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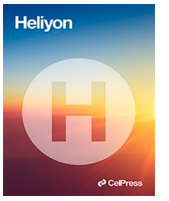

Research article

Effects of Berberine on glucolipid metabolism among dehydroepiandrosterone-induced rats of polycystic ovary syndrome with insulin-resistance

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a set of endocrine disorder syndrome characterized by ovulation disorder. Increased insulin resistance (IR) and compensatory hyperinsulinemia play a vital role in the pathogenesis of PCOS. Therefore, insulin sensitizing agents have been studied in the treatment of PCOS. Berberine (BBR) has been proved to alleviate IR in patients with PCOS, but the mechanism remained unclear. This study was aimed to verify the regulatory mechanism of BBR on PCOS-IR rats. Firstly, we established a female rat PCOS-IR model induced by dehydroepiandrosterone (DHEA) and found that estrus cycle was disrupted in the PCOS-IR group, serum fasting insulin (FINS) level and the homeostasis model assessment of insulin resistance (HOMA-IR) index were significantly higher than normal control group. BBR treatment could recover estrous cycle, reduce abnormal serum hormone levels like luteotropic hormone (LH) and testosterone (T). Most importantly, BBR could concentration-dependently reduce serum FINS level in PCOS-IR rat model. Meanwhile, BBR may improve the abnormal lipid metabolism levels in PCOS-IR group by decreasing low density lipoprotein (LDL), total cholesterol (TC) and triglyceride (TG). Histological results showed that BBR can also protect normal histological structures of ovaries in PCOS-IR rats. Our results indicated that BBR plays a protective role in PCOS-IR, increasing insulin sensitivity, improving hyperandrogens and recovering abnormal blood lipids. Therefore, Our research provides novel insights for therapeutic treatment of BBR in patients with glucolipid metabolic disturbances.

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1. Introduction

Polycystic ovary syndrome (PCOS) is a set of endocrine disorder syndrome characterized by ovulation disorder, which is seriously affecting women's physical and mental health and the over all life quality. It has been reported that about 15 % of childbearing women were suffering from PCOS (Rotterdam standard) [1]. Persistent anovulation, high androgen and insulin resistance (IR) are the main clinical features of PCOS. With the development of PCOS, type 2 diabetes (T2DM), dyslipidemia, cardiovascular disease, and endometrial cancer are the most common clinical complications [1,2]. According to a large-scale epidemiological survey in 10 provinces of China, the prevalence of PCOS is 5.6 % in Chinese Han ethnic women aged 19–45 years [3].

The abnormal physiological phenomenon of reduced peripheral tissue sensitivity to insulin is known as IR. Burghen et al. first reported the involvement of IR in PCOS in 1980, which not only impaired the formation of follicles and affected the development of follicles, but also further aggravated the phenomenon of anovulation [4,5]. In 1989, Dunaif found that approximately 20 % of obese women with PCOS also had IR [6]. Another study also confirmed that thin PCOS patients would accompany with IR as well, mainly revealed in normal fasting insulin level and more likely to have postprandial hyperinsulinemia [7]. Further research confirmed that PCOS patients with IR (PCOS-IR) are more likely to have long-term complications such as glucose metabolic abnormalities, type 2 diabetes, cardiovascular disease, unopposed estrogen effects on the endometrium and so on, which indicated that IR and hyperinsulinism may play important roles on the pathophysiology of PCOS [8]. About 85 % (75 % of lean and 95 % of overweight) women with PCOS were affected by IR, which indicated that IR may be the initiating factor and key player leading to PCOS [9]. Thus, therapeutic strategies aimed to improve insulin sensitivity may prevent the development of metabolic complications associated with PCOS. Hyperinsulinemia is reported to be associated with excessive androgens levels and anovulation [10]. Insulin exerts effects on the ovary via its own receptor and interacts with gonadotrophins to regulate steroidogenesis. IR and hyperandrogenism are associated with a defective PI3K signaling cascade [11,12]. Insulin-sensitizing agents, such as metformin and thiazolidinediones, are used to improve insulin sensitivity in women with PCOS [13–15]. Unfortunately, these two agents have severe adverse effects, such as gastrointestinal disturbance and liver injury [16]. Hence, research and development of more effective and safely insulin sensitizers for PCOS-IR remains to be a challenge in the field.

Berberine (BBR), a type of isoquinoline alkaloid extracted from traditional Chinese medicine (TCM) Rhizomacoptidis. It has been reported that BBR has a wide range of pharmacological activities, such as antibacterial, hypoglycemic and lipid lowering and so on [17–19]. In addition, BBR can also improve metabolic disorders and protect the cardiovascular system. These pharmacological activities make BBR promising to be an attractive option for the treatment of metabolism-related diseases such as PCOS and prevention of cardiometabolic sequelae [20–22]. A double-blinded clinical research which involved 120 patients with PCOS-IR, showed that BBR can significantly reduce the IR of PCOS-IR patients [23]. The in vivo animal experiments confirmed that BBR showed a metformin similar effect, which can reduce the free fatty acid level of insulin resistance and increase the activity of liver glucokinase (GK) in rat model [24,25]. Ko BS et al. demonstrated that BBR improved IR by increasing glucose transporter 4 (GLUT-4) protein expression, promoting the phosphorylation of insulin receptor substrate 1 (IRS-1), initiating PI3K/Akt signaling pathway to increase the insulin-stimulated glucose uptake [26]. In the present study, we established a PCOS-IR rat model to evaluate the effect of BBR on PCOS-IR, and further separated ovarian tissue of animal to verify the effect of BBR on insulin signaling. Our study will provide experimental evidence for the clinical treatment of BBR in PCOS-IR.

2. Materials and methods

Dehydroepiandrosterone (DHEA, Guangzhou Dreampharm Co, Ltd, Guangdong, China); BBR (Guangdong Huanan Pharmaceutical Group Co, Ltd, China); metformin (Salvage Pharmaceutical, Guizhou, China); 0.9 % saline (Henan New Century Pharmaceutical Co, Ltd, China); Rat follicle-stimulating hormone (FSH), Luteotropic hormone (LH), Testostrone (T), Insulin ELISA KIT (ZhongshengSuifeng, Shenzhen, China); High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Total Cholesterol (TC), Triglyceride (TG) ELISA KIT (Nanjing Jiancheng Bioengineering Institute, China); Glucose meter, Optium (sinocare, Hunan, China).

