Original Article

The Therapeutic Effect of Methanolic Extract *Bryonia dioica* Jacq. in a Female Rat Model of Polycystic Ovary Syndrome

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases that affects 5%–10% of women of childbearing age. Several factors contribute to the development of PCOS such as dysfunction of the hypothalamic–pituitary axis and ovarian function, as well as increased insulin levels. The manifestations of the disorder include a wide range of symptoms, including menstrual disorders, acne, infertility, and increased body fat. Currently, the most well-known treatments for PCOS are clomiphene, metformin, letrozole, and tamoxifen. Due to their side effects, the identification of substitute drugs is essential. One of the traditional medicines, which is usually used in different parts of the world, particularly in Western Europe, is *Bryonia dioica* Jacq. (*B. dioica*). This plant is used in the treatment of disease due to its active ingredients like polyphenols. **Materials and Methods:** Induction PCOS in a female rat (3 weeks old) was performed through subcutaneous injection of testosterone enanthate (1 mg/100g) daily for 35 days. The effects of *B. dioica* (30 and 60 mg/kg) root methanolic extract on PCOS-induced was evaluated after 28-day treatment. On the last day, the serum levels of follicle-stimulating hormone (FSH), glucose, low-density lipoprotein/high-density lipoprotein (LDL/HDL), luteinizing hormone (LH), and testosterone and histological studies (hematoxylin and eosin [H&E] staining) were measured. **Results:** Results showed that FSH and LH levels ($P < 0.05$) as well as glucose ($P < 0.001$) in the *B. dioica* groups normalized significantly compared to the PCOS group. LDL levels decreased in rats and the LDL/HDL ratio decreased in all treatment groups. In histologic assay, metformin and *B. dioica* restricted the effects of testosterone in the ovaries of rats. **Conclusion:** The data indicate that methanolic extract of *B. dioica* recovers hormonal factors in PCOS.

Keywords: *Bryonia dioica Jacq., methanolic extract, polycystic ovary syndrome, rat, testosterone*

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disease that affects 5%–10% of women of childbearing age. This disease, which is an ovarian disorder, leads to adrenal–pituitary–hypothalamic disorder.[1,2] In this disease, the secretion of luteinizing hormone (LH) increases compared to follicle-stimulating hormone (FSH). Increased LH stimulates ovarian follicular cells and increases androgen synthesis and secretion.[3,4] Diagnosis of PCOS is based on criteria such as hyperandrogenism (clinical manifestations of androgen enhancement such as hair loss and obesity around menstruation), decreased or no chronic ovulation, and measurement of testosterone, prolactin, dehydroepiandrosterone (DHEA), and hemostasis levels. Common symptoms of PCOS include insulin resistance, hyperandrogenism, anovulation, and subsequent infertility.[4,5] The most common treatments for this disease are clomiphene, metformin, letrozole, and tamoxifen. These medications have side effects, including hot flashes, arthritis, muscle aches, and psychological side effects such as irritability, mood swings, and depression. Treatment is done to alleviate symptoms, correct sexual cycles and ovulation, restore fertility, and prevent long-term complications.[6] Due to the side effects of using many chemicals, the use of medicinal plants is recommended. The active ingredients in plants have a biological equilibrium state due to their association with other substances. These substances do not accumulate in the body and do not produce side effects, which in this regard have a significant advantage over chemical drugs.[7,8]

Bryonia dioica Jacq. (*B. dioica*) belongs to the *Cucurbitaceae* family. The Persian name of the plant is Hazar Goshan, Fashra, or Qira.

