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Integrative Medicine Research

journal homepage: www.imr-journal.com

Original Article

Licorice ethanol extract improves symptoms of polycystic ovary syndrome in Letrozole-induced female rats

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ARTICLE INFO

Article history:

Received 4 May 2018

Received in revised form

18 May 2018

Accepted 25 May 2018

Available online xxx

Keywords:

Glycyrrhizae radix et rhizoma

Letrozole

LH/FSH ratio

Licorice

Polycystic ovary syndrome

ABSTRACT

Background: Licorice (*Glycyrrhizae radix et rhizome*, GRR) has long been used as an ingredient in Korean traditional medicinal herbal formulas for various metabolic and reproductive diseases. Polycystic ovary syndrome (PCOS) is a common endocrine disorder in premenopausal women. In the present study, we examined the effects of GRR extract on PCOS-like symptoms in female rats.

Methods: Symptoms of PCOS were induced by Letrozole treatment for 4 weeks in 6-week-old female SD rats, after which the effects of GRR extract on recovery of normal hormonal levels and polycystic ovaries were assessed. Serum levels of luteinizing hormone (LH), follicular-stimulating hormone (FSH), LH/FSH ratio, and follicular cysts were evaluated, followed by the expression levels of known follicular phase markers such as *Kitl*, *Cyp11a1*, and *Ptgs2*.

Results: The serum level of FSH was reduced only in the Letrozole treatment group (PCOS), whereas significant recovery of FSH level was observed in the Letrozole and GRR co-treatment group (PCOS + GRR). Serum LH levels were not altered in any of the groups. Furthermore, the LH/FSH ratio (known biomarker for PCOS) was elevated only in the Letrozole treatment group (PCOS), whereas it was significantly reduced in the Letrozole and GRR co-treatment group (PCOS + GRR). For histological changes, follicular cysts, antral follicles, and increased thickness of the theca- and granulosa layers were observed in the PCOS group, whereas these alterations were remarkably reversed by GRR treatment.

Conclusion: These results suggest that GRR extract inhibits the symptoms of PCOS by regulating imbalanced hormonal levels and irregular ovarian follicles.

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<https://doi.org/10.1016/j.imr.2018.05.003>

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1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common causes of hyperandrogenism, infertility, and anovulation, and in women, which affects at least 5–10% of women of reproductive age.¹ PCOS is characterized by irregular menses, miscarriage, dysfunction of follicular maturation, hyperandrogenism and dysregulation of hormones such as luteinizing hormone (LH) and follicular-stimulating hormone (FSH), resulting in acne and hirsutism.^{1,2} At present, significant advancements in understanding the pathogenesis of PCOS have been made in addition to treatment modalities for PCOS symptoms.³

Licorice (*Glycyrrhizae radix et rhizome*, GRR) is prescribed in many Korean medicinal formulas for its potential effects in the regulation of inflammation, immune response, hepatic failure, spasms, and women's metabolic disorder.^{4,5} This herbal remedy has long been used as a medicine in ancient documents such as *Donguibogam* as well as in Oriental traditional medicine. GRR is usually harvested during spring and autumn, although autumn is typically preferred. After harvesting, its fibrous roots are removed, dried under the sun, sliced thickly, and stored in a cool place prior to use. In Korean medicine, GRR is indispensable in many medicinal herbal formulas since it is able to harmonize with as well as moderate the characteristics of other medicinal herbs.^{6,7}

To date, rodents are the most widely used animal to study PCOS due to their benefits, including smaller size, short lifespan, high reproduction rate, and variety of genetic strains.⁸ PCOS studies using experimental rat models have focused on neonatal androgenization, injection of steroidal analogs, and endocrine-disrupting chemicals such as aromatase inhibitor.³ Although there are numerous animal models available for the development of PCOS-like symptoms, no model has yet been established due to difficulties in testing the efficacies of variable substances. Androgens have been used to induce acute PCOS conditions in rodents through daily injection or s.c. implantation.⁹ Hence, re-establishment of normal reproductive and/or ovarian cycling occurs after withdrawal of androgens.¹⁰ Subcutaneous implantation includes the rat PCOS model induced by Letrozole, a non-steroidal aromatase inhibitor that blocks the conversion of androgens into estrogen.¹¹ Letrozole-induced PCOS rat models present many features of human PCOS.^{3,11} The Letrozole-induced model is ideal for the study of aromatase deficiency-induced classic PCOS, and Letrozole may be effective in co-treated with natural or medicinal substances.