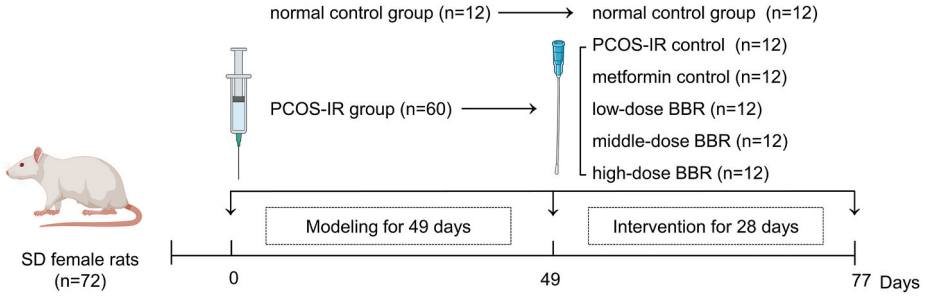

Fig. 1. The experiment design. PCOS-IR: Polycystic ovary syndrome-insulin resistance; BBR: Berberine.

3. Induction of PCOS-IR experimental animal model

A total of 72 21-day-old female SD rats, were purchased from the Guangdong Medical Laboratory Animal Center (Guangzhou, Guangdong, China), with reference No. SYXK2017-0125. The rats were acclimatized at room temperature (25 ± 2 °C) with controlled light/dark cycle to be switched on and off at 06:00 and 18:00, respectively. They were kept in the Animal Laboratory, College of Traditional Chinese Medicine, Guangzhou Pharmaceutical University. The rats were given free access to food and water. All procedures were carried out in accordance to the Guide for the Care and Use of Laboratory Animals as approved by Ethics Committee of Guangdong Pharmaceutical University (00240,578).

All female SD rats were randomly divided into two experimental groups (Fig. 1): The Control group (n = 12) and the PCOS-IR model group (n = 60). Sixty PCOS-IR model group rats were given a high-fat diet as well as subcutaneously injected daily with DHEA (6 mg/100 g body weight dissolved in 0.2 ml sesame oil) for 49 continuous days (PCOS-IR control group). The dose of DHEA was chosen according to previous study [25]. Twelve female rats of the same age and weight were injected with sesame oil as well as given a general diet for 49 continuous days (normal control group).

3.1. Administration of BBR and metformin

Once PCOS rat model was established, five DHEA-groups of rats were gavaged with different concentrations of BBR (162, 81, or 40.5 mg/kg, as high, middle and low dose BBR group respectively) or 45 mg/kg metformin (metformin control group) for 28 days. For normal control group rats were treated with 0.9 % saline for 28 days.

3.2. Assessment of estrous cycle

The estrous cycle was determined daily by vagina smear within 10 days. 0.9 % saline-wetted cotton swabs were used to collect vaginal cells, and the liquid was applied to slides to dry. The sections were stained with Wright-Giemsa dye. Breifly, the smear was stained with solution A of Wright-Giemsa's staining for 30–60 s and then solution B was added for 10 min. After staining, smear was washed twice (1 min each) in ddH2O and observed with microscope equipped with camera. The proestrus stage was defined by the presence of clusters of round, well-formed nucleated epithelial cells; the estrus stage was defined by the presence of predominantly cornified squamous epithelial cells; the diestrus stage was defined by the presence of rare cornified squamous epithelial cells and predominately leukocytes; metestrus could be distinguished from diestrus by the appearance of nucleated epithelial cells in diestrus. Anucleated cornified cells were observed within 10 days means that PCOS rat model was successfully established.

3.3. Determination of the oral glucose tolerance test (OGTT), insulin and glucolipids levels

After PCOS rat model successfully established and 27 days after drug treatment, rats were fasting for 12 h, orbital venous bloods of rats were collected to detect glucolipid and hormones. The concentrations of serum hormones include Luteotropic hormone (LH), and Testostrone (T) were examined by specific ELISA kit strictly according to the manufacturer's protocol. Serum glucolipid include fasting insulin (FINS), fasting blood-glucose (FBG), high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC) and triglyceride (TG). After blood collection, rats were fed with 50 % glucose at the dose of 2 g/kg, blood samples were collected from the tail at 30, 60, and 120 min after glucose administration. Fasting and postprandial blood glucose concentrations were measured with a glucose meter to drawn OGTT curve, and calculate the area under the curve. Homeostasis model assessment of insulin resistance (HOMA-IR) index were calculated by the formula FBG (mmol/L) × FINS (mU/L)/22.5.

3.4. Hematoxylin and eosin staining for ovarian histology

The left ovary of rats was fixed in 4 % paraformaldehyde and embedded in paraffin. Subsequently, 5 μm sections were cut and mounted on slides. The sections were stained using a hematoxylin and eosin staining kit (Beyotime Institute of Biotechnology, China) and performed according to the manufacturer's instructions. The images were obtained by a light microscope (BX51; Olympus Corporation, Tokyo, Japan).

3.5. Statistical analysis

The software SPSS 25 (IBM Corp., Armonk, NY) was used. Parametric variables were analyzed using analysis of variance (ANOVA). The Newman-Keuls' test was applied for multiple comparisons. $P < 0.05$ indicated significant differences. Data are presented as mean ± standard error of mean (SEM).

4. Results

4.1. Compared with normal group, the estrus cycle of rats in PCOS-IR group was obviously destroyed

Daily assessment of estrous cycle was performed by Wright-Giemsa Staining and the slides were investigated under microscope. Nucleated epithelia cells and cornified epithelia cells were observed in normal control group. However, in the PCOS-IR control group,

the cycle was halted at estrus stage (Fig. 2A). Fasting blood glouse (FBG) levels between normal control group and PCOS-IR control group have no significant difference (Fig. 2B, $P > 0.05$). However, serum fasting insulin (FINS) level in PCOS-IR control group was significantly higher than in normal control group (Figs. 2C and 22.78 ± 5.41 VS 11.04 ± 3.42 mU/L, $P < 0.01$). The Homeostasis model assessment of insulin resistance (HOMA-IR) index in PCOS-IR control group was about 2.2 folds higher than that of the normal control group (Fig. 2D, $P < 0.01$). All these results confirmed that we have successfully established PCOS-IR rat model by using DHEA and high-fat diet.

