Original Article

The effect of the aqueous extract of Orchid roots on the serum concentration of progesterone and luteinizing hormone in adult female rats

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Abstract

Introduction:
Orchid roots are commonly used in traditional medicine as an energizing drug and sexual enhancer. The present study was conducted to investigate the effect of the aqueous extract of Orchid roots on the serum concentrations of progesterone and luteinizing hormones in female rats.

Materials and Methods:
The present experimental study was conducted on 40 adult female Wistar rats randomly divided into 5 groups of equal sizes, including the negative control group (receiving no drugs), the sham control group (receiving 1 ml of distilled water) and experimental groups 1, 2 and 3 (receiving 20, 40 and 80 mg/kg aqueous extract of Orchid roots, in respective order). The extract was injected intraperitoneally to the experimental groups for 28 days. At the end of the experimental period, blood samples were taken from the rats to examine their serum levels of progesterone and LH ($p<0.05$).

Results:
Injecting the aqueous extract of Orchid roots to the experimental groups at 40 and 80 mg/kg doses increased the serum levels of progesterone and LH significantly compared to in the negative and sham control groups ($p <0.05$).

Conclusion:
The aqueous extract of Orchid roots can have a positive effect on ovulation in females through increasing their serum concentrations of progesterone and LH.

Keywords: Orchid, Progesterone, Luteinizing Hormones, Rat

Introduction

Fertility and infertility count as the most complicated issues in medical sciences (1). Nearly 13% of the world's population is infertile and approximately 35% of infertilities are rooted in the female reproductive system (2). Medicinal plants and traditional medicine are used experimentally in the treatment of many diseases. Considerable attention has been devoted in recent years to the study of the effects of different plants on fertility in

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laboratory mammals and these studies have produced valuable information on the subject (3).

Orchid or moorland spotted orchid (Lancibracteata [C.koch] Renz Dactyl-orhiza) with the ancient name of Orchis maculata L. belongs to the Orchidaceae family and has different species that grow almost all across the world. The tubers of this plant tend to be harvested in early summer and preserve their medicinal properties for up to two years (3 and 4). This plant contains ingredients such as glucomannan fiber, nitrogen materials, starch, protein, sugar, hydroxylb-enzaldehyde, ferulic acid, quercetin, daucosterol, cirsilineol and steroids (5&6). In traditional medicine, Orchid is prescribed as a treatment for chest injuries and disorders, gastrointestinal disorders, tuberculosis, diarrhea, Parkinson's disease, cancer and fever, and more importantly, as an energizing, performance enhancement and sexual enhancement drug also used for the treatment of erectile dysfunction. This plant is also used in ice cream, beverage and confectionery industries (7&8). Several studies conducted on the effect of Orchid on the male reproductive physiology have reported the plant's positive effects on male reproductive factors (9-11); however, no studies have investigated the effect of this plant on female reproductive factors. The present study was conducted to investigate the effect of the aqueous extract of Orchid roots on the pituitary-gonadal axis hormones and oogenesis in adult female rats.

Materials and Methods

Sampling and extraction

Orchid samples were collected in early summer from around the city of Yasouj. After washing and removing any soil, the tuberous roots of the plants were dried in the shade in the laboratory and then powdered by an electric mill. The produced powder was mixed with ethanol 96% at a rate of 5 times the volume of the plant and stirred well in a Roto-Mix mixer for 24 hours at room temperature to produce a uniform solution. After filtering the solution, it was dried for 48 hours under ambient conditions to turn into a solid alcohol-free extract. The solid extract was dissolved (at doses of 20, 40 and 80 mg) in 1 mL of double distilled water and refrigerated until use (12).

Animal Groupings

The present study observed all the ethical principles of maintaining and working with laboratory animals. The study was approved by the ethics committee of Jahrom University of Medical Sciences under 2991/p/d dated 4 March, 2014. The present experimental study examined 40 adult male Wistar rats with an average weight of 200-180 g. The rats were kept in the Animal Care Facility of Jahrom University of Medical Sciences for a week in order to adapt themselves to the environment.

The rats were kept in a 12-12 light dark cycle at a room temperature of 20-25 °C and were allowed free access to food and water at all times. The rats were randomly divided into 5 groups of 8, including a negative control group that received no substances, a sham control group that received intraperitoneal injections of 1 ml of distilled water per body weight, and experimental groups 1, 2 and 3, which received intraperitoneal injections of 20 mg/kg (minimum dose), 40 mg/kg (medium dose) and 80 mg/kg (maximum dose) per body weight of the aqueous extract of Orchid roots for 4 weeks.