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Fashra is a perennial herbaceous plant with a height of about 3 m. This plant is fast growing and has fleshy and grooved stems, and it grows in a twisting and ascending manner. The male flowers are in clustered inflorescences and the female flowers are in umbrella inflorescences, and all flowers are yellow with green or white stripes. The root of the plant is thick and fleshy with gray skin and white and viscous inside. This plant is one of the native plants of our country and grows in Kermanshah province. In traditional medicine, various effects of this plant have been mentioned, including the effects of emetic, local analgesic, and laxative.[9,10] Antidiabetic activities, antibacterial activities, anticancer activities, and antioxidant activities are among the important biological and therapeutic effects of *B. dioica*.[10]

There are two active ingredients in this plant called bryodine and cucurbitacine, whose antitumor effects have been proven. In terms of chemical composition, the plant's root contains a bitter substance called glucoside called brionine. This glucoside is soluble in water and alcohol, and in addition, an amount of essential oil and resin called brioresin and a large amount of starch are found in the roots.[11,12] In addition, *B. dioica* contains active compounds such as alkaloids, polyphenols, saponins, and flavonoids.[13] It has been indicated that polyphenols showed protective effects in PCOS via reducing inflammation, reactive oxygen species (ROS) generation, insulin resistance, and serum level of testosterone.[14] The other active ingredient in *B. dioica* is saponins that previous studies have been shown saponins reduced PCOS symptoms by reducing nerve growth factor (NGF) expression in ovaries and brain tissues.[15]

As no study was conducted on the *B. dioica* extract in PCOS, in this study the effect of methanolic extract to of *B. dioica* root on testosterone enanthate-induced PCOS rats was examined.

Materials and Methods

Preparation of *Bryonia dioica* extract

First, the plant was collected in spring from Kermanshah province and then identified by a botanist at Razi University and compared with herbarium specimens (voucher number: 128). The plant was thoroughly washed and placed on a wire rack in a room away from direct sunlight to dry. The dried plant was powdered with a special mill and then 100 g powder are infused in 100-mL methanol 70% and the extract was prepared by soaking. After 3 days, the extract was filtered using filter paper and dried by a rotary operator.[16] The dried extract was stored in the refrigerator until use (yield 15%).

Animals

Immature female Wistar rats (21 days old) (40–70 g) were procured from the central animal house, Kermanshah University of Medical Sciences and maintained at standard conditions. All the experimental procedures were performed according to the Ethical Committee of Kermanshah University of Medical Sciences (Protocol no. IR.KUMS.REC.1399.349).

Experimental design

Androgenized Rodent Model of PCOS is used to induce PCOS.[17,18] Twenty Immature female rats (21 days old) received 1 mg/100g of testosterone enanthate (obtained from Iran Hormone Company, Tehran, Iran) subcutaneously daily for 35 days. After PCOS induction, the animals are divided into four groups containing five animals each group.

1. Negative control group: normal saline recipient, for 28 days, orally.
2. Positive control group: metformin 20 mg/kg/day for 28 days, by oral.
3. Treatment group: *Bryonia* extract 30 mg/kg/day for 28 days, by oral (B1)
4. Treatment group: *Bryonia* extract 60 mg/kg/day for 28 days, by oral (B2)

After treatment, the animals were subjected to biochemical, hormonal, and histopathological examination.

Measurement of follicle-stimulating hormone, glucose, low-density lipoprotein/ high-density lipoprotein, luteinizing hormone, and testosterone levels

On the last day of study, euthanasia was performed with an overdose of sodium pentobarbital. The blood samples were collected, and FSH, glucose, low-density lipoprotein/ high-density lipoprotein (LDL/HDL), LH, and testosterone levels were measured. The serum levels of testosterone, FSH, and LH were measured by using the mouse enzyme-linked immunosorbent assay (ELISA) kit.[19]

Histological examination of ovaries

Histopathological studies were performed in ovarian tissues. Hematoxylin and eosin (H&E) staining following the method of Earl L. Parr[20] was used for slide preparation. The samples were then viewed and evaluated under a light microscope at ×40 magnification.

Data analysis

Data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) software program, version 21.0. One-way analysis of the variance (ANOVA) and Tukey's *post hoc* test were used to statistically analyze the data and compare the means of the groups. Results were reported as mean ± standard error of the mean (SEM). A value of $P < 0.05$ was considered significant.

