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Therapeutic effects of puerarin on polycystic ovary syndrome

A randomized trial in Chinese women

Wenjing Li, MS*, Hongbo Hu, MS, Guofang Zou, BD, Zhanzhong Ma, MS, Jing Liu, MS, Fanxiang Li, MS

Abstract

Background: This study aims to assess the therapeutic effects of a well-known component (puerarin) obtained from a Chinese herb root in patients with polycystic ovary syndrome (PCOS).

Methods: Women with premature ovarian failure (POF) were assigned to the obese group (body mass index [BMI] ≥24 kg/m^2 and waist hip ratio [WHR] >0.85) or non-obese group (group 3, n=21). Obese patients were further randomly assigned to the obese treatment group (group 1, n=15) and obese control group (group 1, n=15). All patients received standard treatment (Diane-35, 1 tablet/d, orally, plus metformin, 1.5 g/d, orally). In addition to the standard modality, patients in group 1 and group 3 also orally received 150 mg/d of puerarin tablets for 3 months. Venous blood was drawn before and after treatment. Then, the metabolic and antioxidant biomarkers were measured. The normality of distribution of the data was tested using the Kolmogorov–Smirnov method. The baseline characteristics were analyzed using one-factor analysis of variance (ANOVA), and post-hoc was performed using the least significance difference (LSD)-t test.

Results: Significantly improved blood levels of sex hormone binding globulin (SHBG) and superoxide dismutase (SOD) were observed in patients who received the additional treatment of puerarin, regardless of their lean or obese status, while these were not observed in patients who did not receive puerarin. Furthermore, obese patients with PCOS had significantly lower systolic blood pressure, total cholesterol, and testosterone blood levels, when compared with before treatment.

Conclusion: The addition of puerarin to the present treatment protocol can be considered for the management of metabolic disorders and hyperandrogenism in PCOS patients.

Abbreviations: BMI = body mass index, GPX = glutathione peroxidase, HOMA-β = Homeostasis model assessment of β-cell function, PCOS = polycystic ovary syndrome, PRL = prolactin, WHR = waist hip ratio.

Keywords: antioxidant, metabolic disorders, polycystic ovarian syndromes, puerarin

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine/metabolic disorders in women worldwide.[1] Due to its multifaceted etiology, the present management of PCOS mainly targets individual components of the syndrome (hirsutism, oligomenorrhea, infertility, obesity, and glucose intolerance), depending upon the patient's goals. The evidence-based guideline for the first-line non-pharmacologic intervention for most PCOS women is weight loss, which can restore ovulatory cycles and improve metabolic risk.[2,3]

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Data Availability Statement: The datasets analyzed in the present study are available from the corresponding author upon reasonable request.

Ethics approval: The present study was approved by the Ethics Committee of Yuebei People's Hospital, Shaoguan, Guangdong, China (IRB number: ChiCTR 190002220).

Patient consent: Written informed consents were obtained from patients for publication.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Department of Obstetrics and Gynecology, Yuebei People's Hospital, Shaoguan, Guangdong Province, China.

** Correspondence: Wenjing Li, Department of Obstetrics and Gynecology, Yuebei People's Hospital, No.133 HuiMinNan Road, Wu Jiang Area, Shaoguan, Guangdong Province 512025, China (e-mail: maya8301112@163.com).*

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Combined estrogen-progestin oral contraceptives are the mainstay of pharmacologic management for hyperandrogenism and menstrual dysfunction in women with PCOS.[2,4] In contrast to Western countries, Diane-35 (35 μg of ethinyl estradiol and 2 mg of cyproterone acetate) has been widely used to treat hyperandrogenism in patients with PCOS in China.[5,6] Metabolic syndrome is another common phenotype in patients with PCOS. Metformin is an insulin-lowering drug that can improve metabolic disorders in PCOS, and this has gained increasing interest for this group of patients. However, metformin has many side effects, such as gastrointestinal symptoms, and the interference of the intestinal absorption of vitamin B12 and lactic acidosis[7] limit its use in PCOS patients.

Increasing studies have revealed the effectiveness of alternative/traditional Chinese medicine in treating patients with PCOS.[8–10] Puerarin (4′-7-dihydroxy-8-beta-D-glucosylisoflavone) is the major bioactive ingredient of the root of plant Pueraria lobate (Wild) Ohwi, which is known as Gegen (Chinese medicine name). Gegen has also been used for the management of metabolic disorders[11–13] in traditional Chinese medicine since ancient time.[14] These aforementioned studies suggest that puerarin has potential therapeutic effects on metabolism-related disorders, such as those in PCOS patients. However, little is known about its therapeutic efficacy in PCOS patients.

