



Capacity of *Mentha spicata* (spearmint) Extract in Alleviating Hormonal and Folliculogenesis Disturbances in Polycystic Ovarian Syndrome Rat Model

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ABSTRACT

Polycystic ovary syndrome, a common cause of infertility among women in the reproductive age, is associated with high levels of androgens. Recognizing the anti-androgenic effects of spearmint, the present study aimed to evaluate the effects of its hydroalcoholic extract on the levels of luteinizing hormone, follicle-stimulating hormone, and testosterone and ovarian folliculogenesis in normal and letrozole-induced polycystic ovary syndrome rats. Female mature rats were divided into six groups (n=8 per group), as follows: Normal rats (I or Control), normal rats which received 250 mg/kg spearmint extract (II) or 500 mg/kg spearmint extract (III), and PCOS-induced rats (IV), PCOS-induced rats which received 250 mg/kg spearmint extract (V), or 500 mg/kg spearmint extract (VI). At the end of the experiment the animals were euthanized, and then mentioned parameters were evaluated. Administration of spearmint extract to PCOS rats resulted in a decrease of body weight and testosterone level, higher corpus luteum, and lower ovarian cysts and atretic follicles, compared to PCOS rats which received no spearmint. Accordingly, the spearmint can attenuate polycystic ovarian syndrome-related problems, such as a high testosterone level and ovarian cysts.

Keywords: Folliculogenesis, *Mentha spicata*, Ovary, PCOS, Rat

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common hormonal disorder among women in the reproductive age. It has been demonstrated that genetic factors, hormonal disorders, lifestyle, environmental factors and stress contribute to the development of this syndrome (Shaikh et al., 2014a; Krishnapillai et al., 2015). PCOS is often associated with a high level of androgen hormones, obesity, insulin resistance and oligomenorrhea or anovulation (Hatirnaz et al., 2015). Since a high level of androgens is considered the essential factor in PCOS, the animal model of PCOS is frequently created by androgenizing of animals (Van Houten and Visser, 2014). Medicinal plants have been traditionally used as natural medications, and played vital roles in disease prevention and their promotion (Monsefi and Masudi, 2014; Sharangouda et al., 2015; Namavar Jahromi et al., 2019). *Mentha spicata*, known as spearmint, is a medicinal plant which in Iran's traditional medicine is mainly recommended for digestive system disorders as a carminative and antispasmodic agent, and also for alleviating hirsutism and menstrual pain (Vejdani et al., 2006). The antioxidant, anticancer, anti-inflammatory, antifungal, antimicrobial, and antidiabetic properties of *Mentha spicata* have been shown in some studies (Guimarães et al., 2011; Alae et al., 2016).

It is known that spearmint is beneficial in decreasing free testosterone level and hirsutism in women with mild hirsutism with PCOS, and its adverse histopathological effects on kidney, liver and uterine tissue in animals were observed (Akdogan et al., 2003; Akdogan et al., 2004; Akdogan et al., 2007; Guney et al., 2006; Grant, 2010). The effects of this herbal plant on folliculogenesis of ovarian tissue in normal and PCOS conditions were not determined. Present study was designed to evaluate the effects of spearmint extract on follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone hormones and ovarian folliculogenesis in the animal model of PCOS induced by letrozole.

MATERIALS AND METHODS

Preparation of *Mentha spicata* hydroalcoholic extract

Mentha spicata was purchased from Pursina Pharmaceutical Company, Tehran, Iran. Hydroalcoholic extract was prepared using the maceration method (Monsefi et al., 2015). The dried leaves of the plant were powdered and

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macerated in ethanol for 3 days. Then the solution from the total extract was filtered with filter paper, concentrated by evaporation and stored in refrigerator until being used for the experiments. The yield (w/w) of the solution was 13% (g/g).