Recently, herbal remedies for PCOS have received attention as a form of lifestyle management in traditional medicine clinics, in which the menstrual cycle and normal serum hormones levels can be recovered.¹² Herbal remedies are known to be effective in reducing testosterone as well as increasing FSH and 17 β -estradiol levels,^{13,14} and they have been shown to reduce polycystic ovaries and ovarian volume, improve insulin sensitivity, and normalize reproductive cycles.^{15–17} Additionally, clinical investigations have reported no adverse effects for herbal medicines.^{12,18} However, conclusive evidence regarding absolute therapy could not be obtained in these clinical

studies due to the absence of pre-clinical data explaining the mechanism of PCOS therapy.^{12,13,18}

Natural substances are becoming more common in replacing established medications for the treatment of PCOS. Considering our interest in GRR extract as an alternative medicine and therapy for PCOS, we investigated whether or not GRR extract regulates hormonal imbalances, irregular follicular phase, and abnormal histologic changes in Letrozole-induced female PCOS rats. Additionally, genetic markers for follicular phase were examined using quantitative real-time PCR in ovaries of PCOS rats.

2. Materials and methods

2.1. Plant material and reagents

The GRR was purchased from Backjaedang, an Oriental medicine pharmacy in Daejeon, South Korea. The GRR extract was prepared by adding 10 times 70% (v/v) ethanol to the dried GRR (200 g), leaching at 80°C for 3 hours, and then obtaining a GRR ethanol extract. The GRR 70% ethanol extract was filtered through a 5 μ m filter paper, concentrated by a vacuum rotary vacuum concentrator, and lyophilized using a freeze dryer. The final yield of GRR ethanol ext. was 22.41% w/w (44.85 g).

2.2. Animals

Sprague Dawley rats were obtained from Daehan Biolink (Eumseong, South Korea) and divided into three groups of six rats each. Rats were allowed to adapt to laboratory conditions (temperature: 20 \pm 2°C, relative humidity: 45 \pm 5%, light/dark cycle: 12 h) for 1 week. All animal experimental procedures were approved by the Ethics Committee of the Korea Institute of Oriental Medicine (approved no. 17-031). For the PCOS rat model, a 90-day release pellet (IRA; Innovative Research of America, OH, USA) containing Letrozole (1.8 mg/pellet) was implanted subcutaneously for 4 weeks under anesthesia [Zoletile (30 mg/kg)–Rompun (5 mg/kg)–saline mixture (2:1:2)] in 6-week-old female rats. For the acceleration of PCOS symptoms, a high dose of Letrozole (1 mg/kg) as prepared in 0.2% CMC (carboxymethyl cellulose) and administered by p.o. at 2 weeks after pellet implantation. After Letrozole (1 mg/kg) treatment, GRR extract (300 mg/kg) was prepared in 0.2% CMC and administered by p.o. treatment for 2 weeks. All rats were euthanized at 24 hours after the final injection.

2.3. Serum hormone analysis

Blood samples were collected directly from the inferior vena cava using a 1-mL syringe at the end of the experiment. Serum was obtained by centrifugation at 2000 \times g for 10 min and stored at –70°C until use. Serum luteinizing hormone (LH) and follicular-stimulating hormone (FSH) were measured using ELISA kits (Cusabio Biotech, Wuhan, China). All hormone levels were measured according to the manufacturer's instructions.

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