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Comparison of ginseng extract and metformin on improvement of Polycystic Ovary Syndrome (POS)

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Abstract
Today, the global approach for treatment of many diseases, particularly diseases related to survival of generation and infertility is toward use of medicinal plants instead of consumption of chemical drugs. So, this study will compare the effect of metformin and ginseng extract on improvement of polycystic ovary syndrome based on changes in LH, FSH, estrogen, and progesterone hormones. 63 adult female rats were used in this research and divided into 9 groups of 7 each as healthy control (without receiving any substance), patient control (estradiol valerate), patient sham (estradiol valerate + distilled water), healthy sham (distilled water), experimental1 (estradiol valerate + metformin), experimental2 (estradiol valerate + ginseng), experimental3 (estradiol valerate + metformin + ginseng), experimental4 (estradiol valerate + ginseng), and experimental5 (estradiol valerate + metformin + ginseng). The results showed that simultaneously dose-dependent use of metformin and ginseng has better effect on improvement of hormonal changes in polycystic ovary syndrome.

Keywords: Ginseng, Metformin, Sexual Hormones, PCOS

1. Introduction
Inability to get pregnant for a year without any contraceptive method is called infertility [1, 2]. Approximately 85-90% of healthy young couples become pregnant within a year and 10-15% of them suffer from infertility. Approximately 43% of infertile couples seek treatment and just 24% of them require special cares [1, 2]. Finally, almost ovulation can be induced in all infertile women with anovulation [1, 3]. Half of the infertility cases are due to ovulatory dysfunction of ovaries [1, 2]. Polycystic Ovary Syndrome (PCOS) is one of the most important causes of ovulation disorders [3, 4]. This syndrome, which its clinical symptoms have widely range, is the most common endocrine disorder in women [1, 2]. Metformin belongs to anti-diabetic drugs family such as phenformin and buformin that is prepared from French lilac (French lile) or goat's rue (galea officinali) [5]. Metformin is sold under various brands names such as: Riomet, Glucophage, Diabex, Dianben, Gluformin, Obimet, Fortamet Glumetza, and Diaformin. Metformin was first used for diabetes type 2, but increasingly used in treatment of multi-cystic ovarian syndrome [6]. Use of metformin in individuals with PCOS was first reported in a small study which performed at University of Andes in Venezuela [7].
Ginseng is a plant which its name is derived from a Greek word meaning treatment of all diseases (Pana kos) [8]. Ginseng is an aromatic, perennial medicinal plant related to *panax* genus and *Araliaceae* family (Ivy) and is common in traditional medicine of Asian countries [10]. The medicinal properties of ginseng plant are related to its roots. The root of the plant contains triterpene saponins, essential oils, alkaloids, aminoglycosides, poly acetylene, polysaccharides, peptidoglycan, nitrogen compounds, sesquiterpenes, fatty acids, carbohydrates, and phenolic compounds. Chemical and pharmacological investigations have performed on this plant [11, 12]. The main active component of the plant is ginsenosides (saponins) which has triterpenoid structure and pharmacological activities of ginseng plant relates to these compounds [13]. Given to the importance of fertility and polycystic ovary role in infertility as well as different effects of metformin and ginseng on various diseases; as well as medicinal plants applications for reducing consumption of chemical medicines in treatment of diseases, the present study is performed with the purpose of metformin and ginseng extract effects on improvement of polycystic ovary syndrome.

2. Material and Methods

The study was performed from October 2014 to March 2015. All ethical use of laboratory animals has been respected in this study according to Shiraz University Animal Welfare Committee guidelines. A number of 63 adult female Wistar rats aged approximately from 7 to 8 weeks and weighing approximately 170-200 g were used in this experiment, and they were purchased from Breeding and Maintenance Center of Laboratory Animals in University of Shiraz Medicinal Sciences. All the adult female rats should be placed on one sexual cycle, to do experiments on them. The estrous cycle in female rats is lasted 4 up to 5 days. Since, the used rats in this experiment were in different phases of estrous cycle, a method should be adopted so that all rats to be placed in one phase of estrous cycle. Vaginal smears were prepared from all rats to make in phase their sexual cycles and to ensure that they were all in one phase of estrous cycle.