PCOS is associated with a variety of abnormalities, which range from the endocrine system to the antioxidant system. For example, sex hormones (luteinizing hormone [LH] and testosterone) are elevated in women with PCOS, when compared with healthy women.[15] Women with PCOS have insulin resistance and hyperinsulinemia.[16] Hence, they can be predispose to metabolic disorders and hyperadrogenism.[17–19] Oxidative stress is closely correlated to the high incidence of PCOS. It has been reported that PCOS patients present with decreased antioxidant concentrations, and consequently, with increased protein oxidation and an elevated total oxidative state.[20–22] Therefore, it is important to measure biomarkers associated with PCOS, because this may help identify women with PCOS, who would benefit mostly from intervention. The present pilot study investigates the effects of the addition of puerarin to the Diane-35 plus metformin treatment protocol on various biomarkers (sex hormones, metabolic indices, and antioxidant parameters) in patients with PCOS.

Although the majority of women with PCOS are overweight or obese (body mass index [BMI] $\geq 25\ kg/m^2$), a small but significant proportion of patients have normal or low BMI. Furthermore, although most of the clinical signs (e.g., hormonal profiles and insulin resistant rate) are comparable between lean and overweight patients with PCOS,[23–25] lean/non-obese patients may or may not have some of the phenotypes, such as irregular menstrual cycles and acne.[26,27] Therefore, there is clinical relevance to differently consider lean/non-obese and obese patients with PCOS. Accordingly, lean/non-obese and obese patients with PCOS were considered as different subgroups in the present study.

2. Materials and methods

2.1. Patients

All patients with PCOS were of the Han ethnic group. Each participant voluntarily provided an informed consent. The present study was approved by the Ethics Committee of Yuebei People's Hospital, Shaoguan, Guangdong, China (IRB number: ChiCTR 190002220). The diagnosis of PCOS was based on the Rotterdam criteria 2003.[28] Patients with PCOS from January 2014 to December 2016 were recruited for the present study, and their average age was 25.29 ± 6.0 years old (range: 18–39 years old).

Exclusion criteria: adrenal diseases or ovarian tumors that induced elevated testosterone levels; a history of taking oral contraceptives within the recent 3 months; a history of taking antioxidative-related drugs, medication that could affect reproductive endocrine functions, or medication that could affect the metabolism of glucose and lipids; the use of hormonal treatment for ovulation induction in the past 2 months. A total of 84 patients were screened. Among these patients, 9 patients did not meet the above criteria, and 5 patients declined to participate in the trial. Hence, a total of 70 patients were recruited for the study. These patients were assigned into 2 groups, based on their BMI: obese group and non-obese group. If the patient's BMI was $\geq 24\ kg/m^2$ and the waist hip ratio (WHR) was >0.85, the patient was diagnosed as obese. Otherwise, the patient was diagnosed as non-obese. Obese patients were further randomly assigned into 2 groups, based on the order they visited the hospital (even or odd number): obese treatment group (group 1, even number) and obese control group (group 2, odd number). There were 20, 20, and 30 patients in the obese treatment group, obese control group, and non-obese group (group 3), respectively, at the beginning of the study. However, 5, 5, and 9 patients, respectively, withdrew from the study before the end of the trial. The main reason for their withdrawal was that the patient changed her mind, and did not want to continue the study. There was no relationship between patient withdrawal and adverse events. However, one patient without puerarin treatment presented with mild liver injury, based on the blood biomarkers [alanine aminotransferase (ALT) and asparatate aminotransferase (AST)]. This patient was recorded, and withdrawn from the study. Then, this patient was managed for liver recovery. The liver function of this patient was in the trial. Thus, 15, 15, and 21 patients with PCOS in group 1, group 2, and group 3, respectively, completed the study. All participants and healthcare providers were informed of the treatment modality.

2.2. Treatment protocols

All patients received standard treatment for PCOS with Diane-35 (1 tablet/d, orally) plus metformin (1.5 g/d, orally). In addition to the standard modality, patients in group 1 and group 3 orally received 150 mg/d of puerarin tablets. The dose was chosen based on a previous report,[29] which analyzed the results obtained from 32 randomized clinal trials. The treatment lasted for 3 months. After the treatment, these patients were routinely followed up, and were instructed to return to the hospital anytime when they did not feel well, or when any new symptom appeared. Patients had the freedom to withdraw from the study anytime.

Diane-35 was purchased from the Pharmaceutical Division of Bayer in Guangzhou (J20200003, Guangdong, China). Metafomin was purchased from Tianyunshan Pharmaceutical Company (H44023514, Guangzhou, China), and Puerarin was obtained from Guorun Pharmaceutical Company (H14020875. Shanxi, China).