4.2. BBR and metformin treatment recovered the estrous cycle of PCOS-IR rats

After 28 days' treatment of BBR metformin, the vaginal cytology of rats were assessed using Wright-Giemsa staining, and changes were observed. The estrus cycle assessment was conducted during period of BBR and metformin administration (Fig. 3). In BBR and metformin groups, all estrous cycle stages were observed, which means BBR and metformin treatment can obviously recovered PCOS-IR rat's estrous cycle.

4.3. Alterations of serum hormone levels between control and BBR treatment groups

Abnormal serum hormone levels such as follicle-stimulating hormone (FSH), luteotropic hormone (LH) and testosterone (T), are the main serological features of PCOS. In our study, serum LH, FSH and T levels were detected by ELISA kit. Our results demonstrated that the serum levels of LH (Fig. 4A, 1659.0 ± 416.99 VS 903.11 ± 316.59 ng/L, $P < 0.01$), FSH (Figs. 4B and 6.042 ± 1.43 VS 4.055 ± 1.083 IU/L, $P < 0.01$) and T (Figs. 4C and 2.29 ± 0.28 VS 1.33 ± 0.24 ng/ml, $P < 0.01$) were significantly increased in the DHEA-induced PCOS-IR rat model, comparing to normal control group, which were consistent to the previous reports that DHEA-induced PCOS-IR could increase serum hormone levels [25]. After 4 weeks BBR and metformin treatment, the serum levels of LH, FSH and T were significantly decreased compare to PCOS-IR control group. Most importantly, BBR treatment was concentration-dependent and reversed the increment of LH, FSH and T levels induced by DHEA in serum. The reversal effects of serum LH (Fig. 4A) FSH (Fig. 4B) and

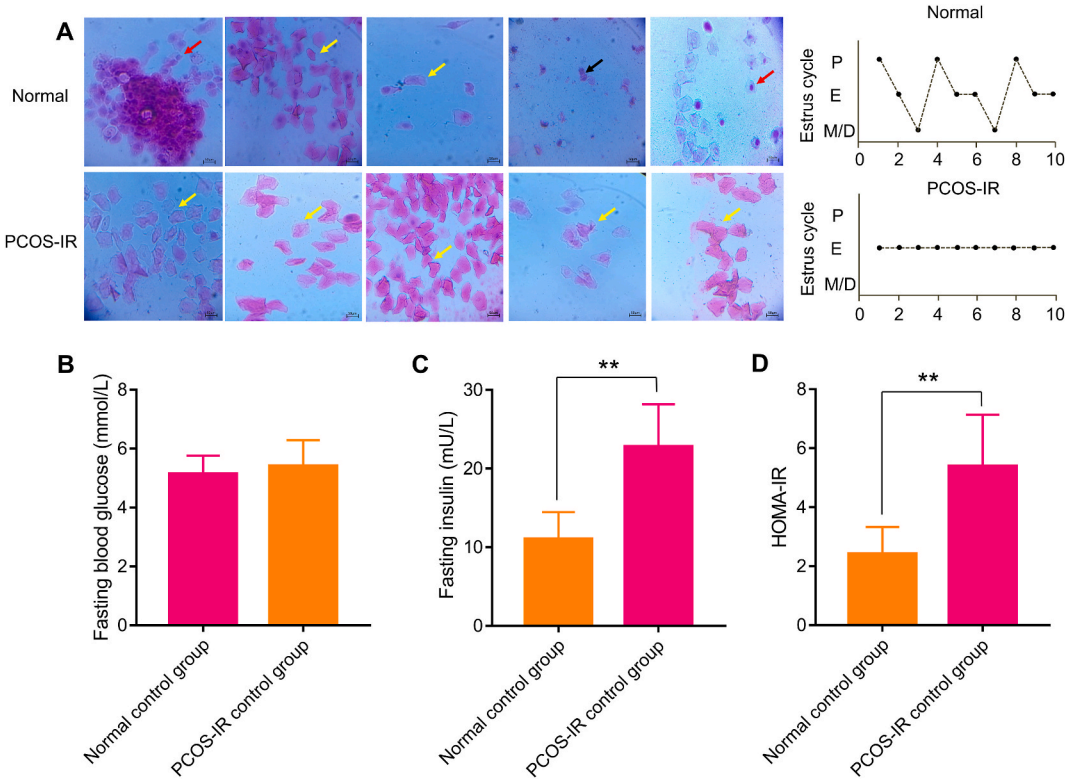

Fig. 2. PCOS-IR rats model were successfully established. (A) Three main cell types detected in vaginal smear samples: nucleated epithelial cells (red arrows); cornified squamous epithelial cells (yellow arrows); and leukocytes (black arrows). The ratio of these cell types presented in the smear can be used to identify rat in proestrus (P), estrus (E), metestrus (M), or diestrus (D) as described in the methodology. Two or three regular estrous cycles were observed in the rats of normal control group (n = 10 per group) by vaginal smearing within 10 days, while prolonged estrus cycle, disappeared estrus cycle, and persistent anovulatory cycle were revealed in the rats of PCOS-IR (n = 10); (B) Fasting blood-glucose (FBG); (C) Fasting insulin (FINS); and (D) Homeostasis model assessment of insulin resistance (HOMA-IR) levels in normal (n = 10) and PCOS-IR groups (n = 10). **: compare with the PCOS-IR control, $P < 0.01$.