Results

Effects of treatment on serum levels of hormones

The effect of *B. dioica* on hormones is shown in Figures 1–3. Testosterone levels were significantly decreased in metformin group ($P < 0.001$) as well as in B1 ($P < 0.05$) and B2 groups ($P < 0.001$) as compared with PCOS group. LH and FSH levels were significantly higher in the metformin group than in the PCOS group ($P < 0.01$). *Bryonia dioica* at 30 mg/kg showed a significantly greater level of FSH and LH than the PCOS group ($P < 0.05$).

Effect of *Bryonia dioica* on serum low-density lipoprotein and low-density lipoprotein/high-density lipoprotein ratio

As shown in Figures 4 and 5, the LDL level was significantly decreased in B1 ($P < 0.05$) and B2 ($P < 0.01$) groups as compared to the PCOS group. In addition, the ratio of LDL/HDL markedly decreased in B1 ($P < 0.05$) and B2 ($P < 0.01$) groups.

Effect of *Bryonia dioica* on serum glucose

The effect of *B. dioica* on serum glucose is shown in Figure 6. The glucose levels were significantly decreased in B1 group as compared with the PCOS group ($P < 0.001$). Also serum glucose was markedly lower in B2 group than in the PCOS group ($P < 0.0001$).

Bryonia dioica restricted abnormal ovarian morphology in polycystic ovary syndrome rats

Figure 7A (normal group without induction of PCOS) shows too many corpus luteum (CL) cells—mature follicles (Graafian follicles) seen with normal granulosa cells. Numerous primary and secondary (antral) follicles. All cell's structures were normal. In the PCOS group [Figure 7B], no CL was seen. Follicular cyst with a thin layer of granulosa cells was also seen. Absence of primary follicles and mature follicles was seen. In metformin-treated group [Figure 7C],, pre-antral and primary follicles were present. The CL was seen. Granulosa cells went back to normal.

In B1-treated group [Figure 7D], relative improvement in structure of cells was observed as compared to PCOS group. The walls of the follicles and the granulosa cell layer were normal.

Graafian follicles contain ovum, and primary and secondary follicles were present, indicating normalization of the ovary. In the B2-treated group [Figure 7E], mature follicles with normal granulosa layer were observable. Ovum, primary follicles, and CL were present.

Discussion

PCOS is an intricate metabolic and endocrine syndrome described by irregular ovulation and hyperandrogenism.[21,22] In PCOS, increased production of androgens and estradiol is an early sign of abnormal steroidogenesis. Conversely, increased secretion of anti-Müllerian hormones (AMH) and LH, increased Gonadotropin-releasing hormone (GnRH) pulse rate, and decreased FSH levels are signs of the hypothalamic–pituitary–malfunctioning axis.[23] Treatment for this syndrome includes lifestyle changes, surgery, and taking some medicines, including clomiphene citrate and metformin.[24] Due to the unwanted effects of these drugs, the use of herbal medicines and their derivatives, which are less expensive, less invasive, and more valuable than other methods, has been considered.[25] As the identification of side effects of these medicines is momentous in the control of PCOS, in the various studies, the focus has been on the study of herbal medicines.[26] Having biologically active compounds and no significant side effects has caused special attention to be paid to the consumption of medicinal plants. For example,

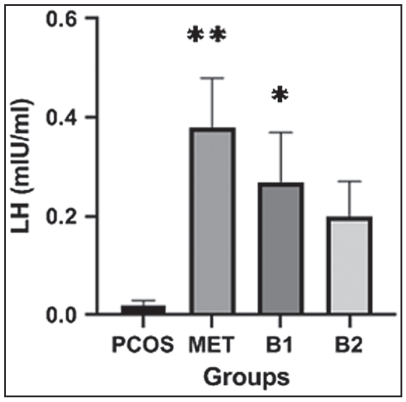

Figure 2: Serum levels of LH in PCOS rats. *$P < 0.05$ and **$P < 0.01$ vs. PCOS group. LH = luteinizing hormone, PCOS = polycystic ovary syndrome group, MET = metformin group, B1 = *Bryonia* extract group (30 mg/kg/day), B2 = *Bryonia* extract group (60 mg/kg/day)