2.3. Biomarkers in blood and measurements

All patients were instructed to fill-in the same questionnaire before and after the 3-month treatment, which included the basic

demographic information, medical history, and items correlated to PCOS symptoms and their health status.

Venous blood was drawn from each participant after fasting for 12 hours within the 2nd to 4th day of their menstrual period. Blood was drawn twice, such as before and after the study. The serum was separated by centrifugation at 4°C. The following parameters were measured in serum: sex hormones, including follicle stimulating hormone, luteinizing hormone and testosterone, prolactin, estrone, estradiol, and sex hormone binding globulin, were measured by fluorescence immunoassay using the Roche Cobas e602, according to manufacturer's instructions (Shanghai RuiQi Biotech, Minhang District, Shanghai); metabolic indices (e.g., fasting blood glucose, fasting insulin, triglyceride, total cholesterol, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol) were measured by ELISA (Shanghai Weimeng Biotech); antioxidant parameters, including superoxide dismutase, glutathione peroxidase, catalase, vitamin C, and vitamin E, were measured by ELISA (Shanghai Weimeng Biotech). The intra- and inter-assay coefficients of variation were <5%. The homeostasis model assessment-insulin resistance (HOMA-IR), insulin sensitivity index (HOMA-IS), and insulin secretion (Homeostasis model assessment of β-cell function [HOMA-β]) were calculated according to the following formula: HOMA-IR = FBG (mM) × FINS (μIU/mL)/22.5; HOMA-IS = 1/(FBG [mM] × FINS [μIU/mL]); HOMA-β = 20 × FINS/(FBG − 3.5). If the HOMA-IR value was >1.47, the patient was diagnosed as insulin resistance.

2.4. Statistical analysis

All data were analyzed using the SPSS 18.0 software (International Business Machines Corporation, Armonk, New York, USA). The normality of distribution of the data was tested using the Kolmogorov–Smirnov method. The baseline characteristics were analyzed by one-factor analysis of variance (ANOVA), and post-hoc was performed using the least significance difference (LSD)-t test. Non-normally distribution data were analyzed using the Kruskal-Wallis H-test. The differences between post-treatment and pre-treatment were analyzed by *t* test or Wilcoxon test, depending on the normality of the data. $P < .05$ was considered statistically significant.

3. Results

3.1. The percentage of patients who withdraw during the study period were similar among the different groups

During the 3-month treatment period, the patient withdrawal rate was 25% (5/20), 25% (5/20), and 30% (9/30) for group 1, group 2, and group 3, respectively. There was no significant difference in withdrawal rate among these groups (chi-square = 0.217, $P = .897$).

3.2. Patient baseline characteristics

In addition to the BMI, the WHR, prolactin (PRL), HOMA-β, and glutathione peroxidase (GPX) levels were significantly different in the obese groups (group 1 and group 2), when compared with group 3 ($P < .05$, Table 1). When compared with group 3 (4.99 ± 0.65 mM), patients in group 1 had significantly higher fasting blood glucose (FBG) levels (5.56 ± 0.57 mM, $P < .05$). In contrast, patients in group 2 had significantly lower levels of vitamin E (VE), when compared with patients in group 3 ($P < .05$, Table 1). The comparisons between group 1 and group 2 revealed that patients in group 1 had significantly increased levels of sex hormone binding globulin (SHBG), FBG, and total cholesterol (TC) ($P < .05$, Table 1).

3.3. Comparisons between groups after treatment

After the 3-month treatment with different protocols, patients with PCOS, who were treated with the addition of puerarin, exhibited significant differences in biomarkers related to hormone levels and antioxidative activities, when compared with patients in group 2 (Table 2). For example, obese patients treated with the addition of puerarin had significantly lower T levels (median, 10.40 nM), when compared with patients in group 2 (mean, 16.25 nM) who did take puerarin, ($P < .001$). Patients in groups 1 and 3 had significantly higher levels of high-density lipoprotein-cholesterol (HDL-C) and GPX, when compared with patients in group 2 ($P < .001$).

3.4. Comparison of changes caused by the treatment among the different groups of patients with PCOS

Compared with patients in group 2, patients in group 1 and group 3 were treated with the addition of puerarin to the standard modality (Diane-35 plus metformin). As shown in Table 3, patients in group 1 and group 3 exhibited significant changes in SHBG, TC, and superoxide dismutase (SOD) levels, when compared with those before treatment, while these were not observed in patients in group 2.

The investigation of the effects of puerarin in obese PCOS patients revealed that the addition of puerarin in the treatment protocol significantly decreased the systolic blood pressure (SP) and testosterone (T) blood levels, when compared with those before treatment (indicated with a superscripted letter c, Table 3). However, these effects were not observed in patients in group 2. The therapeutic effects of the addition of puerarin between obese and non-obese PCOS patients were also compared. Significantly increased PRL levels were observed in non-obese patients after puerarin treatment, when compared with before treatment, but this was not observed in patients in group 1 (Table 3).