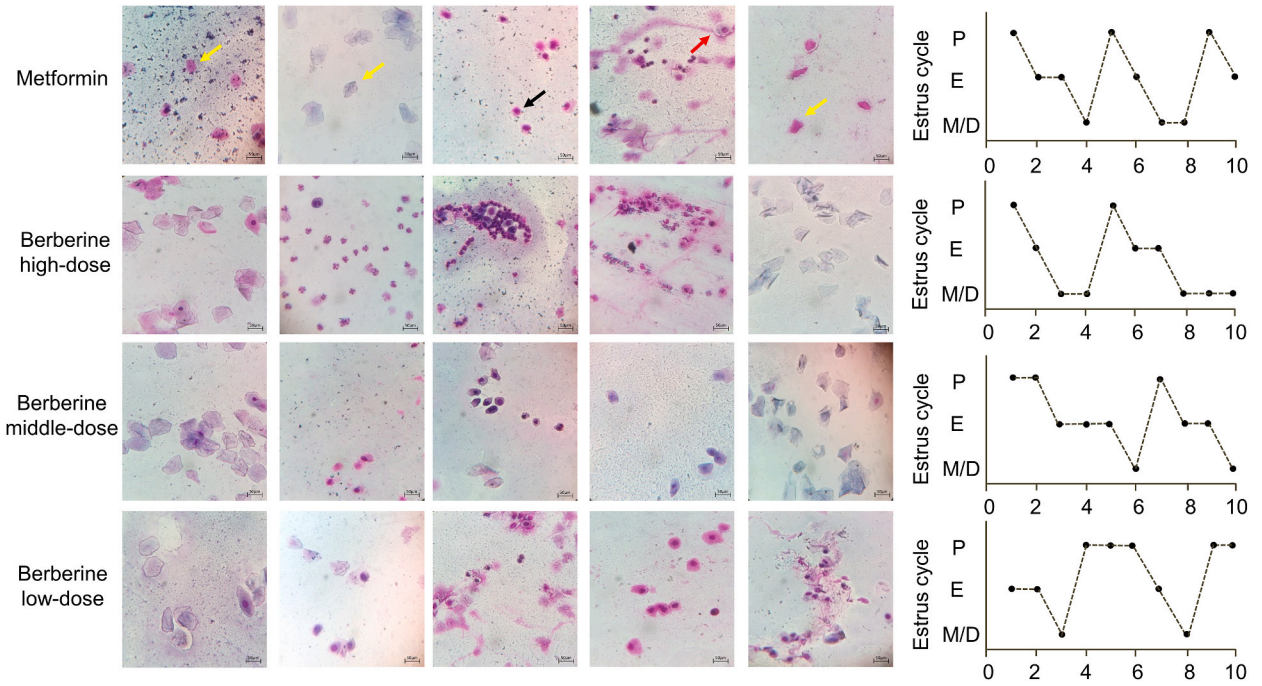

Fig. 3. Vaginal smear of rats in BBR and metformin treatment groups. 28 days after administration of BBR and metformin, the changes of vaginal cytology in rats were observed through the method of Wright-Giemsa's staining. Three main cell types detected in vaginal smear samples: nucleated epithelial cells (red arrows); cornified squamous epithelial cells (yellow arrows); and leukocytes (black arrows). The proestrus (P) stage was defined by the presence of clusters of round, well-formed nucleated epithelial cells; the estrus (E) stage was defined by the presence of predominantly cornified squamous epithelial cells; the diestrus (D) stage was defined by the presence of rare cornified squamous epithelial cells and predominately leukocytes; metestrus (M) could be distinguished from diestrus by the appearance of nucleated epithelial cells in diestrus. It was found that the estrous cycle of rats was recovered with different dosage of BBR and metformin treatment (n = 10 per group).

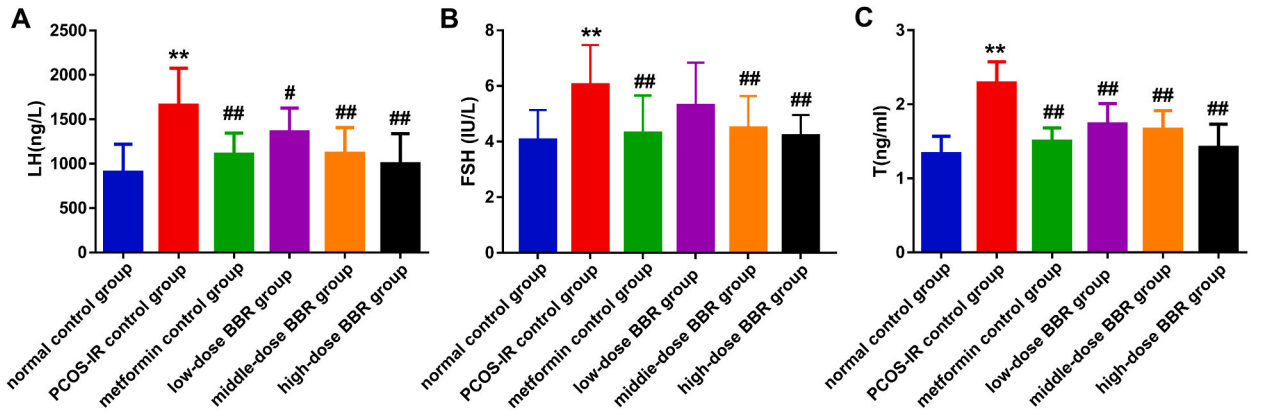

Fig. 4. Serum levels of LH and T in each group. The serum levels of LH (A), FSH (B) and T (C) were significantly higher in the PCOS-IR control group than in the normal control group (**, $P < 0.01$). Both BBR and metformin could reduce the serum levels of LH, and T. BBR revealed similar effect with positive control metformin and showed concentration-dependent manner (n = 10 per group). Luteotropic hormone (LH), Follicle-stimulating hormone (FSH), Testostrone (T). # and ##: compare with the PCOS-IR control, $P < 0.05$ and $P < 0.01$, respectively.

T (Fig. 4C) levels in high-dose BBR treatment group was similar to metformin.