Animals

Forty-eight mature Wistar albino female rats were obtained from animal house of Shiraz University of Medical Sciences, Shiraz, Iran. Prior to use in the study, rats were kept in cages in temperature-controlled rooms with constant humidity and 12 hr/12 hr light/dark cycle with free access to standard diet and water. For selection of rats with normal estrus cycle, daily vaginal smears were carried out and immediately evaluated with a light microscope (Monsefi et al., 2013). Wistar female rats with two normal estrus cycles were weighed and allocated into the six groups (n: 8) as below; Group I (control): Received 1 ml distilled water orally for 20 days; Group II: Received spearmint extract (250 mg/kg) for 20 days; Group III: Received spearmint extract (500 mg/kg) for 20 days; Group IV: Received letrozole orally for 28 days; Group V: Received letrozole orally for 28 days, and then received spearmint extract (250 mg/kg) for 20 days; Group VI: Received letrozole orally for 28 days, and then received spearmint extract (500 mg/kg) for 20 days. Polycystic ovary syndrome induction was carried out by treating rats daily with letrozole (Femara®, made by Novartis Pharmaceuticals Corp., Basel, Switzerland) orally (1 mg/kg) for 28 days, and confirmed by persistent estrus phase and high number of ovarian cysts in ovarian sections via hematoxylin and eosin staining (Neisy et al., 2019).

After treatment duration, animals were weighed, euthanized by inhalation of ether, and a blood sample was taken for hormonal analysis. In addition, the ovarian tissues of all rats were removed and prepared for the histological evaluation (Sadeghi et al., 2017).

Hormonal assay

Blood samples were collected from the heart, and were centrifuged at 3000 rpm for 15 min. Serum portions were separated and frozen until being evaluated. Serum concentrations of testosterone (Padtan Elm Company, Tehran, Iran), LH and FSH were measured with their specific kits (Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China) (Sadeghi et al., 2017).

Histological analysis

Ovarian tissues were removed, fixed in 10% buffered formalin solution, and the paraffin blocks were prepared. The blocks were sectioned at 5µm thickness, and were stained in the hematoxylin and eosin method (Alaee et al., 2014). The number of primordial follicles, primary follicles, secondary follicles, Graafian follicles, atretic follicles, corpus luteum and cysts were counted in ovarian sections using a light microscope (Olympus, Japan).

Statistical analysis

Statistical analysis was performed using SPSS 16 software (IBM, Armonk, USA). For data analysis, the One-Way ANOVA test was used, followed by the Tukey test to compare the means. P value of ≤ 0.05 was considered statistically significant.

Ethical approval

The study protocol was approved by the Animal Ethical Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1396.S1066), and was carried out in accordance with the university's Guideline for the Care and Usage of Laboratory Animals.

RESULTS

The results are presented in two parts; A: Evaluation of the effects of spearmint extract on normal female rats (comparing groups I, II and III). At the end of the experiment, the body weight of the animals that received spearmint extract was not different from that of the control group ($p > 0.05$, table 1). The level of LH, FSH and testosterone did not change among spearmint extract-administered groups in comparison to the control group ($p > 0.05$, table 1). Administration of spearmint extract in two doses of 250 and 500 mg/kg (groups II and III) led to a significant decrease in the number of primordial follicles ($P < 0.001$). In addition, the number of primary follicles, secondary follicles, Graafian follicles and corpus luteum decreased in these groups compared to the control group, but it was not statistically significant ($p > 0.05$). However, the number of atretic follicles was significantly higher in the groups administered spearmint extract compared to the control group ($p < 0.001$, table 2). B: Evaluation of the effects of spearmint extract on PCOS-induced rats comparing with groups C (control), IV, V and VI. At the end of the study, the body weight in the PCOS-induced group was significantly higher than those in the control group ($P: 0.031$).

At the end of the experiment, in both PCOS-induced groups that received spearmint extract, the rats' weights were not different from that of the control group ($P > 0.05$). In the PCOS-induced group that was administered high doses of spearmint extract, weights were significantly less in comparison to those of PCOS-induced rats ($P: 0.001$, table 1). The level of LH and FSH showed no significant alteration among control, PCOS-induced groups and PCOS-induced groups that received spearmint extract ($p > 0.05$), but the level of testosterone in the PCOS-induced group was significantly higher in comparison to the control group ($p < 0.001$). In PCOS-induced groups that took two doses of spearmint extract, testosterone level was significantly lower in comparison to the PCOS-induced group ($p < 0.001$, table 1).