4. Discussion

To the best of our knowledge, the present study is the first clinical trial to assess the therapeutic efficacy of puerarin in Chinese women with PCOS. It was found that the addition of puerarin to Diane-35 plus metformin significantly increased the blood levels of SHBG and SOD, when compared with those before treatment, in both obese and non-obese PCOS patients. In addition, obese patients with PCOS had significantly lower SP, total cholesterol, and T blood levels, when compared with those before treatment. The findings of the present study suggest that puerarin can be an adjunctive to the standard treatment protocol for Chinese women with PCOS.

Although most patients with PCOS were overweight/obese, a significant number of patients were within the normal body weight. These 2 populations of patients may or may not have different symptoms.[26,27] In the present study, the baseline demographics revealed that obese patients had significantly higher levels of SHBG, but had lower levels of HOMA-IS and GPX, when compared with lean patients. Furthermore, after

Table 1

Baseline characteristics of patients with PCOS (N = 51).

Variables		Group 1 (n = 15)	Group 2 (n = 15)	Group 3 (n = 21)
Basic health status				
Age, y	$\overline{X} \pm SD$		26.40 ± 6.38	24.48 ± 5.70
Marital status n (%)	Single	6 (40.00)	5 (33.33)	13 (61.90)
	Married	9 (60.00)	10 (66.67)	8 (38.10)
BMI, kg/m^2	$\overline{X} \pm SD$	**29.22 ± 4.63**[a]	**28.06 ± 5.25**[a]	19.45 ± 2.17
WHR	$\overline{X} \pm SD$	**0.94 ± 0.06**[a]	**0.93 ± 0.03**[a]	0.78 ± 0.04
Clinical signs/symptoms				
Infertility n (%)	Yes	5 (33.33)	4 (26.67)	3 (14.29)
	No	10 (66.67)	11 (73.33)	18 (85.71)
Menarche age, y	Median (P_{25}, P_{75})	14.00 (12.00, 15.00)	13.00 (12.00, 13.00)	13.00 (13.00, 14.00)
Menstrual cycle n (%)	Normal	1 (6.67)	3 (20.00)	5 (23.81)
	Abnormal	14 (93.33)	12 (80.00)	16 (76.19)
Menstrual period n (%)	Normal (3–7 days)	9 (60.00)	8 (53.33)	8 (38.10)
	Abnormal (>7 days)	6 (40.00)	7 (46.67)	13 (61.90)
Amenorrhorea n (%)	Yes	**8 (53.33)**[a, b]	2 (13.33)	5 (23.81)
	No	7 (46.67)	13 (86.67)	16 (76.19)
Irregular vaginal bleeding	Yes	3 (15.00)	9 (45.00)	10 (33.33)
n (%)	No	17 (85.00)	11 (55.00)	20 (66.67)
Oligomenorrhea n (%)	Yes	18 (90.00)	16 (80.00)	17 (56.67)
	No	2 (10.00)	4 (20.00)	13 (43.33)
SP, mm Hg	$\overline{X} \pm SD$	113.10 ± 14.46	112.90 ± 10.24	106.53 ± 10.50