Blood collection and hormonal testing

At the end of the study, the rats were weighed, anesthetized with ether and their blood samples obtained directly from their heart using a 5 cc syringe. The blood samples were centrifuged at 3000 rpm for 15 min and their serum was then frozen at -20 °C until testing. Rat ELISA kits (made by BioVendor, the Czech Republic) were then used to measure the

rats' luteinizing hormone (LH) and progesterone levels.

Statistical analysis

Data were analyzed using the one-way ANOVA. Since the Kolmogorov-Smirnov test showed the distribution of the data to be normal, parametric tests were used for their analysis. The Duncan test was used to determine the difference between the means whenever there was a statistically significant difference between the groups. Statistical analyses were performed in SPSS-18 and the level of statistical significance was set at $P<0.05$. The results obtained are expressed as Mean ± SEM. Excel was used to draw the figures.

Results

The effect of Orchid root extract on serum LH concentrations

There were no statistically significant differences in serum LH concentrations between the negative and sham control groups. Serum LH concentrations showed a significant increase in the experimental groups receiving the medium and maximum doses of the aqueous extract of Orchid roots compared to in the control groups ($P<0.05$); however, no such difference was observed between the control groups and the experimental group receiving the minimum dose of the extract (Figure 1).

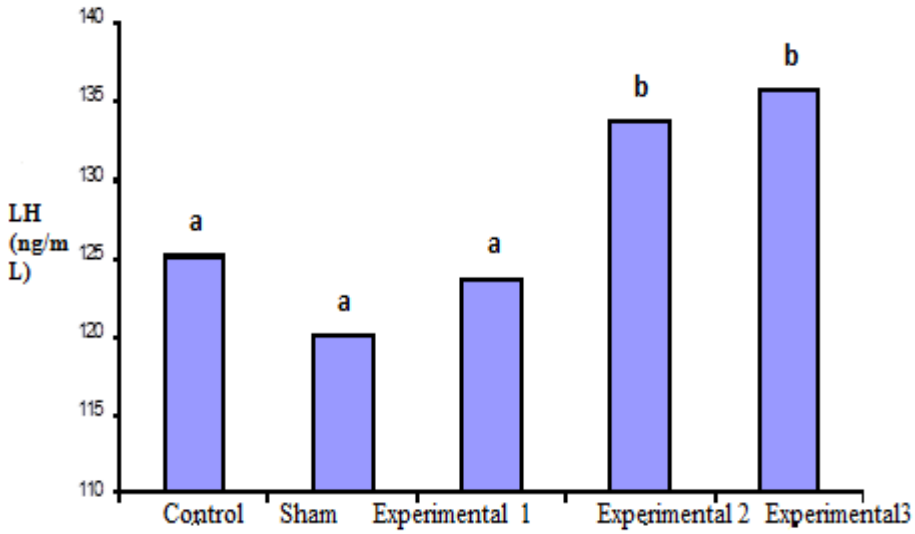

Figure1: Comparing the changes in serum LH concentrations between the experimental groups receiving different doses of Orchid extract and the negative and sham control groups. According to the Duncan test, means with a different letter were significantly different.

The effect of Orchid root extract on serum progesterone concentrations

There were no significant differences in serum progesterone concentrations between the negative and sham control groups. The serum concentrations of this hormone increased significantly in the experimental groups receiving the medium and maximum doses of the aqueous extract of Orchid roots compared to in the control groups ($P<0.05$). A significant difference was also observed between the two experimental groups receiving the maximum and medium doses of the extract; however, no differences were observed between the group receiving the minimum dose of the extract and the negative and sham control groups (Figure 2).

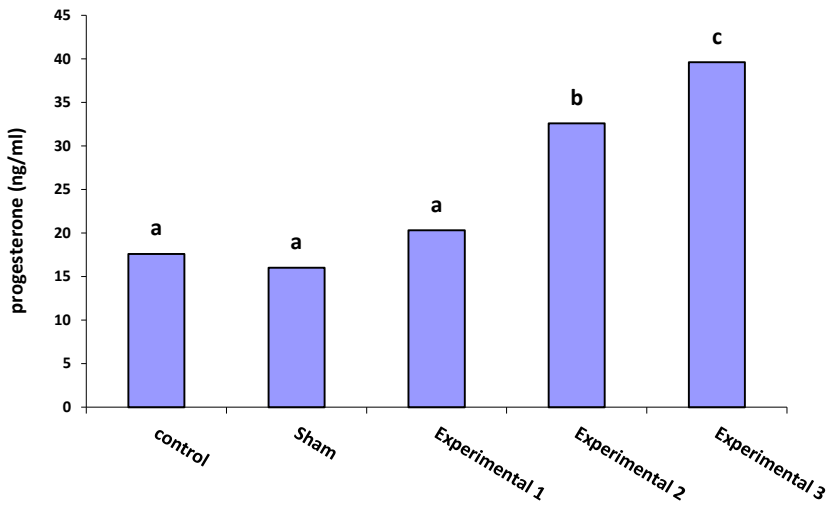

Figure 2: Comparing the changes in serum progesterone concentrations between the experimental groups receiving different doses of Orchid extract and the control and sham groups. According to the Duncan test, means with a different letter were significantly different.