2.1 Induction Method of Polycystic Ovary

The hormonal induction method with estradiol valerate was used in the study. Animals were selected that after daily vaginal esmear test, had 2 to 3 consecutive and regular period of estrous cycle within 12 to 14 days. A dose of 4 mg/kg body weight of estradiol valerate, which was solved in 2 mg of sesame oil as a solvent, was intramuscularly and once injected to groin at abdominal area of rats in all groups except the healthy control [14, 15]. The vaginal smear test was obtained daily, after ensuring induction of polycystic ovary syndrome in the experimental groups and after achieving to the stage of sustained horny vaginal smear [16, 17].

2.2 Preparation of Ginseng Extract

At first the roots of the plant were powdered and the percolation method was used for extraction. Thus, the dried powder was poured in cylindrical portion of percolator device, which was filled up to two-third with alcohol (80%) and the rest with water. The valves of the device were closed, when the first solution had been exited from the last valve and after passing of 24 hours, they opened again and the extract collected. Then it was dried at a temperature of 40-30 ˚C under a microbe-free environment [18].

The rats were randomly divided into 9 groups of 7 each following ensuring of presence of polycystic ovaries in the subjects:

Healthy control group: the rats were kept in normal conditions without receiving of any substances.

Patient control group: the rats were daily and intramuscularly received 0.2 ml of water and alcohol within 28 days, following the injection of estradiol valerate and formation of ovarian cysts.

Patient sham group: the rats were daily and intramuscularly received 0.2 ml of water and alcohol within 28 days, following the injection of estradiol valerate and formation of ovarian cysts.

Healthy sham group: the rats were daily and intramuscularly received 0.2 ml of water and alcohol within 28 days.

Experimental group 1: the rats were daily and intramuscularly received metformin drug at a dose of 100 mg/kg within 28 days, following the formation of ovarian cysts.

Experimental group 2: the rats were daily and intramuscularly received ginseng at a minimal dose of 100 mg/kg within 28 days, following the formation of ovarian cysts.

Experimental group 3: the rats were daily and intramuscularly received metformin at a dose of 100 mg/kg and ginseng at a dose of 100 mg/kg within 28 days, following the formation of ovarian cysts.

Experimental group 4: the rats were daily and intramuscularly received ginseng at a maximal dose of 200 mg/kg within 28 days, following the formation of ovarian cysts.

Experimental group 5: the rats were daily and intramuscularly received metformin at a dose of 100 mg/kg and ginseng at a dose of 200 mg/kg within 28 days, following the formation of ovarian cysts.

After the end of the experiment and following the anesthesia blood samples were directly taken with a 5 cc syringe from animals' hearts and abdomen areas in order to measure the levels of LH, FSH, estrogen, and progesterone hormones. The results were analyzed using SPSS software and one-way analysis of variance (ANOVA) for comparison between groups followed by Duncan's test for multiple comparison between various groups were used in the study.

3. Results

The obtained results from measuring of FSH hormone among various groups show that there was a significant reduction in patient control, patient sham and the other experimental groups compared to the healthy control.

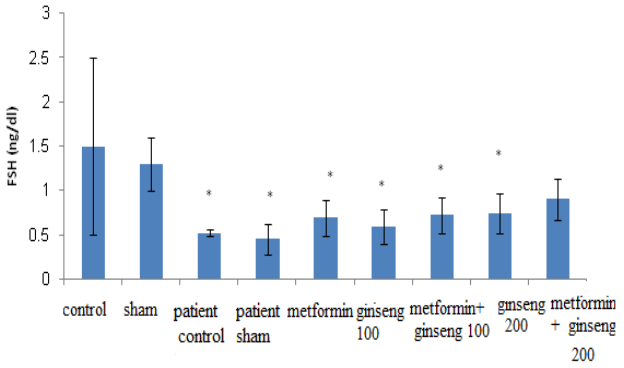

Chart 1: related changes to FSH hormone

The obtained results from measuring of LH hormone show that there was no significant difference in experimental groups compared to the healthy and patient control groups.