DP, mm Hg	$\overline{X} \pm SD$	74.00 ± 9.81	70.45 ± 8.67	70.20 ± 7.10
Hirsutism n (%)	Yes	**11 (55.00)**[b]	18 (90.00)	21 (70.00)
	No	9 (45.00)	2 (10.00)	9 (30.00)
Acne n (%)	Yes	5 (25.00)	5 (25.00)	16 (53.33)
	No	15 (75.00)	15 (75.00)	14 (46.67)
Acanthosis nigricans n (%)	Yes	11 (55.00)	8 (40.00)	7 (23.33)
	No	9 (45.00)	12 (60.00)	23 (76.67)
Hormones				
Testosterone (T), nM	Median (P_{25}, P_{75})	19.26 (11.05, 47.87)	18.22 (13.03, 33.77)	16.24 (13.09, 19.94)
SHBG, nM	$\overline{X} \pm SD$	64.62 ± 15.67	66.18 ± 21.49	67.62 ± 21.40
PRL, ng/L	Median (P_{25}, P_{75})	**797.44**[a,b] (714.94, 873.41)	**710.81**[a] (614.87, 779.47)	1644.00 (1378.55, 1877.85)
E1, nM	Median (P_{25}, P_{75})	21.82 (20.00, 199.93)	20.00 (20.00, 93.63)	25.43 (20.00, 707.42)
E2, nM	Median (P_{25}, P_{75})	24.52 (17.35, 64.71)	24.97 (22.17, 31.42)	45.65 (21.69, 118.19)
E1/E2 n (%)	>1	12 (60.00)	10 (50.00)	16 (61.54)
	≤1	8 (40.00)	10 (50.00)	10 (38.46)
LH, mM	Median (P_{25}, P_{75})	13.77 (5.31, 33.19)	11.00 (7.29, 22.39)	14.19 (7.24, 45.81)
FSH, mM	Median (P_{25}, P_{75})	10.85 (7.59, 30.20)	10.56 (7.67, 21.01)	12.21 (6.30, 34.03)
LH/FSH	2 to 3	2 (10.00)	4 (20.00)	3 (10.00)
	other	18 (90.00)	16 (80.00)	27 (90.00)
Metabolic status				
FBG, mM	Mean ± SD	**5.56 ± 0.57**[a, b]	5.20 ± 0.41	4.99 ± 0.65
FBI, mIU/L	Median (P_{25}, P_{75})	14.29 (12.09, 22.55)	13.92 (11.24, 19.19)	19.81 (10.94, 25.98)
HOMA-IR	Median (P_{25}, P_{75})	3.49 (2.85, 6.31)	3.40 (2.75, 4.53)	4.24 (2.26, 5.99)
HOMA-IS	Median (P_{25}, P_{75})	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)
HOMA-β	Median (P_{25}, P_{75})	**160.50**[a] (131.13, 236.68)	**163.79**[a] (124.20, 201.63)	261.32 (165.88, 505.55)
TC, mM	Mean ± SD	**3.89 ± 1.76**[b]	2.77 ± 1.22	4.33 ± 1.42
TG, mM	Median (P_{25}, P_{75})	3.22 (3.06, 6.04)	3.30 (2.87, 4.93)	4.215 (2.89, 7.27)
HDL-C, ng/L	$\overline{X} \pm SD$	556.43 ± 258.31	625.92 ± 256.90	712.76 ± 279.85
LDL-C, ng/L	$\overline{X} \pm SD$	134.09 ± 37.65	93.34 ± 55.48	137.00 ± 94.15
Antioxidative				
SOD, U/mL	$\overline{X} \pm SD$	34.66 ± 17.32	48.26 ± 23.07	47.46 ± 20.55
CAT, U/L	$\overline{X} \pm SD$	7.26 ± 2.91	5.63 ± 2.35	9.27 ± 7.50
GPX, ng/L	$\overline{X} \pm SD$	**636.98 ± 278.05**[a]	**574.28 ± 295.91**[a]	1066.01 ± 458.33
VC, ng/L	$\overline{X} \pm SD$	1169.68 ± 491.98	1185.00 ± 414.31	1261.19 ± 459.33
VE, mM	$\overline{X} \pm SD$	855.12 ± 287.12	**765.91 ± 357.25**[a]	1080.97 ± 532.48