4.4. BBR significantly reduced serum level of FINS in PCOS-IR

Insulin resistance and hyperinsinticemia are the most common endocrine metabolic disorders in PCOS patients. In our study, we found that the FINS level in PCOS-IR control group is higher than in normal group (Fig. 5B, 22.78 ± 5.41 VS 11.04 ± 3.42 mU/L, $P <$ 0.01). High-dose BBR treatment not only significantly decreased the FINS level (Figs. 5B and 12.45 ± 4.36 VS 22.78 ± 5.41 mU/L, $P <$

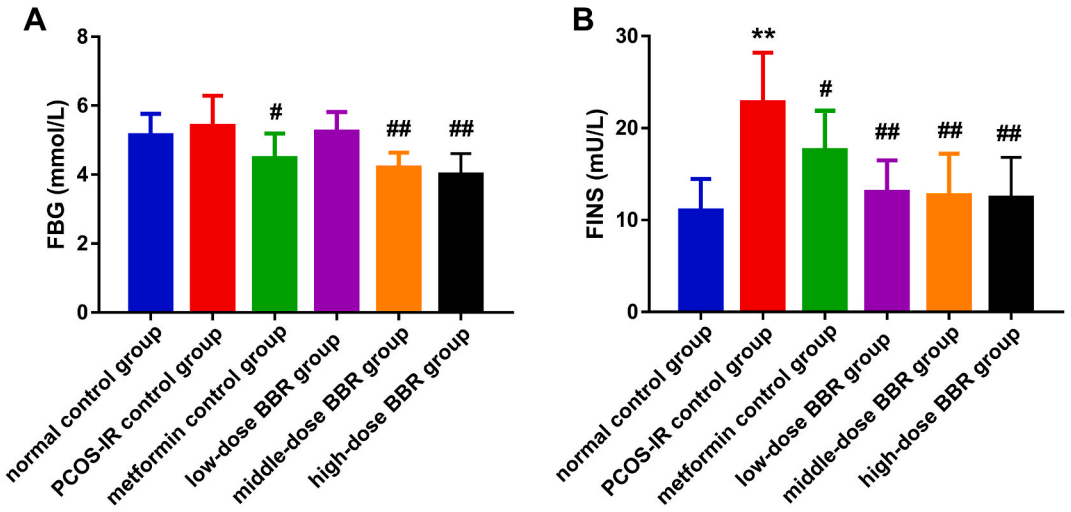

Fig. 5. Serum levels of FBG and FINS in each group. Both BBR and metformin could reduce the serum levels of FBG and FINS and BBR exhibited similar effect with positive control metformin (A). The serum level of FINS was significantly higher in the PCOS-IR control group than in the normal control group (B) (n = 10 per group). FBG: fasting blood-glucose; FINS: fasting insulin. **, $P < 0.01$ compare with the normal control; # and ##: compare with the PCOS-IR control, $P < 0.05$ and $P < 0.01$, respectively.

0.01), but also reduced FBG level (Figs. 5A and 4.00 ± 0.61 VS 5.41 ± 0.88 mmol/L, $P < 0.01$) compare to PCOS-IR control group. Meanwhile, different concentrations of BBR could reduce the FINS level of rats, and the effect of improving insulin resistance is better than that of metformin (see Table 1).

Furthermore, oral glucose tolerance test (OGTT test) showed that the blood glucose levels were significantly lower in the metformin control and high-dose BBR groups than in the PCOS-IR control group at 30 min (Table 1. 8.138 ± 1.978 VS 9.59 ± 1.138 mmol/L, 7.578 ± 1.432 VS 9.59 ± 1.138 mmol/L, $P < 0.01$) after glucose feeding. The HOMA-IR index was significantly decreased in all BBR groups and metformin group compared to the PCOS-IR control group ($P < 0.01$; Table 2) (see Table 2).

4.5. BBR significantly improved the abnormal lipid metabolism in DHEA-induced PCOS-IR rats

In PCOS-IR rats, the metabolism of serum high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC) and triglyceride (TG) was disordered. Our results confirmed that serum LDL (Fig. 6B, 0.67 ± 0.36 VS 0.36 ± 0.17 mmol/L, $P < 0.01$), TC (Figs. 6C and 2.25 ± 0.51 VS 1.45 ± 0.33 mmol/L, $P < 0.01$) and TG (Figs. 6D and 0.55 ± 0.13 VS 0.34 ± 0.07 mmol/L, $p < 0.01$) levels in PCOS-IR group were significantly higher than those in normal control group, while HDL (Figs. 6A and 0.39 ± 0.13 VS 0.70 ± 0.16 mmol/L, $P < 0.01$) was significantly lower than that in normal control group. BBR treatment significantly reduced the abnormally elevated serum levels of LDL, TC and TG, and reversed the abnormally decreased level of HDL in PCOS-IR group. These results indicated that BBR could improve DHEA-induced abnormal lipid metabolism in model rats.

4.6. Protective effects of BBR on the follicular development in PCOS ovaries as determined by histological examination

The present study examined histological changes in ovaries by hematoxylin and eosin staining. The results demonstrated that healthy structures of follicles in different development stages and the corpus luteum were observed in the normal control group (Fig. 7A). Compared with the control group, more early development of small follicles, atresia follicles, interstitial cells were observed

Table 1
The influence of berberine on blood glucose level in PCOS-IR rats in oral glucose tolerance test ($\bar{X} \pm S$, n = 10) .

Groups	Glucose blood (mmol/L) ($\bar{X} \pm S$, n = 10)			
	0min	30min	1 h	2 h
Normal control	5.15 ± 0.62	9.16 ± 1.00	5.02 ± 0.71	4.71 ± 0.59
PCOS-IR control	5.41 ± 0.88	9.59 ± 1.14	4.97 ± 0.72	4.47 ± 0.44
Metformin	4.48 ± 0.72△	8.14 ± 1.98△	5.76 ± 1.02△	5.22 ± 0.72
Low-dose BBR	5.25 ± 0.57	8.21 ± 0.93△	4.29 ± 1.02	4.15 ± 0.85
Middle-dose BBR	4.20 ± 0.437△△	8.51 ± 1.49	5.45 ± 0.82	4.41 ± 0.58
High-dose BBR	4.00 ± 0.612△△	7.59 ± 1.43△△	5.92 ± 0.59△	4.39 ± 0.71

Oral glucose tolerance test (OGTT test) showed that the blood glucose levels were significantly lower in the metformin control and high-dose BBR groups than in the PCOS-IR control group at 30 and 60 min after glucose feeding ($P < 0.05$). PCOS-IR : polycystic ovary syndrome with insulin resistance. △and △△: compare with the PCOS-IR control, P<0.05 and P<0.01 respectively.

Table 2
HOMA-IR indexes of PCOS-IR rats ($\bar{X} \pm S$, n = 10).