The number of primordial follicles was significantly lower in the PCOS-induced group, and in the PCOS-induced groups which received spearmint extract compared to control group ($P < 0.001$). The number of primary follicles was not different in PCOS-induced rats by comparing to the control group and the PCOS-induced rats which took spearmint extract ($P > 0.05$). The number of secondary follicles was meaningfully lower in the PCOS-induced group and those which received spearmint extract as compared to the control group ($P: 0.033$). There were no Graafian follicles in the PCOS-induced group, and in those which had spearmint extract, but this was not statistically significant comparative to the control group ($P > 0.05$). The number of atretic follicles and cysts was considerably higher in the PCOS-induced rats in contrast to the control group ($P < 0.001$), and in the PCOS-induced group compared to PCOS-induced groups which received spearmint extract ($P < 0.001$) (Figure 1). The number of corpus lutea in PCOS-induced rats was also meaningfully lower than the control group ($P: 0.001$), and its number increased significantly in PCOS-induced rats who took the spearmint extract (250 and 500 mg/kg) in comparison to PCOS-induced rats ($P < 0.001$).

Table 1. Body weight at the beginning and end of the experiments, and the level of luteinising hormone, follicle stimulating hormone and testosterone of female rats in studied groups

Groups	Weight at the beginning (g)	Weight at the end (g)	LH (ng/dl)	FSH (ng/dl)	Testosterone (mIU/ml)
(I) Control	152.37 ± 10.04	199.25 ± 13.54	23.00 ± 1.90	15.80 ± 1.30	0.40 ± 0.09
(II) Extract (250 mg/kg)	158.00 ± 12.66	207.87 ± 17.23	22.50 ± 1.70	13.41 ± 2.04	0.29 ± 0.08
(III) Extract (500 mg/kg)	160.37 ± 13.00	195.12 ± 12.71	22.90 ± 1.9	14.90 ± 2.29	0.30 ± 0.06
(IV) PCOS	158.12 ± 9.17	223.00 ± 4.035* †	24.70 ± 1.50	13.86 ± 0.60	3.70 ± 0.90* † ‡
(V) PCOS + Extract (250 mg/kg)	154.12 ± 7.19	210 ± 19.27	23.00 ± 1.80	13.23 ± 0.43	1.27 ± 0.43
(VI) PCOS+ Extract (500 mg/kg)	151.75 ± 5.99	190.25 ± 17.63	22.40 ± 1.40	14.90 ± 1.20	1.05 ± 0.2

Data are shown as mean ± SD. $P \leq 0.05$ is considered statistically significant. *: Significant differences between PCOS-induced group (IV) and control group. †: Significant differences between PCOS-induced group (IV) and group VI. ‡: Statistically significant differences between PCOS-induced group (IV) and groups V and VI.

Table 2. The number of primordial, primary, secondary, Graafian and atretic follicles, corpus lutea and cysts in ovarian tissue of the studied groups

Groups	Primordial follicles	Primary follicles	Secondary follicles	Graafian follicles	Atretic follicles	Corpus luteum	Ovarian cysts
(I) Control	7.81±3.22* † ‡	8.62±1.08	5.56±1.09 [§]	0.31±0.40	2.12±1.14* †	5.93±1.52 [†]	0 [†]
(II) Extract (250 mg/kg)	3.12±1.99	7±2.06	4.18±2.07	0.25±0.44	10.06±3.60	4.93±1.84	0
(III) Extract (500 mg/kg)	0.87±0.61	7.18±1.79	4.65±2.06	0.43±0.72	7.18±4.47	4.37±1.70	0
(IV) PCOS	2.93±1.34 [†]	7.37±1.50	3.31±1.50	0	12.56±1.96 [*]	1.31±1.07 [*]	10.18±3.01 [*]
(V) PCOS + Extract (250 mg/kg)	2.12±1.08	6.43±1.78	2.25±1.73	0	7.93±2.73	4.06±1.98	0.18±0.54
(VI) PCOS + Extract (500 mg/kg)	2.18±2.1	8.00±1.15	2.5±1.54	0	7.56±2.73	5.18±1.79	0.12±0.34

Data are shown as mean ± SD. $P \leq 0.05$ is considered statistically significant. *: Significant differences between group II, III and control group. †: Significant differences between PCOS-induced group (IV) and control group. ‡: Significant differences between control and groups V and VI. §: Significant differences between control and groups IV, V and VI. *: Significant differences between PCOS-induced group (IV) and groups V and VI.