BMI = body mass index, CAT = catalase, DP = diastolic blood pressure, FBG = fasting blood glucose, FINS = fasting insulin, FSH = follicle stimulating hormone, GPX = glutathione peroxidase, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = homeostasis models assessment-insulin resistance index, LDL-C = low-density lipoprotein-cholesterol, LH = luteinizing hormone, SOD = superoxide dismutase, SP = systolic blood pressure, T = testosterone, TC = total cholesterol, TG = triglyceride, VC = vitamin C, VE = vitamin E, WHR = waist hip ratio.

[a], significant difference compared with group 3 (non-obese treatment group), $P < .05$; b, significant difference between group 1 and group 2 (obese control group), $P < .05$.

Table 2

Comparisons of markers between the different groups of patients with PCOS after treatment.

Variables		Group 1 (n = 15)	Group 2 (n=15)	Group 3 (n=21)	F/χ^2		P
Basic health status and symptoms							
BMI	$\overline{X}\pm$SD	29.22±4.43	27.91±5.20	19.25±1.82	26.410	a	<.001
WHR	$\overline{X}\pm$SD	0.93±0.08	0.94±0.04	0.80±0.04	32.702	a	<.001
Menstrual cycle	Normal(21–35 d)	14 (93.33)	13 (86.67)	15 (100.00)	2.143	d	.343
	Abnormal	1 (6.67)	2 (13.33)	0 (0.00)			
Menstrual period	Normal(3–7d)	13 (86.67)	10 (66.67)	9 (60.00)	2.813	d	.245
	Abnormal	2 (13.33)	5 (33.33)	6 (40.00)			
Amenorrhorea	No (n, %)	15 (100.00)	15 (100.00)	15 (100.00)			
Irregular vaginal bleeding	No (n, %)	15 (100.00)	15 (100.00)	15 (71.43)			
SP, mm Hg	$\overline{X}\pm$SD	108.87±12.36	111.27±11.33	107.00±13.86	0.435	a	.650
DP, mm Hg	$\overline{X}\pm$SD	76.07±7.97	72.60±5.11	73.67±10.21	0.732	a	.487
Hirsutism (n, %)	Yes	8 (53.33)	**13 (86.67)**	**5 (33.33)***	8.927	d	.012
	No	7 (46.67)	2 (13.33)	10 (66.67)			
Acne n (%)	Yes	5 (33.33)	4 (26.67)	3 (20.00)	0.682	d	.711
	No	10 (66.67)	11 (73.33)	12 (80.00)			
Acanthosis migrican n (%)	Yes	6 (40.00)	4 (26.67)	2 (13.33)	2.727	d	.256
	No	9 (60.00)	11 (73.33)	13 (86.67)			
Hormones							
T, nM	Median (P_{25}, P_{75})	**10.40 (9.74, 12.75)****	**16.25 (12.95, 22.61)**	**16.63 (14.46, 21.59)**	17.034	c	<.001
SHBG, nM	$\overline{X}\pm$SD	77.61±19.98	76.97±10.37	88.64±16.13	3.115	a	.053
PRL, ng/L	Median (P_{25}, P_{75})	**814.36 (535.19, 955.22)**	**782.87 (557.39, 954.59)**	**1015.17 (911.13, 1230.51)***	16.267	c	<.001
E1, nM	Median (P_{25}, P_{75})	65.70 (20.00, 123.00)	80.50 (20.00, 443.50)	28.77 (20.00, 178.30)	1.203	c	.548
E2, nM	Median (P_{25}, P_{75})	**22.06 (20.36, 41.53)****	**39.84 (25.23, 60.23)**	**37.77 (24.83, 95.78)**	7.164	c	.028
E1/E2 n (%)	>1	11 (73.33)	9 (60.00)	10 (47.62)	2.401	d	.301
	≤1	4 (26.67)	6 (40.00)	11 (52.38)			
LH, mM	Median (P_{25}, P_{75})	7.97 (5.95, 19.27)	13.22 (4.61, 21.17)	19.91 (12.49, 34.76)	7.477	c	.024
FSH, mM	Median (P_{25}, P_{75})	13.95 (11.46, 16.94)	13.93 (9.24, 18.81)	21.21 (8.45, 35.84)	1.457	c	.483
LH/FSH	2–3	3 (20.00)	2 (13.33)	6 (28.57)	1.232	d	.540
	other	12 (80.00)	13 (86.67)	15 (71.43)			
Metabolic status							
FBG, mM	$\overline{X}\pm$SD	5.32±0.45	5.32±0.49	4.93±0.72	2.702	a	.077
FBI, mIU/L	Median (P_{25}, P_{75})	12.75 (12.02, 15.74)	11.46 (8.22, 14.44)	14.00 (11.04, 20.40)	4.095	c	.129
HOMA-IR	Median (P_{25}, P_{75})	2.95 (2.77, 3.55)	2.94 (1.86, 3.30)	2.99 (2.52, 4.20)	2.268	c	.322
HOMA-IS	Median (P_{25}, P_{75})	0.02 (0.01, 0.02)	0.02 (0.01, 0.02)	0.01 (0.01, 0.02)	2.252	c	.324
HOMA-β	Median (P_{25}, P_{75})	149.97 (127.10, 183.42)	102.75 (95.50, 206.27)	270.20 (144.23, 323.24)	10.564	c	.005
TC, mM	$\overline{X}\pm$SD	**2.59±1.37****	2.49±6.93	**2.94±1.61**	0.585	a	.561
TG, mM	Median (P_{25}, P_{75})	**3.68 (2.48, 3.79)****	**5.58 (2.97, 7.93)**	**5.10 (3.64, 8.12)***	10.539	c	.005
HDL-C, ng/L	$\overline{X}\pm$SD	**921.67±274.59**	**790.32±226.95**	**960.76±369.99**	1.409	a	.254
LDL-C, ng/L	$\overline{X}\pm$SD	**60.89±27.86****	**149.60±24.82**	**101.08±58.56***	16.139	a	<.001
Antioxidative							
SOD, U/mL	$\overline{X}\pm$SD	58.24±22.97	**47.52±18.23**	**70.02±17.52**	5.932	a	.005
CAT, U/L	$\overline{X}\pm$SD	14.91±4.39	16.70±9.80	11.28±8.01	2.292	a	.112
GPX, ng/L	$\overline{X}\pm$SD	**713.40±353.65**	**594.42±257.67**	**1183.84±329.10***	17.704	a	<.001
VC, ng/L	$\overline{X}\pm$SD	1569.17±539.97	1653.55±432.10	2011.93±658.16	3.181	a	.050
VE, mM	$\overline{X}\pm$SD	1419.15±351.59	**1191.63±365.73**	**1769.24±816.43***	4.317	a	.019