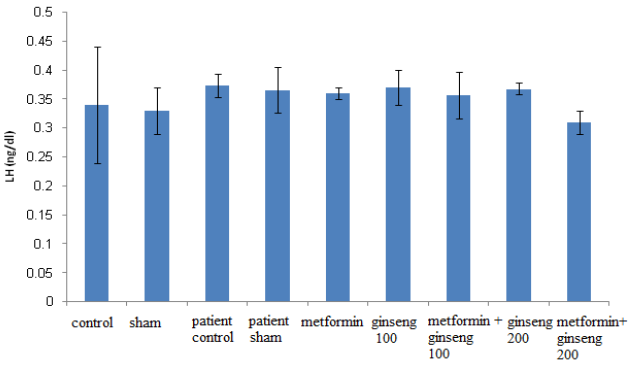

Chart 2: related changes to LH hormone

The obtained results from measuring of estrogen hormone among different groups show that there was a significant reduction in patient control and patient sham groups compared to the healthy control group. The ginseng groups, which received 100 mg/kg and 200 mg/kg of the substance, had a significant reduction compared to the healthy control group. The metformin group, which received 100 and 200 mg/kg of metformin with ginseng, had a significant increase compared to the patient control group.

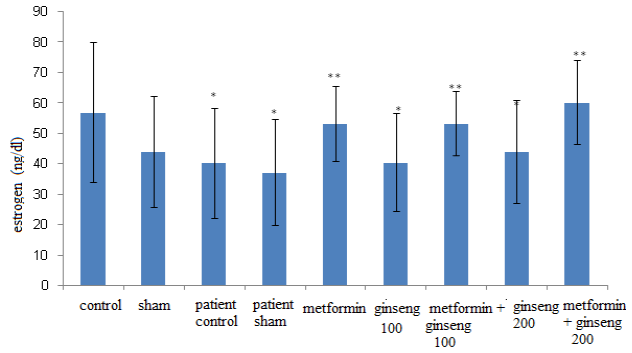

Chart 3: related changes to estrogen hormone

The obtained results from measuring of progesterone hormone among different groups show that there was a significant reduction in patient control and patient sham groups compared to the healthy control group. Also the metformin group, which received 200 mg/kg of metformin with ginseng, had a significant increase compared to the healthy and patient control groups.

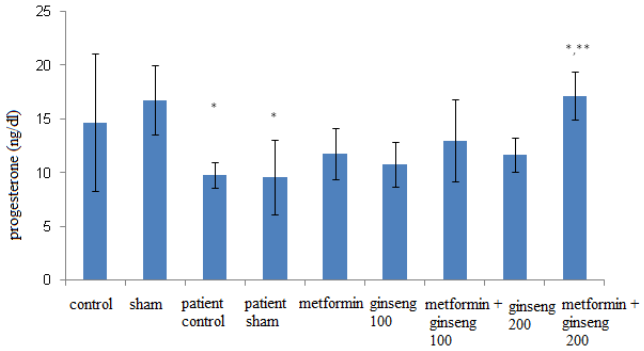

Chart 4: related changes to progesterone hormone

* indicates a significant difference compared to healthy control group
** indicates a significant difference compared to patient control group