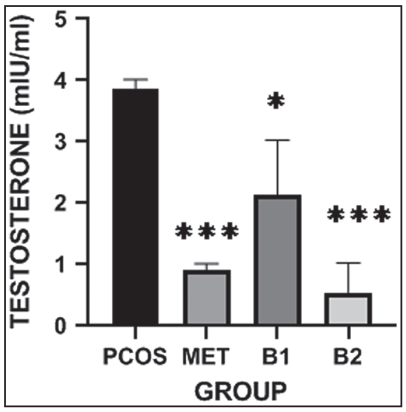

Figure 1: Serum levels of testosterone in PCOS rats. *$P < 0.05$ and ***$P < 0.001$ vs. PCOS group. PCOS = polycystic ovary syndrome group, MET = metformin group, B1 = *Bryonia* extract group (30 mg/kg/day), B2 = *Bryonia* extract group (60 mg/kg/day)

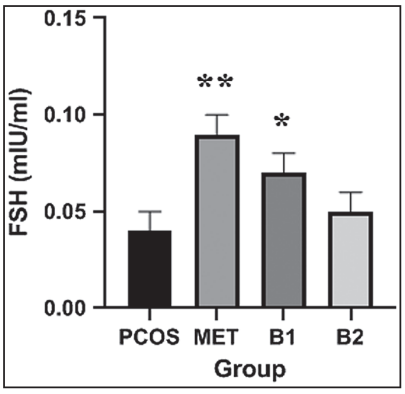

Figure 3: Serum levels of FSH in PCOS rats. *$P < 0.05$ and **$P < 0.01$ vs. PCOS group. FSH = follicle-stimulating hormone, PCOS = polycystic ovary syndrome group, MET = metformin group, B1 = *Bryonia* extract group (30 mg/kg/day), B2 = *Bryonia* extract group (60 mg/kg/day)

plants such as Red clover (*Trifolium pratense*), genistein, and soy have antiandrogenic properties due to the presence of specific phyto-estrogenic compounds such as biochanin A and daidzein, which can be used therapeutically in patients with PCOS.[27-29] It has been shown that flavonoid compounds in plants with antioxidant activity can reduce oxidative stress in ovarian tissue and reduce the number of cystic follicles in this tissue.[30] Plant antioxidants also reduced the effects of hyperandrogenism following PCOS induction in mice.[31] The antiandrogenic properties of plants also reduce the secretion of androgens by inducing negative feedback on LH. Decreased androgens lead to less LH synthesis and reduce the dominant effect of LH on FSH, which in turn may be a reason for the restart of the natural pathway of sex cycle hormones and the occurrence of ovulation in PCOS.[32,33] *Bryonia dioica* is one of the traditional medicines, which is widely used in different parts of the world, especially in Western Europe. The root of *B. dioica* is enriched in bioactive compounds such as kempferol 3, 7-di-O-rhamnoside, and polyphenols that show pharmacological effects including antioxidant activity, analgesia, hepato-protective, antihypercholesterolemia and hyperglycemia, and improving fertility disorders.[34-36] Previous studies have shown that aqueous extract of *B. dioica* in low concentrations (50 µg/mL) in MDA MB-231 cells can cause apoptosis and stop the cell cycle in the G2/M phase.[37] This study intended to establish an animal model of PCOS using DHEA. DHEA was used in some studies to induce PCOS in rats. Histological analysis of ovarian tissues clearly showed the treated cystic follicles.[37,38] The first androgen to appear in the blood of adult women is DHEA,[39] which also increases in 25% of PCOS cases.[40] DHEA administration during fertility causes complications of PCOS, such as menstrual disorders and polycystic ovaries.[41,42] The results of our research showed that *B. dioica* normalized the effect of testosterone on ovary of rats as well as the serum levels of LH and FSH. A study by Chauhan and Dixit[43] found that the ethanolic extract of *B. laciniosa* Linn. seeds increased FSH and LH levels. Our data showed that the levels of FSH and LH in the treatment group were improved compared with untreated group. In previous studies, the effect of *B. laciniosa* on glucose homeostasis has been reported in the streptozotocin (STZ) diabetic model of rats. Therefore, the antidiabetic effect of this plant can be used in the treatment of diabetic patients.[13,44] In our study, glucose levels were significantly reduced in the *B. dioica* groups (30 and 60 mg/kg) when compared with the PCOS group. Significant reductions in LDL and LDL/HDL ratio were observed in rats treated with the ethanolic extract of *B. laciniosa* and the saponin component of *B. laciniosa*.[45] In this study, *B. dioica* (60 mg/kg) decreased LDL levels in PCOS rats and the LDL/HDL ratio was decreased in all treatment groups compared to the PCOS group.