Table 1: Results of the Kolmogorov-Smirnov test

	LH	PROGESTERON
Kolmogorov-Smirnov Z	0.550	1.035
P-Value	0.923	0.234

Table 2: Comparing serum LH and progesterone concentrations between the experimental groups receiving different doses of Orchid extract and the negative and sham control groups.

Group / Variable	Negative Control	Sham Control	Experimental Group 1	Experimental Group 2	Experimental Group 3
Progesterone (Ng/ml)	17.6±1.1a	16.03±0.78a	20.3±1.9a	32.6±1.4b	39.6± 2.5 C
(IU/L)LH	125.1±1.4	120.2 ±2.04	123.8±1.6a	123.7±1.8b	13.7±2.1b

Discussion

The results of the present study showed that all the three doses of the aqueous extract of Orchid roots increase serum LH and progesterone concentrations in female rats.

Other studies have also reported an increase in serum LH concentrations with the administration of the aqueous extract of Orchid roots. Studies conducted by Thakur et al. and Faraj et al. on the effect of the aqueous extract of Orchid on the hypothalamic-pituitary gonadal axis in male subjects have also shown that the aqueous extract of the roots of this plant improved spermatogenesis and boosted sexual stamina through increasing LH and progesterone concentrations (9&12).

The increase in LH (a gonadotropin) is potentially caused by three substances in the Orchid roots, including quercetin, daucosterol (a natural phytosterol-like compound derived from beta Sitosterol) and cirsilineol, which can produce estrogen (13&14). Estrogen affects LH concentrations by enabling the self-inhibition of gamma-aminobutyric acid neurons in the preoptic area. These

neurons decrease LH through negative feedback. In other words, the inhibition of gamma-aminobutyric acid neurons is expected to increase LH; to conclude, when estrogen is present and gamma-aminobutyric acid neurons are inhibited, LH concentrations increase (15-17).

A study conducted on the effect of Orchid root extract on body weight showed that the plant extract increases serum concentrations of leptin and decreases food intake in rats (18). Leptin has a major role in controlling the release of LH from the pituitary gland (19) through increasing nitric oxide in the hypothalamus and the pituitary gland (20); increasing leptin concentrations can therefore be another mechanism used for increasing the release of LH through the administration of Orchid extract.

The ovarian cycle consists of two phases; first, the follicular phase, in which the follicles mature and get ready to release an egg, and second, the luteal phase, in which the corpus luteum forms and ultimately regresses (21). In the luteal phase, LH binds with its receptor in the theca cells of the corpus luteum and stimulates the production of progesterone (21&22). The increase in progesterone concentrations in this study was due to the effect of Orchid extract on the luteal phase, as ovulation increases with the serum concentrations of LH and progesterone.

In vitro and in vivo studies have shown that using insulin and insulin-like growth factor 1 stimulate the synthesis of progesterone in the ovarian luteal cells (23&24). The ferulic acid and quercetin present in Orchid root extract contribute to the increase in insulin and IGF-1 concentrations (25&26), which can then be effective in stimulating the synthesis of progesterone.

Progesterone is synthesized from cholesterol through an enzymatic reaction in two stages. First, cholesterol is converted to pregnenolone in the mitochondrion catalyzed by the cholesterol side chain cleavage enzyme, cytochrome P450. Pregnenolone then leaves the mitochondrion and is transformed into progesterone in the endoplasmic reticulum by 3 beta-hydroxysteroid dehydrogenase and is then instantly released through diffusion due to the impossibility of its storage in the body (27). Despite the large amount of steroids in Orchid roots and the presence of of cytochrome P450 enzymes in the quercetin content of the root, this enzyme is potentially able to convert large amounts of cholesterol to progesterone and thus contribute to a significant increase in progesterone concentrations (14).

Conclusion

The aqueous extract of Orchid roots can have a positive effect on ovulation in females through increasing their serum concentrations of progesterone and LH. Further studies are recommended be conducted on the effective compounds of Orchid root extract on the ovarian tissue and the process of ovulation.

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Conflicts of Interest

The authors have no conflicts of interest to declare with regard to the compilation or publication of the present research.

References:

1. Oliva A, Spira A, Multigner L. Contribution of environmental factors to the risk of male infertility. Hum Reprod 2001;16(8):1768-76.
2. Aryanpur M, Heydari Gh, Tarahomi M, et al. Prevalence of tobacco smoking among infertile couples in Tehran.J Reprod Infertil 2009;9(4):20-3.

