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The Effect of Palm Pollen Extract on Polycystic Ovary Syndrome (POS) in Rats

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ABSTRACT

The positive effects of Palm pollen extract on the potential of fertility and folliculogenesis process in female genus have been reported in several studies. So, this is study is aimed to evaluate the effects of Palm pollen extract on induced- polycystic ovary syndrome (PCOS) in rats. A number of 48 adult female Wistar rats were divided in this experimental study into 6 groups of 8 each. Control group: the rats in this group were kept in a normal condition until the end of the experiment. They did not receive any substances. Sham group: sesame oil (the solvent of estradiol valerate) was injected to the rats in this group at the first day of the experiment.After 60 days, they were given distilled water (the solvent of Palm pollen) per the body weight for 21consecutive days. 4 mg estradiol valerate in 2 ml sesame oil was injected intramuscularly to the rats in the other groups. Moreover they experienced the special conditions of each group, including: PCOS 1 control group: the rats in this group were killed after 60 days. PCOS 2 control group: the rats in this group were killed after 81 days. The experimental 1 group: after 60 days, the rats in this group were orally administered 200 mg/kg of Palm pollen extract for 21 days. The experimental 2 group: after 60 days, the rats in this group were orally administered 400 mg/kg of Palm pollen extract for 21 days. At the end of the experiment period, blood samples were obtained to evaluate serum levels of FSH, LH, estrogen and progesterone. The ovaries were also removed and studied with light microscopy after weighing and cutting of the tissues. LH and estrogen levels in the PCOS group + 400 mg/kg of Palm pollen extract showed a significant decrease compared to the PCOS group ($p<0.05$). FSH and progesterone levels in the PCOS group + 400 mg/kg of Palm pollen extract showed a significant increase compared to the PCOS 1 and PCOS 2 groups ($p<0.05$). A decreased number of cystic follicles and an increased number of primary, antral and graaffian follicles as well as corpus luteum werealso seen in the PCOS group + 400 mg/kg of Palm pollen extract compared to the PCOS group. Palm pollen extract can improve tissue symptoms and adjust the levels of sex hormones in polycystic ovary syndrome.

Keywords: Palm Pollen, Polycystic Ovary Syndrome, Rat

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common hormonal disorders in women in childbearing ages, which includes 5 to 10 percent of disorders in these ages [1]. Polycystic ovary syndrome is seen in 75% of women who are infertile due to the lack of ovulation. Among the important symptoms of the syndrome can note to lack of ovulation, infertility, increase of weight gain, polycystic ovaries, dysfunction in the hypothalamus and pituitary (increased LH levels and decreased FSH levels) glands, increased production of androgens and many other identifying factors [2, 3]. Type 2 diabetes, high blood pressure and cardiovascular diseases are also considered as the long-term complications of the syndrome [4]. The main goal in the treatment of PCOS is restoringnormal ovulation and ultimately restoring of the fertility. Treatment of the syndrome includes low-calorie diet, exercise, medication (spironolactone, glitazones, clomiphene and metformin) therapy as well as surgery [5].

Evidence suggests medicinal plants have been taken into consideration from years away for treatment of various diseases; so thatnowadays due to adverse complications and side effects of synthetic medicines on the body, the subject of many researches over the world is still investigation on therapeutic effects of various plants.

Palm tree is a plant that its fruits and its various parts are used for different subjects. The plant is native to the tropical regions such as Africa and Saudi Arabia, and of course, it is planted in Iran in some regions (e.g. in Fars, Khuzestan, Kerman and Sistan and Baluchestan provinces and in the other central areas). Palm pollen ingredients contain various vitamins (e.g. vitamin A, E and C)as well as elements (e.g. zinc, copper, selenium, cobalt, iron, nickel and manganese); it also contains essential and non-essential amino acids, fatty acids, flavonoids, sterols, estradiol, estrone, beta sitosterol and cholesterol [6, 7].

Palm pollen in traditional medicine has been recommended to treat infertility and it was used in ancient Egypt to treat infertility in women. In addition, results of recent studies have also indicated the effectiveness of Palm pollen on female reproductive process. Results of study of El-Desoky et al. on mice that had removed their ovaries showed that Palm pollen increases significantly the levels of estradiol and progesterone hormones and also a non-significant increase was observed in the levels of LH and FSH hormones [9]. Results of study of Moshtaghi et al. on the effects of Palm pollen extract on the levels of sex hormones in female rats showed that Palm pollen seed increases the levels of estrogen and progesterone hormones [10].

Therefore according to the effectiveness of Palm pollen on female reproductive system, the current study isconducted to evaluate the effects of Palm pollen extract on polycystic ovary syndrome in rats.