BMI = body mass index, CAT = catalase, DP = diastolic blood pressure, FBG = fasting blood glucose, FINS = fasting insulin, FSH = follicle stimulating hormone, GPX = glutathione peroxidase, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = homeostasis models assessment-insulin resistance index, LDL-C = low-density lipoprotein-cholesterol, LH = luteinizing hormone, SOD = superoxide dismutase, SP = systolic blood pressure, T = testosterone, TC = total cholesterol, TG = triglyceride, VC = vitamin C, VE = vitamin E; WHR = waist hip ratio; a, one-way ANOVA test; b, Pearson chi-square test; c, Kruskal–Wallis H test. *, significantly different from group 1 or group 2, or both if in bold. **, significantly different from group 2 or group 3 or both groups, if in bold.

treatment with the same protocol, obese patients exhibited different responses to the drugs, when compared with lean patients. Therefore, the present study and others studies[26,27] suggest that clinicians should consider that obese patients with PCOS differ from patients with normal body weight, when initiating pharmaceutical interventions.

Metabolic disorders and hyperandrogenisms are very common features in patients with PCOS. Metabolic disorders can be managed with metformin and thiazolidinediones, which decrease insulin and androgen levels in patients.[30] However, the known side effects associated with these drugs have encouraged the exploration of alternative medicines for treating PCOS patients. Thus, the present clinical trial was conducted on a small number of patients with PCOS, and these patients were treated with puerarin in conjunction with metformin and Diane-35. It was found that the addition of puerarin to the present treatment protocol significantly increased the SHBG levels, when compared with patients without the addition of puerarin during treatment. SHBG is an important biomarker for predicting PCOS risk, with lower SHBG levels significantly associated to increased risk of PCOS.[31] PCOS women with low levels of SHBG would more likely have hyperandrogenism, diabetes mellitus 2 (DM2), insulin

Table 3

Comparisons of the differences between after treatment and before treatment, with or without the addition of puerarin.