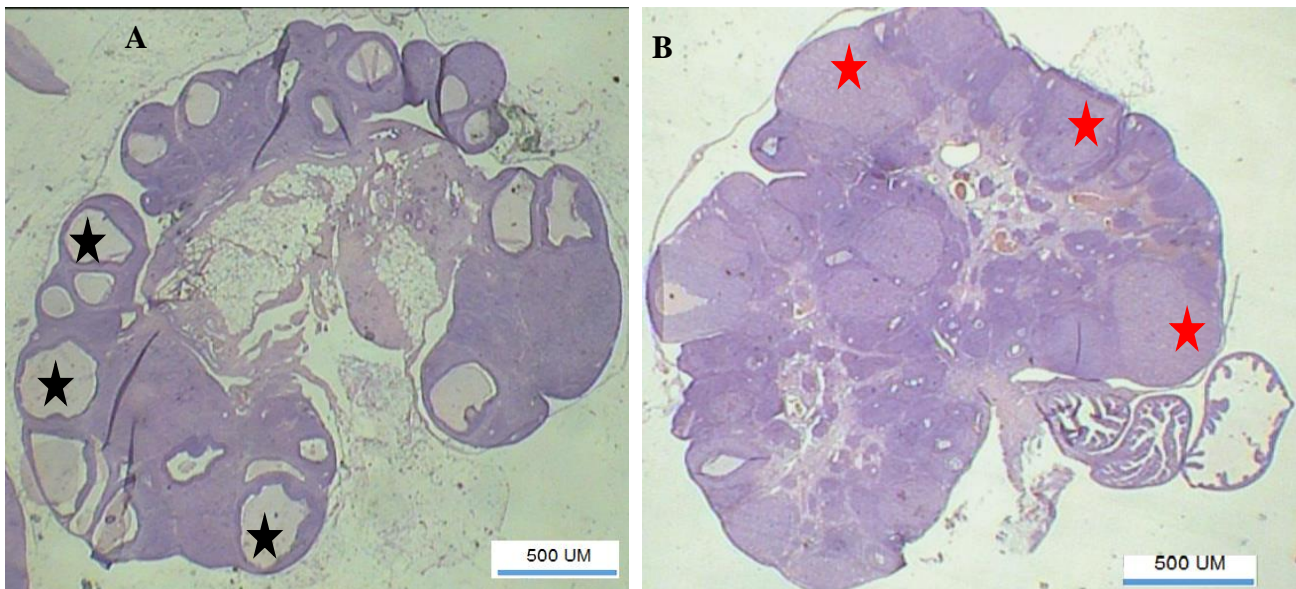


Figure 1. Light photomicrograph of ovarian tissue of the rats. Hematoxylin and eosin staining, 40× magnification. **A:** Ovarian tissue of polycystic ovary syndrome rats displays a considerable number of ovarian cysts. **B:** Ovarian tissue of polycystic ovary syndrome rats received spearmint extract (500 mg/kg) demonstrates no ovarian cyst. Treatment with spearmint extract has increased corpora lutea in this group compared with the polycystic ovary syndrome group. The red stars show corpora lutea, and the black stars show cystic follicles.