3. Parandin R,Ghorbani R, Sadeghipour Roodsari HR. Effects of Alcoholic Extract of Achillea Millefolium Flowers on Fertility Parameters in Male Rats. J Shahid Sadoughi Univ of Med Sci 2011:19(1): 84-93.
4. Kashani HH, Malekzadeh Shiravani S, Hoshmand F. The effect of aqueous extract of Salep prepared from root-tubers of Dactylorhiza maculate (Orchidaceae) on the testes and sexual hormones of immature male mice. J Med Plants Res 2012; 6(24):4102-4106.
5.Cozzolino S, Widmer A. Salep diversity: an evolutionary consequence of deception? Trends Ecol Evol 2005; 20(9): 487-494.
6. Baronelumaga MR, Cozzolino S, Kocyan A. Exine micromorphology of Salepinae (Salepoideae, Salepaceae): phylogenetic constraints or ecological influences? Ann Bot 2006; 98(1): 237-244.
7. Farhoosh R, Riazi A. A compositional study on two current types of salep in Iran and their rheological properties as a function of concentration and temperature. Food Hydrocolloids 2007; 21(3): 261-265
8. Tekinsen KK, Guner A. Chemical composition and physiocochemical proprtiies of tubera salep produced from som Prchidacea species. Food Chem 2010; 121(2): 468-471.
9. Thakur M, Dixit VK. Aphrodisiac activity of Dactylorhiza hatagirea (D.Don) Soo in male Albino rats. Evid Based Complement Altern Med 2007; 4(Suppl 1): 29-31.
10. Thakur M, Chauhan NS, Bhargara S, et al. A comparative study on aphrodisiac activity of some ayurvedic herbs in male albino rats. Arch Sex Beha 2009; 38(3): 1009-15.
11.Thakur M, Thompson D, Connellan P, et al. Improvement of penile erection, sperm count and seminal fructose levels in vivo and nitric oxide release in vitro by ayurvedic herbs. Andrologia 2011; 43(4): 273-7.
12. Faraji Z, Nikzad H, Parivar K, et al. The effect of aqueous extract of Salep Tubers on the structure of testis and sexual hormones in male mice. J Jahrom Univ Med Sci 2013; 11(1): 71-76.[Persian]
13. Lund TD, Lephart ED. Dietary soy phytoestrogens produce anxiolytic effects in the elevated plus-maze. J Brain Res 2001; 913: 180-4.
14. Bruce J. Grattan Jr. Plant Sterols as Anticancer Nutrients: Evidence for Their Role in Breast Cancer. Nutrients 2013; 5: 359-387
15.Jereny P. Spencer E. The intraction of flavonoids within neural signaling pathways. Rev 2007;3:257-73.
16.Bown R. Gonadotropins LH and FSH. Endocrinol Biol 2004;5: 720-8.
17. Edward J. Wagner, Oline K, et al.Estrogen Biphasically Modifies Hypothalamic GABAergic Function Concomitantly with Negative and Positive Control of LuteinizingHormone Release. J Neurosci 2001; 21(6):2085–2093.
18. Hajiani M, Kargar Jahromi H , Kargar Jahromi Z,et al . Study on the effect of 4-weeks treatment of the aqueous extract of the roots of Salep plants on the serum concentration of leptin hormone and body weight in male rats. J Jahrom Univ Med Sci 2015;12(4). [Persian]
19.Khodaii H, Chamani M, Sadeghi A, et al. Effects of conjugated linoleic acid on mouse factors and hormones in the process of ovulation in miceintint. J Fertil 2009; 2: 101-9.
20.Squires Ey. Applied animal endocrinology. 1st ed. Cambridge: CABI; 2003.
21. Gayton A. Medical Physiology: Endocrinology and Reproduction. 2nd.Tehran: Teymourzadeh Publ; 2006: 867-75.
22.Zamiri MJ. Physiology of reproduction. Tehran: Haghshenas Pub; 2005.
23-Armstrong DG, Gong JG, Gardner JO, et al. Steroidogenesis in bovine granulosa cells: the effect of short-term changes in dietary intake. Reprod 2002; 123:371–378.
24. Butler ST, Pelton SH , Butler WR. Insulin increases 17 β-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. Reprod 2004; 127:537–545.
25. Asgary S, Naderi GA, Zadegan NS, et al.The inhibitory effects of pure flavonoids on in vitro protein glycosylation. J Herb Pharmacother 2002; 2(2):47-55.
26. Kaningahom H, Lono G,Blovm V,Translation Ghazi Gahani B, Mansoori aghazi M, Pregnancy and parturition viliamz, 2 nd. Tehran: Publicment golban. 2005:95-102.