treated groups, the total number of fetuses was 137 which in average amounts to 5.7 fetuses per animal. In groups 1 and 2, 34 and 36 fetuses were counted in total with average numbers per animal of 5.6 and 6, respectively. In group 3, the total number of fetuses was 20 (3.33 fetuses in average) and 47 in group 4 (7.8 fetuses in average). In the 2 control groups 5 and 6, total fetuses were 48 corresponding to an average of 4 fetuses/animal. The average serum progesterone concentration in animals of the treated groups was 45 ± 3.2 mg/ml and the difference between treated and control groups, was significant ($P < 0.05$). In this study, the length and the weight of alive fetuses were investigated and the results showed that the differences in mean length and weight of alive fetuses between treated and control groups (excluding group 4) were significant and the length and weight of treated groups decreased ($P < 0.05$). The mean of aborted fetuses in the treated groups (extract doses of 50, 100, 150 and 200mg/Kg body weight) are shown in Table 2. In treated groups, the percent of absorbed fetuses was 20, while, no abortion was seen in the two control groups. Kruskal-Wallis test showed a significant difference between treated groups ($P < 0.05$).

The mean difference of abortion between treated groups showed that the mean difference of abortion in group 1 **(**$P < 0.01$) and group 2 ($P < 0.05$) was significantly different from group 3. The difference between other groups was not statistically significant. There were no aborted fetuses in the two control groups and there was no significant difference between other groups. The amount of flavonoid components and total flavonol were 124.6 ± 7.99 mg/ml and 51.08 ± 7.81 mg/ml, respectively.

Table 1: The effect of *S. lavandulifolia* Vahl extract on reproductive parameters

Group	Dose (mg/kg)	Progesterone (mg/ml)	Mean of fetus weight (mg)	Mean of fetus length (mm)	Percent of abortion	Number of degenerated/dead fetuses	Number of alive fetuses	Number of total fetuses
1	50	47 ± 2.1	0.2 ± 0.04	0.9 ± 0.04	14.7	5	29	34
2	100	43 ± 4.2	0.2 ± 0.01	0.9 ± 0.07	25	9	27	36
3	150	44 ± 3.1	0.1 ± 0.03	0.9 ± 0.01	45	9	11	20
4	200	46 ± 1.6	0.9 ± 0.07	1.6 ± 0.06	29.8	14	33	47
	Total	46 ± 1.6	0.35 ±0.02	1.07	27	37	100	137
5	distilled water	61 ± 1.8	1.1 ± 0.06	1.7 ± 0.09	0	0	24	24
6	control	65 ± 2.8	1.1 ± 0.04	1.55 ± 0.05	0	0	24	24

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Table 2: The mean of aborted fetuses in animals treated with different doses of the extract

Group	Dose (mg/kg)	The mean of aborted fetuses to all fetuses for each mouse (mean ± SD)
1	50**	0.136 ± 0.108
2	100*	0.151 ± 0.263
3	150	0.391 ± 0.583
4	200	0.172 ± 0.353

**The mean difference of abortion was significant between 150 and 50 (mg/Kg body weight) groups ($p < 0.01$).
*The mean difference of abortion was significant between 150 and 100 (mg/Kg body weight) groups ($p < 0.05$).

DISCUSSION

S. lavandulifolia is popularly claimed as an abortifacient agent by Iranian women; the effect of its extract on fertility control, however, had not previously been systematically investigated. This study was designed to provide scientific evidence to show the abortifacient potential of the hydroalcoholic extract of *S. lavandulifolia* (different doses) in pregnant mice.

Many mechanisms such as stimulation of uterus constriction and harmonic mechanisms may cause abortion. The work of Hajhashemi et al. (2006) showed that *S. lavandulifolia* had an antispastic effect, so, other abortifacient mechanisms should be considered in this study.

Safaei's work showed that the apigenin content of hydroalcoholic extracts of *S. lavandulifolia* is higher than in its aqueous extract and it could be claimed that the existence of this estrogenic compound in the hydroalcoholic extract is effective on abortion (Safaei, 2004).

Apigenin has a lower estrogenic activity than isoflavonoid homologues but some studies showed that its estrogenic properties are effective on abortion (Le Bail et al., 1998). This compound decreases the formation of estrogenic receptors on the surface of mouse uterus (Breinholt et al., 2000). Many studies have been done on the metabolism of these flavonoids. Romanova and coworkers injected apigenin to rats and detected it in plasma after 30 min (Romanova et al., 2000). Also, Gradolatto and coworkers (2005) investigated the apigenin metabolism after first injection. The results showed that apigenin is poorly metabolized, so that accumulation in the body is possible.

Some flavonoids have an important role in functional regulation of hypothalamus-pituitary adrenal axis. Since, ovary and uterus functions are controlled by hypothalamus-pituitary gland-gonadal axis and their discharged hormones, tissue effects and harmonic changes are possible.

Based on the direct and indirect effects of flavonoids on nervous system and neurotransmitters via receptor sensitivity and its regulation, it is possible that progesterone decreases due to receptor desensitization in corpus luteum (Rabbani et al., 2005; Butterweck et al., 2004). Elbaum and coworkers (1975) reported that the reduction of progesterone concentration to 20 % of natural quantity in mouse had no effect on fetus survival and growth.

According to the above considerations, we suggest that *S. lavandulifolia* changes the activity level of hypothalamus-pituitary-gonadal axis due to its flavonoid compounds. Also, *S. lavandulifolia* decreases progesterone concentration and leads to the disability of fetus maintenance and causes abortion.

After day 11^{th} of pregnancy, progesterone alone is not sufficient for the maintenance of pregnancy alone, and a little estradiol is needed, in addition. Estradiol requirement begins in day 11^{th} of the pregnancy and takes 3-4 days (Milligan and Cohen, 1994). In this study, the extract was injected between 7^{th} to 12^{th} days of pregnancy and since the plant metabolic rate is low, therefore, this plant extract may affect the hypothalamus-pituitary gland-gonad axis through decreasing estradiol levels.

In treated groups, the injected extract of *S. lavandulifolia* decreased the weight of

fetuses and showed that this plant has affected growth. The results of this study showed that 150 mg of the extract has more abortifacient activity than 200 mg of the extract. An explanation can be that flavonoids can be more effective in lower concentrations. This is also reflected by the decrease in length and weight of fetuses which is observed in all treated groups except for the high dose group 4.

CONCLUSION

In conclusion, this study demonstrated that hydroalcoholic extract of *S. lavandulifolia* has a dose dependent abortifacient activity. Therefore, the use of *Stachys lavandulifolia* during pregnancy may cause abortion and consequently the plant should be considered contraindicated or be used with caution.

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