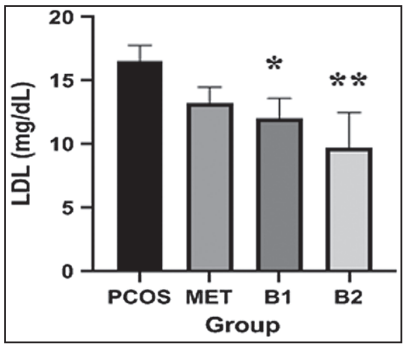

Figure 4: Serum levels of LDL in PCOS rats. *$P < 0.05$ and **$P < 0.01$ vs. PCOS group. LDL = low-density lipoprotein, PCOS = polycystic ovary syndrome group, MET = metformin group, B1 = *Bryonia* extract group (30 mg/kg/day), B2 = *Bryonia* extract group (60 mg/kg/day)

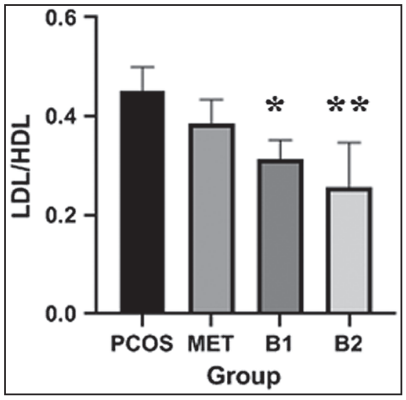

Figure 5: Serum levels of LDL/HDL in PCOS rats. *$P < 0.05$ and **$P < 0.01$ vs. PCOS group. LDL = low-density lipoprotein, HDL = high-density lipoprotein, PCOS = polycystic ovary syndrome group, MET = metformin group, B1 = *Bryonia* extract group (30 mg/kg/day), B2 = *Bryonia* extract group (60 mg/kg/day)

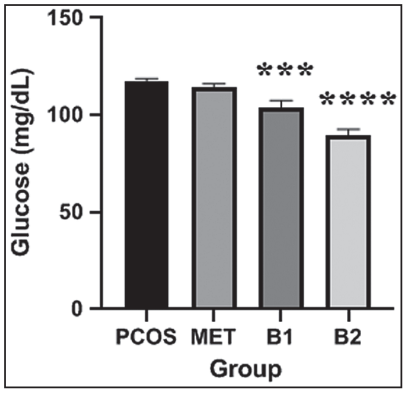

Figure 6: Serum levels of glucose in PCOS rats. ***$P < 0.001$ and ****$P < 0.0001$ vs. PCOS group. PCOS = polycystic ovary syndrome

Conclusion

According to the study's data, *B. dioica* showed the protective effect on PCOS rats and normalized the hormones, glucose, LDL, and LDL/HDL ratio, which are abnormal in PCOS rats. Moreover, *B. dioica* has an improvement effect on the symptoms and markers of PCOS and fertility but more researches to get precise results are needed.

Financial support and sponsorship

Nil.