MATERIALS AND METHODS

48 female Wistar rats aged 2-3 months and weighing 180-200 g were included in this experimental study. A week before beginning of the experiment, the rats were transported to the laboratory to be compatible with the laboratory conditions. The animals were kept in special cages with standard space and under proper environmental conditions with ambient temperature $22\pm1^{\circ}C$ and 12-hour dark-light cycle with free access to daily food and water. To conduct the experiment, the rats that had 2 to 3regular estrous cycles during a period of 12 to 14 days of monitoring their vaginal smearwere selected while the subjects were in estrous phase of their reproductive cycle. Hormonal induction method with estradiol valerate was used for induction of PCOS; so that a dose of 4 mg estradiol valerate in 0.2 ml sesame oil was injected intramuscularly into the groin (behind the leg muscle) of the selected rats for a once. After the injection, daily vaginal smear test was prepared to observe and monitor variation of estrous cycle and its irregularity and toachieve to stable persistent vaginal cornification (PVC)stage, which is one of the symptoms of follicular cysts in ovaries. Induced PCOS by estradiol valerate lasted in aperiod of 60 days.

The control group: the rats in this group were kept in a normal condition until the end of the experiment. They did not receive any substances.

The sham group: based on the body weight, sesame oil (the solvent of estradiol valerate) was injected to the rats in this group at the first day of the experiment; after 60 days, they were given distilled water (the solvent of Palm pollen) per the body weight for 21consecutive days.

After ensuring formation of polycystic ovary, PCOS were randomly divided into 4 groups of 8 each:

PCOS 1 control group: the rats in this group were intramuscularly injected once with 4 mg of estradiol valerate dissolved in 0.2 ml of sesame oil; the PCOS rats were killed after 60 days.

PCOS 2 control group: the rats in this group were intramuscularly injected once with 4 mg of estradiol valerate dissolved in 0.2 ml of sesame oil; the PCOS rats were killed after 81 days.

The experimental 1 group: the rats in this group were intramuscularly injected once with 4 mg of estradiol valerate dissolved in 0.2 ml of sesame oil and the animals were then orally administered 200 mg/kg of aqueous extract of Palm pollen [10] for 21 days.

The experimental 2 group: the rats in this group were intramuscularly injected once with 4 mg of estradiol valerate dissolved in 0.2 ml of sesame oil and the animals were then orally received 400 mg/kg of aqueous extract of Palm pollen [10] for 21 days.

The rats were anesthetized at the end of 3 weeks and blood samples were taken. Blood serum was then isolated and sent to laboratory for measuring the serum levels of estrogen, progesterone, LH and FSH hormones using special kits by enzyme-linked immunosorbent assay (ELISA) method. The ovaries were removed after dissection, placed in formalin (10%) and sent to laboratory for preparation of tissue sections. At least three fields of each slide were then studied under light microscopy using a calibrated graticule. The number of primary, preantral, antral and graaffian follicles as well as corpus luteum and the number of cystic follicles were counted and the mean was determined.

One-way analysis of variance (one-way ANOVA) was used to analyze the data. To understand the differences between means, Duncan test was used in cases that statistical differences of various groups were significant. Statistical analysis was performed using SPSS version 21 and $P<0.05$ wasconsidered as the significance level. The data in the results were calculated and compared as Mean±SEM.

RESULTS

The mean weight of ovaries in all PCOS groups had a significant increase compared to the control group. However, the mean weight of ovaries in the experimental 1 and 2 groups had a significant decrease compared to the PCOS 1 control and PCOS 2 control groups.

Estrogen levels in the PCOS 1 control and PCOS 2 control groups had a significant increase compared to the control group. However, estrogen levels in the experimental 1 and 2 groups had a significant decrease compared to the PCOS 1 control, PCOS 2 control and control groups.

Progesterone levels in the PCOS 1 control and PCOS 2 control groups had a significant decrease compared to the control group. However, the levels in the experimental 1 and 2 groups had a significant increase compared to the PCOS 1 control and PCOS 2 control groups.

LH levels in all PCOS groups had a significant increase compared to the control group. However, LH levels had a significant decrease in the experimental 1 and 2 groups compared to the PCOS 1 control and PCOS 2 control groups.

FSH levels in PCOS 1 and PCOS 2 control groups had a significant decrease compared to the control group. However, FSH levels had a significant increasein the experimental 1 and 2 groups compared to the PCOS 1 control and PCOS 2 control groups.

The average number of primary, preantral, antral and graaffian follicles and corpus luteum reduced in all PCOS groups compared to the control group. However, the number of the follicles and corpus luteum increased in the experimental 1 and 2 groups compared to the PCOS 1 control and PCOS 2 control groups.