Groups	Normal control	PCOS-IR control	Metformin control	Low-does BBR	Middle-does BBR	High-does BBR
HOMA-IR	2.42 ± 0.91	5.39 ± 1.75**	3.51 ± 0.10△△	2.86 ± 0.67△△	2.41 ± 0.97△△	2.41 ± 0.68△△

The HOMA-IR index was significantly decreased in all BBR groups and metformin group than in the PCOS-IR control group. PCOS-IR: polycystic ovary syndrome with insulin resistance. compare with the normal control; **P<0.01 compare with the Normal control, and △△P<0.01: compare with the PCOS-IR control.

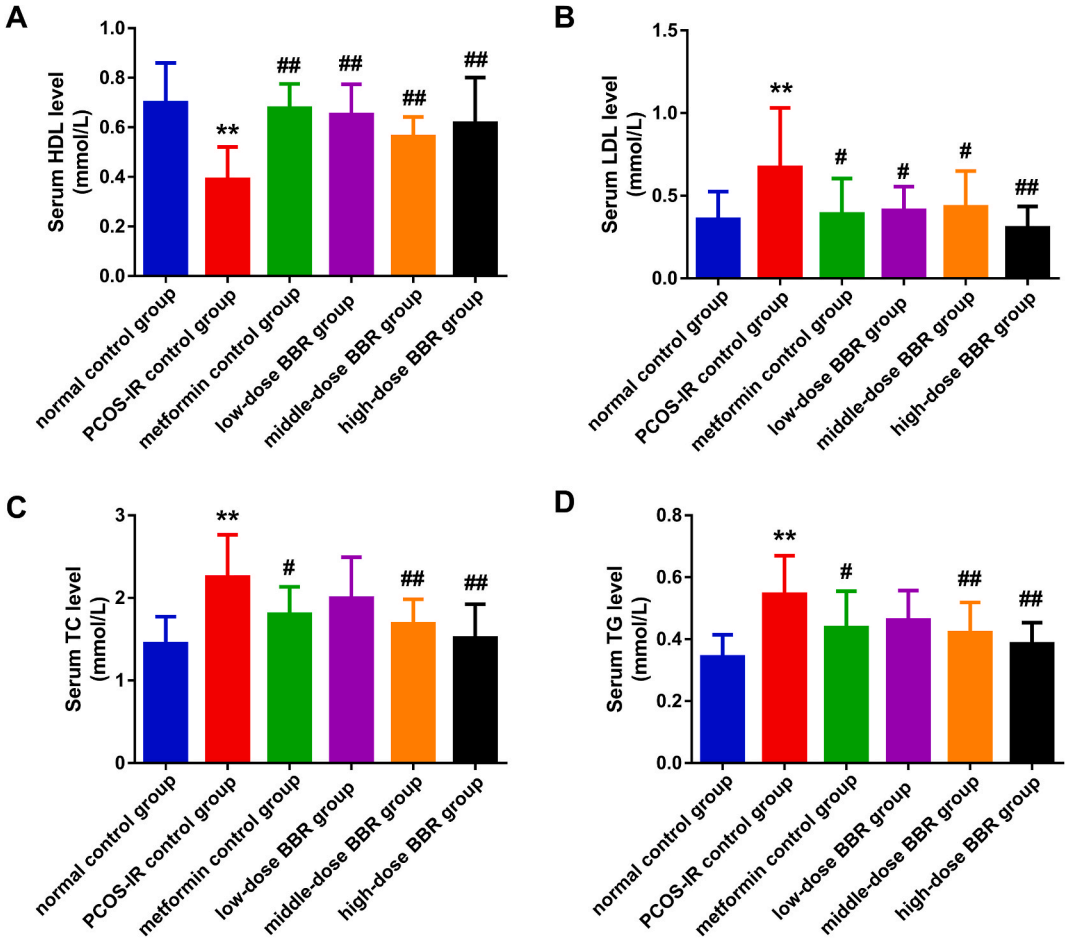

Fig. 6. Serum levels of HDL, LDL, TC and TG in rats after administration with BBR and metformin. After administered with BBR and metformin, the serum level of HDL was significantly increased compare to PCOS-IR group (A). While the serum levels of LDL (B), TC (C), TG (D) were significantly decreased in all BBR groups and metformin control group (n = 10 per group). HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TC: Total Cholesterol; TG: Triglyceride; **: compare with normal control group, $P < 0.01$; #and ##: compare with the PCOS-IR control, $P < 0.05$ and $P < 0.01$, respectively.

in the ovaries of PCOS-IR group. The majority of follicles were expanding cystic follicles with degrading and loosely arranged granulosa cell layers (Fig. 7B). After administration with metformin and BBR (Fig. 7C–F), the number of expanding cystic follicles was decreased significantly in metformin group, middle-dose and high-dose BBR group ($P < 0.05$) (Fig. 7G). While no significant differences among the number of corpora lutea ($P > 0.05$) (Fig. 7H).

5. Discussion

In the present study, we established the PCOS-IR rat model induced with DHEA and high-fat, which was first proposed by Lee et al., in 1991 [25]. Anderson et al. established the PCOS model in SD female rats by subcutaneous injection of DHEA and observed increased serum levels of DHEA, androstendione, T and estradiol, but no obvious change in serum levels of FSH [27]. The results of our animal modeling were similar the previous reports. In this study, the PCOS-IR rats showed prolonged estrus cycle, disappeared estrus cycle,