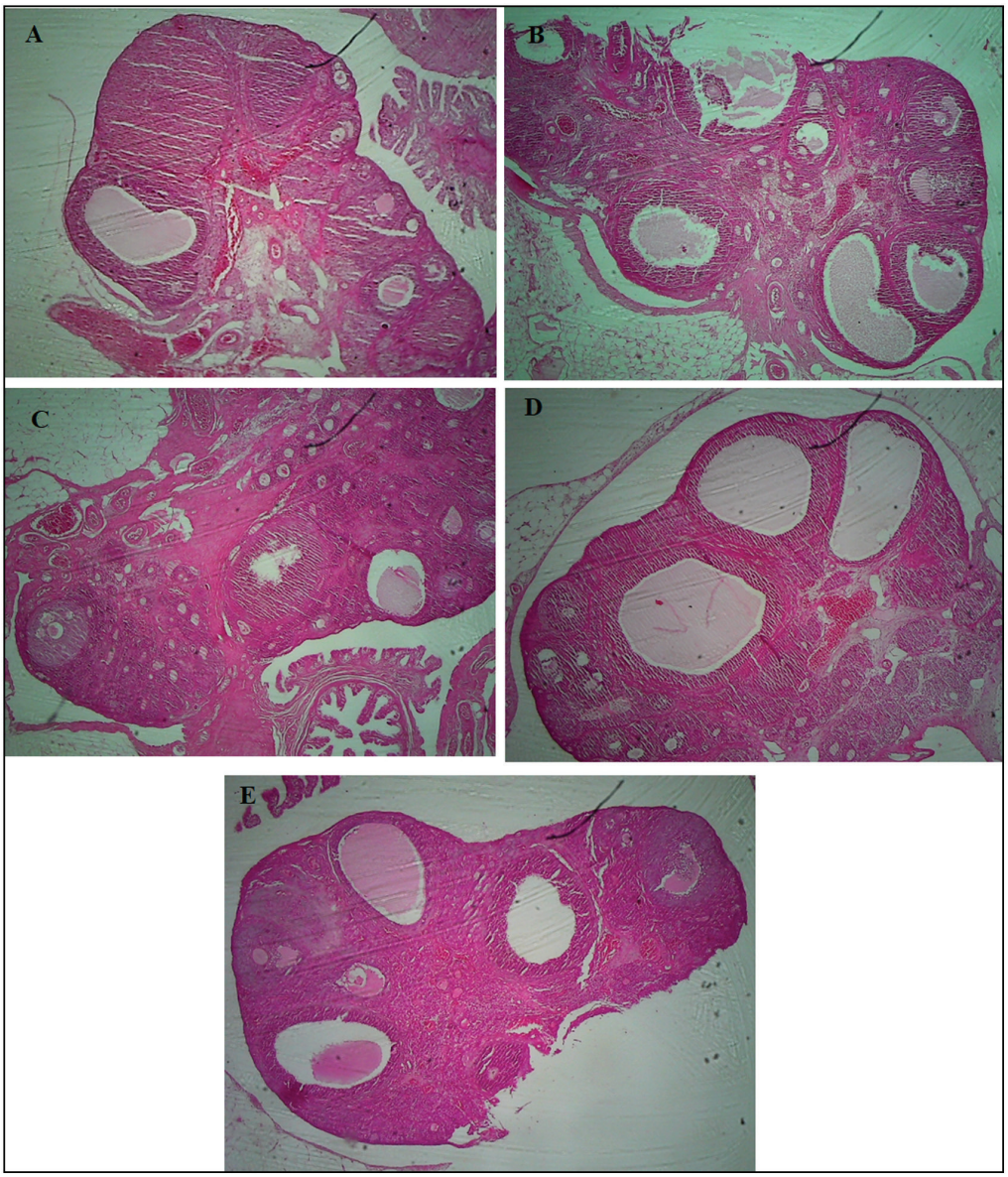

Figure 7: Morphological alterations in rat ovarian tissues. Rat ovarian tissues were stained with H&E, as explained in the "Materials and Methods" section, to observe morphological changes (×40). (A) Normal ovary. (B) PCOS group. (C) Metformin-treated group. (D) B1 (30 mg/kg/day)-treated group. (E) B2 (60 mg/kg/day)-treated group. H&E = hematoxylin and eosin, PCOS = polycystic ovary syndrome group, B1 = *Bryonia* extract group, B2 = *Bryonia* extract group

Conflicts of interest

There are no conflicts of interest.

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