4. Discussion

Studies have found that changes in level of sexual hormones are one of the important diagnostic criteria in polycystic ovary syndrome. The levels of testosterone, estradiol, and LH hormones increase and FSH level decreases in women with this syndrome, however, sometimes the level of FSH does not change [19]. Studies expressed that hyperandrogenism and increased serum levels of LH and testosterone hormones are common in this disease [20, 21]. In general, gonadotropins are glycoproteins that release from pituitary gland in response to gonadotropin releasing hormones. Pulsatile release of GnRH leads to pulsatile production of LH and FSH hormones. The response of LH and FSH hormones to the activity depends on phase of menstrual cycle. So, the phase of menstrual cycle complicates the research about these hormones responses to physical activity. Reduction of body fats that can cause changes in secretion of LH and FSH is another case that creates this complexity [22]. Investigations found that FSH levels at the end of follicular phase are essential for creation of mono follicular growth [23, 24]. Due to increase of sensitivity of follicles to gonadotropin, the growth and multiple follicles development, which is the incidence factor of OHSS and multiple birth syndromes, occurs in patients with polycystic ovarian [25]. Studies found that metformin has a significant impact on the level of blood sexual hormones as well as on the number of fetuses in rats with polycystic ovary syndrome [26]. Consumption of metformin for treatment of this syndrome was first reported by Velazquez. The results of this application is improvement of sensitivity to insulin, reduction of LH, free and total testosterones and increase of FSH and attached sexual hormones to globulin in obese women with polycystic ovary syndrome [27]. Studies also showed that metformin reduces the levels of estradiol, LH and insulin in not overweight women with PCO [28]. The reduction of FSH and progesterone levels was also observed in the groups which received metformin, in the present study. This reflects the positive effects of metformin on improvement of the disease; and agrees with the results of previous researches.

Investigations found that increase of LH levels in hypophysectomized adult rats, which were treated with pregnant mar's serum gonadotropin, is needed for final stages of follicular development [29]. Improvement in secretion of gonadotropin hormones is observed in the ginseng groups. This means that an increase in secretion of these hormones was observed in ginseng groups compared to the patient control group.

It was reported in past studies that use of ginseng extract increase the level of FSH and LH in experimental groups. These results agree with the results of the present study. However, related changes to LH hormone were not significant. Ginseng has also found to have effect on anterior pituitary and on release of nitric oxide, which applies the activity due to the presence of ginsenosides (saponins) compounds. The active molecule (nitric oxide) increases gonadotropins secretion and sexual activity [30]. Except in ovaries, the enzymes producing nitric oxide can be found in total of hypothalamus-pituitary-gonadal axis [31]. Nitric oxide in ovaries is affected by gonadotropins; but effect on them in hypothalamus [32]. The location of neurons producing nitric oxide is in vicinity of GnRH neurons in hypothalamus [33]. It seems that nitric oxide also influences on ovarian arteries. So, by increase of nitric oxide the secretion of LH and FSH is also increased and vice versa [34]. Nitric oxide by influencing on pituitary gland triggers release of LH and FSH hormones and as stated, ginseng extract increases the secretion of nitric oxide, which probably improves polycystic ovary syndrome.

The studies have found that by daily consumption of 1000 mg metformin for 6 month, only 8% of significant improvement was observed in menstrual cycle [36]. As in the present study, changes in metformin groups have been toward polycystic ovary syndrome treatment.

Investigations determined that metformin reduces androgen secretion from ovaries and adrenal glands. Therefore, metformin is an effective medicine for patients with polycystic ovary syndrome, especially in those who are resistant to clomiphene.

Studies also showed that metformin can directly and indirectly influence on uterine blood flow and can be assumed that metformin influences on permeability of uterus septum and reduces androgen and thus applies its tightening effect on vascular tissues [37-40]. In the current study, the levels of progesterone and estrogen hormones were reduced in the patient control group. But increase in concentration of these hormones was observed in metformin groups. Also, an increase in concentration of progesterone and estrogen hormones was observed in ginseng groups. Some studies stated that serum levels of progesterone are increased by ginseng extract [41], which probably related to the gonadotropins negative feedback mechanism and to secretion

of progesterone and estrogen hormones. The results also showed that consumption of high dose of ginseng extract and metformin is more effective in polycystic ovary syndrome treatment.

5. Conclusion

Studies showed that simultaneous use of ginseng extract and metformin medicine is more effective in polycystic ovary syndrome treatment. Metformin as a chemical medicine has a better effect on treatment of polycystic ovary syndrome. And the properties of ginseng extract are due to the presence of effective ingredients.

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