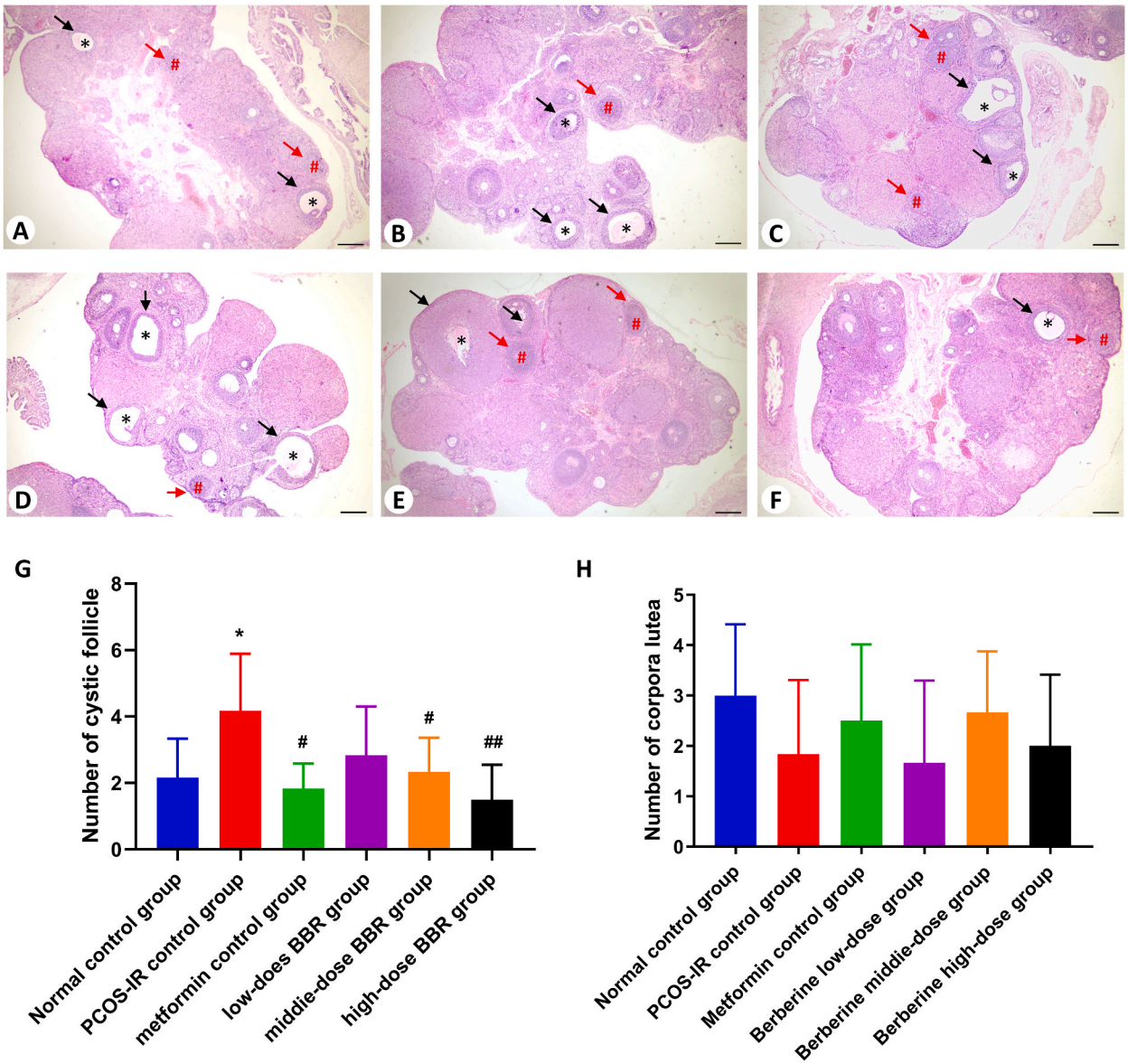

Fig. 7. Ovarian histological examination after administration with BBR and metformin. Histological sections were stained with hematoxylin and eosin (HE × 40). Healthy structures of follicles in different development stages and the corpus luteum were observed in the normal control group (A). Compared with the control group, more early development of small follicles, atresia follicles, interstitial cells were observed in the ovaries of PCOS-IR group, the majority of follicles were expanding cystic follicles with degrading and loosely arranged granulosa cell layers (B). After administration with BBR and metformin, the number of expanding cystic follicles was decreased, meanwhile the presence of a number of follicles at different developmental stages and corpus luteum was observed in the metformin control group (C), low-dose (D), middle-dose (E) and high-dose group (F). Cystic follicle is represented by black arrowheads and black asterisk; corpora lutea are represented by red arrowheads and red pound. Scale bar, 200 μm. Quantitative analysis of cystic follicles (n = 6 per group) (G). Quantitative analysis of corpora lutea (n = 6 rats per group) (H). *: compare with normal control group, $P < 0.05$; #and ##: compare with the PCOS-IR control, $P < 0.05$ and $P < 0.01$, respectively.

and persistent anovulatory cycle. Besides, the serum levels of LH and T were significantly higher in PCOS-IR group than in normal control group, and so did the serum level of FINS and the HOMA-IR index. The increased androgens and hyperinsulinemia, two main characteristics of PCOS, indicated that the PCOS-IR rat model was successfully established.

We subsequently treated PCOS-IR rats with BBR and metformin, the estrous cycle of rats was recovered in both groups; the serum level of FBG was decreased significantly in middle-/high-dose BBR groups and metformin control group, and so did the serum level of FINS and the HOMA-IR index in all BBR group and metformin control group. Our results demonstrated that BBR has a comparable activity to metformin as an insulin sensitizer in reducing FBG and FINS production, and improving glucose homeostasis in the PCOS-IR rat model. In additional, the decrease of serum level of insulin, another beneficial effect of BBR on lipid metabolism was also observed

in the study. The serum levels of TC, TG, and LDL were significantly decreased, and that of HDL was increased after treatment with BBR. In the current study, we noted that BBR reduced the serum levels of LH and T in PCOS-IR rats, reducing hyperandrogenemia, with the effect strengthened with increased dose. As compared with metformin, BBR showed similar metabolic effects of ameliorating insulin sensitivity and reducing hyperandrogenemia. BBR was also demonstrated greater effect on lowering lipid compared with metformin. Several studies suggested that BBR improves insulin sensitivity via activating the AMP-activated protein kinase pathway, which may be the target for regulation of glucose metabolism [28–30]. The PI3K-Akt signaling pathway is another major known molecular mechanism of BBR induced improvement of IR in PCOS at present [11,31], which was also demonstrated in the present study.

IR appears to aggravate the severity of hyperandrogenemia in women with PCOS [32]. This may be due to the ability of insulin in increasing the release of gonadotropins, LH, and FSH and/or re-programming the response of the ovary to these hormones [33–35]. Independently, hyperinsulinemia may affect ovulation by causing dysfunction of granulosa cells and consequently delaying or diminishing follicle growth, leading to follicular arrest [36,37].