Table 1- the average of the research variables in different groups of the test

Variables / Groups	Ovary weight (mg)	Primary follicles	Preantral follicles	Antral follicles	Graaffian follicles	Cystic follicles	Corpus luteum
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control group	41.4±3	5.1±1.2	3.4±0.5	3.8±0.5	3.5±0.5	0±0	3.5±0.7
Sham group	35±5	4.4±1.5	3.3±0.4	3.7±1	3.8±1.3	0±0	3.7±1.1
PCOS 1 control group	104.3±18*	3.7±1.1*	2±0.8*	0.28±0.5*	0±0*	1.7±0.7*	0±0*
PCOS 2 control group	121.4±12*	4±1.7*	1.9±0.8*	0.43±0.5*	0±0*	1.9±0.7*	0±0*
Experimental 1 group (200 mg)	77.1±12*	5.3±1.4	2.4±1	1±0.8*	0.58±0.2*	1±0.6*	0.57±0.05*
Experimental 2 group (400 mg)	68.6±3*	5.9±1.5	2.9±0.75	2.7±0.8	1.1±0.6*	0.17±0.09	0.85±0.6*

The asterisk represents the difference between the groups with the control group.

Table 2- the average levels of estrogen, progesterone, LH and FSH hormones in different experimental groupsof the test

Variables / Groups	Estrogen (ng/l)	Progesterone (ng/l)	LH(IU/l)	FSH(IU/l)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control group	241.6±18	115±5.4	5±1	7.6±1.3
Sham group	239.5±20	113.9±3.5	5±1.2	6.8±1.8
PCOS 1 control group	253.1±21	81.4±6.7*	8.5±0.5*	4.5±0.6*
PCOS 2 control group	263.4±20	79.5±5.9*	8.7±1.2*	4.6±0.64*
Experimental 1 group (200 mg)	236.5±13	101.7±4.8	7.5±1.2*	5.9±0.67*
Experimental 2 group (400 mg)	224.4±14*	109.8±7.7	7.8±0.6*	7.1±0.92

The asterisk represents the difference between the groups with the control group.

The average number of cystic follicles increased in all PCOS groups compared to the control group. However, the number of the follicles reduced in the experimental 1 and 2 groups compared to the PCOS 1 control and PCOS 2 control groups.

DISCUSSION

Based on the results of the current study, estradiol valerate-induced polycystic ovary syndrome increases LH and estrogen levels and on the other hand decreases progesterone and FSH levels compared to the control group. In addition to the hormonal changes listed for PCOS group, increased number of primary, preantral, antral and graaffian follicles and corpus luteum was reported in this study. This is consistent with results of other studies in this area. A significant increase in the number of cystic follicles and apoptotic granulosa cells in rats receiving estradiol valerate was reported in the researches of Ghasem Zadeh et al. (2013) and Tahmasebi et al. (2015) [11, 12]. However, a sudden increase in LH (LH surge) levels is an essential process forovulation, but steady rise of the hormone in PCOS causes the formation of antral follicles without ovulation and thus produces cystic follicles [13]. When the ratio of LH to FSH levels increases, the ovaries increase preferentially the synthesis of androgens. The levels of insulin and insulin-like growth factors (IGFs) are also increased in PCOS, which increase androgen synthesis in follicular sheath cells and as a result improve LH function [14].

The results of this study also showed that oral administration of Palm pollen extract can improve the induced-PCOS symptoms. It seems that reduction of PCOS symptoms by consumption of Palm pollen extract is related to reduced levels of LH and estrogen as well as increased levels of FSH and progesterone hormones, since reduced number of cystic follicles as well as increased number of corpus luteum was observed in the experimental groups treated with Palm pollen extract as a result of the hormonal changes. This reflects return of normal process of folliculogenesis and ovulation in PCOS rats.

The positive effects of Palm pollen extract on folliculogenesis process was reported in a study performed by Hosseini et al. They showed that a dose of 400 mg/kg of Palm pollen extract increases the number of secondary and antral follicles and also increases the levels of ovarian hormones in rats [15]. Stimulatory effects of Palm pollen on ovary have related by researches to the presence of compounds such as glycosidal flavonoids, saponins, alkaloids and steroidal compounds [15, 16, 17].

In addition to the positive effects of establishment of hormonal balance between gonadotropin and ovarian hormones in improving PCOS symptoms, administration of various kinds of antioxidants such as vitamin E and selenium is also considered as another common treatment approach to treat this syndrome [18, 19]. Palm pollen contains a variety of natural antioxidants including different kinds of vitamins and minerals such as zinc and selenium [6, 7]. The results of researches indicated that levels of reactive oxygen species (ROS) in ovarian tissue are increased in polycystic ovary syndrome and the balance between oxidant and antioxidant system is disturbed in this condition [20]. Natural growth of Theca interstitial layer is necessary for normal ovarian function and oxidative substances and free radicals impair regular growth and apoptosis in this layer [21]. It has been known as there is a direct relationship between reduced oxidative stress and increased maturation of oocytes in women with PCOS and infertile women [22]. So antioxidants can improve PCOS symptoms by reduction of oxidative stress [23].

Total Conclusion:

The hormonal and histological results of this study showed that Palm pollen extract can improve PCOS symptoms. Reduced number of cystic follicles and increased number of corpus luteum, which possibly represent the restarting process of ovulation, are related to antioxidant properties of the extract.

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