DISCUSSION

Nowadays, infertility is an important major concern of many couples which affects both men and women. PCOS is a condition that affects a woman's hormone levels that may lead to infertility related problems. Women with PCOS tend to have higher levels of androgens (Akdogan et al., 2007; Alaei et al., 2019). According to anti-androgenic effects of *Mentha spicata* and its beneficial effect in women with mild hirsutism and PCOS (Akdogan et al., 2007; Grant, 2010), in the current study, spearmint extract was administered to an animal model of PCOS to determine the effects of this herbal plant on LH, FSH and testosterone hormone levels, and also on folliculogenesis of ovaries. In addition, the effects of the extract on mentioned parameters were studied in normal rats. Measurement of body weight changes was one of the established methods to evaluate the toxicity of plant extracts (Gupta and Sharma, 2006). Since there was no significant change in the body weight of normal rats after administration of spearmint extract, it seemed that this medicinal plant has no general toxicity effect, which was also confirmed in other studies (Nozhat et al., 2004; Sadeghi et al., 2017). Furthermore, the spearmint at the level of administered doses had no effect on LH, FSH and testosterone levels in normal rats. Given the significant increase in the number of atretic follicles in normal rats which received spearmint extract, this agent may have detrimental effects on the ovarian folliculogenesis. Other studies have also demonstrated detrimental effects of spearmint tea on uterine, kidney and liver (Akdogan et al., 2003; Akdogan et al., 2004; Guney et al., 2006). Androgens are essential drivers of early and intermediate stages of follicular maturation. Locally produced androgens facilitate follicular development and serve as a substrate for estrogen production in the later stages of folliculogenesis (Pan et al., 2015). The optimum level of this hormone is crucial, because an excess level of androgens overrides follicular development, resulting in follicular arrest, follicular atresia and disturbance of ovulation (Gleicher et al., 2011). Therefore, the higher number of atretic follicles may be attributed to anti-androgenic effects of spearmint, but it should be determined whether the levels of testosterone and the number of corpus lutea, which are a manifestation of ovulation, did not decrease in the normal rats receiving spearmint extract. The disruption of follicular development in these groups may be related to the alteration in the level of other kinds of androgens that were not evaluated in our study, such as dihydrotestosterone and androstenedione, which are also involved in the growth and development of ovarian follicles in mammals (Cupisti et al., 2008; Lebbe and Woodruff, 2013). PCOS is a metabolic disease usually accompanied by insulin resistance, visceral obesity and elevated body mass index, all of which are correlated with an elevated level of oxidative stress and androgen production of the ovaries and adrenal glands, thus it contributes to the disturbed follicular development, oocyte maturation, and, ultimately, infertility (Shaikh et al., 2014b; Hussain et al., 2015; Papalou et al., 2016). The results showed that administration of spearmint extract to PCOS rats significantly reduces body weight and testosterone level.

In the present study, the weight did not change in normal rats that received spearmint extract. Current study found that spearmint has no effect on body weight in a normal condition. However, in PCOS condition, it may control and sustain body weight which may occur thorough metabolic mechanisms that were disturbed in the condition of PCOS.

Therefore, spearmint extract may initiate a complicated mechanism that results in control of body weight, and also reduction of testosterone. It was demonstrated that spearmint leaves decrease cholesterol, and in type II diabetes, decrease oxidative stress, and improve activity of antioxidant enzymes (Rajeshwari et al., 2012). Al-Rekabi (2015) showed that administration of phenolic compounds of *Mentha spicata* leaves extract to diabetic male rats can significantly enhance the antioxidant defense system, and reduce body weight and levels of glucose and cholesterol. Grant (2010) showed that administration of spearmint tea for 30 days significantly reduced free and total testosterone in PCOS women. A significant decrease in free testosterone level was also observed in women with hirsutism after receiving spearmint teas (Akdogan et al., 2007). It was shown that in the PCOS condition, ovarian steroidogenic enzyme deficiencies, such as aromatase deficiency, induced a hyperandrogenic environment in the ovary, contributing to follicular maturation arrest and oligoovulation or anovulation (Rajeshwari et al., 2012). Reducing the body weight of anovulatory obese women decreased testosterone concentration, restored ovulation, and improved menstrual function and conception rates (Moran et al., 2003). However, in this study, although administration of spearmint led to a significant decrease in the body weight and testosterone level of PCOS-induced rats, the attenuated number of follicles caused by PCOS induction was not improved. Yet, similar to an identical study in which the effects of spearmint oil on PCOS were evaluated, the number of corpus luteum increased after the spearmint extract was administered, reflecting the higher rate of ovulation in this group (Sadeghi et al., 2017). The high number of atretic follicles and ovarian cysts observed in PCOS-induced rats was thought to be associated with a high level of androgen. In PCOS animals that took spearmint extract, the number of atretic follicles and ovarian cysts decreased considerably, which could be associated with the anti-oxidant and anti-androgenic effects of the spearmint.

CONCLUSION

Spearmint as an anti-androgenic herb is believed to reduce testosterone level in PCOS condition, resulting in destruction of ovarian cysts and restoration of ovulation. It is suggested for future studies to evaluate ovarian antioxidant capacity and apoptosis status, and also fertility potential of PCOS-induced female rats after receiving spearmint extract at the level of mentioned dose used in this study.

DECLARATIONS

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Competing interests

The authors declare that there are no conflicts of interest.

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