It's worth emphasizing that insulin was observed to have a synergistic effect with luteinizing hormone to initiate the steroidogenic pathway and testosterone production by ovarian theca cells [38,39]. Zhao et al. investigated the relationship between hyperandrogenism and IR in theca cells derived from the porcine ovaries. They observed that the levels of 17-hydroxylase (CYP17, a crucial enzyme in androgen biosynthesis) and T were dramatically higher in dexamethasone-treated theca cells in the PCOS-IR group than in control group ($P < 0.01$), suggesting the involvement of IR in the pathogenesis of hyperandrogenism in PCOS [40]. These alterations were partially reversed in PCOS-IR cells treated with BBR. The CYP17 mRNA expression and T level were significantly lower in the BBR group than in the PCOS-IR group ($P < 0.05$). BBR inhibited CYP17 mRNA expression, the precursor of testosterone which was reflected in lower testosterone levels. These results indicate that BBR can both attenuate IR and inhibit androgen production in theca cells [40].

In our study, the rats with PCOS-IR induced by DHEA and high-fat diet exhibited hyperinsulinism and hyperandrogenism. Enzyme defects in estrogen synthesis may be responsible for hyperandrogenemia. Moreover, the lack of negative feedback at the hypothalamus-pituitary axis leads to the hypersecretion of LH. We assumed that, in addition to hyperandrogenemia in rats with PCOS, the increased serum level of LH would result in unfavorable conditions for producing follicles. In the present study, elevated serum levels of T and LH were observed along with prolonged estrus cycle, disappeared estrus cycle, and persistent anovulatory cycle in PCOS-IR rats. The activation of the intracellular PI3K/Akt signaling has been demonstrated to of play an important role in androgen production in theca cells [41,42]. PI3K is involved in LH-induced Akt phosphorylation in theca cells, resulting in an increase in ovarian cytochrome P450 (*CYP17A1*) gene expression [43]. The *CYP17A1* gene is responsible for the increase in the activity of 17-a hydroxylase in patients with PCOS. This enzyme plays a key role in steroid synthesis by increasing the level of androgens [44].

Hyperlipidemia is a component of the metabolic syndrome and often predicts cardiovascular diseases. The pathophysiology is very complex and has been only partially elucidated. IR and obesity are implicated in the development of hyperlipidemia. However, there is debate regarding whether they are the cause or the consequences of metabolic disorder. The most commonly used agents for hyperlipidemia are statins and fibrates (peroxisome proliferator-activated receptor-alpha agonists). These agents, while effective, may cause a markedly increased risk of myopathy and rhabdomyolysis [45]. BBR, a quaternary ammonium salt isolated from Rhizoma-coptidis, possesses an anti-hyperlipidemic potential. Li et al. determined the effect of BBR on high-fat diet-induced hyperlipidemia in male Wistar rats [46]. Treatment with BBR for 4 weeks significantly decreased the serum levels of TG, TC, and LDL in these rats, whereas the serum level of HDL was restored. They concluded that the anti-hyperlipidemic effect of BBR in hyperlipidemic rats is associated with a global change in the metabolism of lipids, carbohydrates, and amino acids, as well as the structure of microbiota. The present study also confirmed the significant lipid-lowering effect of 4-week BBR intervention. These results were consistent with the data obtained by another research [45].

Several clinical trials on the effect of BBR on dyslipidemia have been completed or are in progress. The combination of BBR and pioglitazone for the treatment of non-alcoholic fatty liver disease has entered Phase II clinical trial (NCT00633282). Another clinical trial (NCT01138930) is designed to determine the effect of BBR on lipid metabolism and IR in obese patients with PCOS. The effect of BBR alone (NCT00462046, Phase III clinical trial) or in combination with metformin (NCT00425009, Phase I clinical trial) on dyslipidemia is under study. In a Phase IV clinical trial (NCT01649986), the effects of BBR on lipid and metabolic features (serum levels of total cholesterol, LDL cholesterol, and triglycerides) are evaluated. The present study demonstrated the therapeutic effect of BBR on dyslipidemia in rats with PCOS, but the mechanism remains ambiguous. Kong et al. reported that the mechanism of BBR-moderated lipid metabolism was related to the up-regulation of LDL receptor in both mRNA and protein levels in the liver [47,48].

In present study, we noted that it is important to scrutinize the mechanism of sensitizing insulin and reducing androgen and lipid with BBR. The molecular mechanism of BBR-mediated attenuation of IR in PCOS-IR rats would be preliminarily investigated in our further study.

Some limitations of the present study need to be mentioned. First, only glucose metabolism in the ovaries of PCOS rats was examined, whereas androgen metabolism was not examined. Second, the sample size was too small, and the research was limited to animal experiments. Third, due to COVID-19 pandemic, we can't optimize the Western blot analysis of IRS-1, PI3K-p85, and Glut-4 protein expression, and did not perform immunohistochemical staining for verification. We plan to investigate the safety and efficacy of BBR in treating patients with PCOS-IR and determine optimal dosage of BBR in our future clinical trials. We will also investigate the mechanism of insulin-sensitizing effect of BBR in vitro experiments.

6. Conclusions

In this study, we successfully established DHEA-induced female rat PCOS-IR model. We confirmed that in PCOS-IR group the estrus

cycle was halted at estrus stage, serum FINS level and the HOMA-IR index were significantly higher than normal control group. 4 weeks BBR administration, the estrous cycle was recovered, the serum LH, T and FINS levels were significantly decreased. Meanwhile, BBR significantly increasing insulin sensitivity, improving hyperandrogens, reversing abnormal lipid metabolism and protected normal histological structures of ovaries in DHEA-induced PCOS-IR rats. In our next study, further mechanism study will be shown to explore the signaling pathway in BBR treatment. Therefore, BBR may be used as a potential therapeutic agent for clinical PCOS-IR treatment.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Li Li: Conceptualization. **Yao Xiao:** Conceptualization. **Jiahe Zhou:** Data curation. **Hui Mo:** Data curation. **Xiaofang Li:** Formal analysis. **Yuancheng Li:** Formal analysis. **Youfeng Wang:** Methodology. **Minglin Zhong:** Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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