Variables		Group 1			Group 2			Group 3		
		Change	*t*/*Z*	*P*	Change	*t*/*Z*	*P*	Change	*t*/*Z*	*P*
Basic health status										
BMI, kg/m^2	$\overline{X}\pm$SD	0.01 ± 1.93	0.013	.990	−0.14 ± 0.82	−0.667	.515	0.48 ± 1.07	1.753	.101
WHR	$\overline{X}\pm$SD	−0.01 ± 0.04	−0.840	.415	0.01 ± 0.01	3.836	**.002**[c]	0.01 ± 0.02	1.356	.196
SP, mm Hg	$\overline{X}\pm$SD	−3.67 ± 6.56	−2.163	**.048**[c]	−2.00 ± 7.04	−1.100	.290	1.40 ± 14.41	0.376	.712
DP, mm Hg	$\overline{X}\pm$SD	1.33 ± 6.76	0.764	.457	0.93 ± 5.15	0.702	.494	3.13 ± 12.03	1.008	.330
Sex hormones										
T, nM	$\overline{X}\pm$SD	**−15.36 ± 16.49**[a]	−3.608	**.003**[c]	−4.13 ± 11.93	−1.341	.201	−0.40 ± 9.18	−0.198	.845
SHBG, nM	$\overline{X}\pm$SD	13.17 ± 17.39	2.933	**.011**[c]	8.51 ± 25.72	1.282	.221	19.49 ± 21.57	4.140	**<.001**[c]
PRL, ng/L	$\overline{X}\pm$SD	−36.34 ± 205.59 [a]	−0.685	.505	**13.87 ± 275.76**[a]	0.195	.848	−527.41 ± 385.70	−6.266	**<.001**[c]
E1, nM	$\overline{X}\pm$SD	−76.96 ± 254.07	−1.173	.260	97.99 ± 296.07	1.282	.221	−89.60 ± 330.74	−1.241	.229
E2, nM	$\overline{X}\pm$SD	−18.07 ± 42.46	−1.649	.121	12.93 ± 31.26	1.602	.132	−10.80 ± 63.09	−0.785	.442
LH, mM	$\overline{X}\pm$SD	−16.53 ± 35.51	−1.802	.093	−3.14 ± 21.45	−0.567	.580	−2.73 ± 28.71	−0.436	.667
FSH, mM	Median (P_{25}, P_{75})	0.19 (-19.91,6.02)	−0.227	.820	1.61 (−5.97,4.45)	−0.568	.570	0.00 (−6.28,1.68)	−0.625	.532
Metabolic status										
FBG, mM	$\overline{X}\pm$SD	−0.19 ± 0.75	−0.963	.352	0.07 ± 0.42	0.614	.549	−0.07 ± 0.88	−0.389	.702
FINS, mIU/L	$\overline{X}\pm$SD	−2.70 ± 7.12	−1.470	.164	−3.13 ± 5.40	−2.243	**.042**[c]	−2.93 ± 6.38	−2.103	**.048**[c]
HOMA-IR	$\overline{X}\pm$SD	−0.81 ± 2.21	−1.423	.177	−0.73 ± 1.29	−2.174	**.047**[c]	−0.89 ± 1.67	−2.431	**.025**[c]
HOMA-IS	Median (P_{25}, P_{75})	0.0010 (−0.0005,0.0040)	−1.351	.177	0.0027 (−0.0036,0.0116)	−1.250	.211	0.0020 (−0.0003,0.0049)	−1.494	.135
HOMA-β (%)	$\overline{X}\pm$SD	−10.59 ± 59.81	−0.685	.504	−33.32 ± 89.93	−1.435	.173	−56.34 ± 256.50	−1.006	.326
TC, mM	$\overline{X}\pm$SD	−11.30 ± 18.32	−2.388	**.032**[c]	−3.58 ± 12.69	−1.093	.293	−14.59 ± 23.82	−2.806	**.011**[c]
TG, mM	$\overline{X}\pm$SD	**−6.34 ± 22.01**[b]	−1.115	.284	15.11 ± 24.67	2.372	**.033**[c]	5.45 ± 22.24	1.122	.275
HDLC, ng/L	$\overline{X}\pm$SD	429.46 ± 418.07	3.978	**.001**[c]	186.76 ± 227.33	3.182	**.007**[c]	172.78 ± 311.63	2.541	**.019**[c]
LDLC, ng/L	$\overline{X}\pm$SD	**−70.16 ± 33.88**[b]	−8.020	**<.001**[c]	**55.21 ± 62.40**[a]	3.426	**.004**[c]	−64.84 ± 102.19	−2.907	**.009**[c]
Antioxidant biomarkers										
SOD, U/mL	$\overline{X}\pm$SD	21.15 ± 21.66	3.781	**.002**[c]	−1.98 ± 32.66	−0.235	.817	23.98 ± 25.61	4.290	**<.001**[c]
CAT, U/L	$\overline{X}\pm$SD	**7.96 ± 5.41**[a]	5.702	**<.001**[c]	**10.78 ± 10.71**[a]	3.897	**.002**[c]	0.82 ± 6.21	0.603	.553
GPX, ng/L	$\overline{X}\pm$SD	147.72 ± 492.45	1.162	.265	29.12 ± 268.40	0.420	.681	62.05 ± 490.30	0.580	.568
VC, ng/L	$\overline{X}\pm$SD	330.57 ± 715.97	1.788	.095	426.72 ± 594.55	2.780	**.015**[c]	782.60 ± 680.67	5.269	**<.001**[c]
VE, mM	$\overline{X}\pm$SD	533.14 ± 427.86	4.826	**<.001**[c]	438.36 ± 610.30	2.782	**.015**[c]	696.20 ± 865.67	3.685	**.001**[c]

BMI = body mass index, CAT = catalase, DP = diastolic blood pressure, FBG = fasting blood glucose, FINS = fasting insulin, FSH = follicle stimulating hormone, GPX = glutathione peroxidase, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = homeostasis models assessment-insulin resistance index, LDL-C = low-density lipoprotein-cholesterol, LH = luteinizing hormone, SOD = superoxide dismutase, SP = systolic blood pressure, T = testosterone, TC = total cholesterol, TG = triglyceride, VC = vitamin C, VE = vitamin E, WHR = waist hip ratio. a, significant difference compared with group 3 (non-obese treatment group), $P < .05$; b, significant difference between group 1 and group 2 (obese control group), $P < .05$; c, significant difference after treatment, when compared with before treatment, $P < .05$.

resistance, and obesity.[32,33] A low SHBG level is considered a biomarker of hyperandrogenism and insulin resistance.[34,35] The inverse association between the SHBG level and metabolic syndromes was reported by a study,[36] and this was further observed in patients with or without PCOS.[37,38] Thus, the increased levels of SHBG in PCOS women treated with the addition of puerarin can improve the metabolic disorders and other symptoms associated with PCOS. It is warranted to follow-up these patients for a longer period of time in the future to verify the benefits of the puerarin treatment. A significantly decreased T level was also found in obese patients after treatment with the addition of puerarin, when compared with before treatment. This could be partially explained by the elevated levels of SHBG. Therefore, the addition of puerarin to the present treatment can improve the hormonal profile and metabolic syndromes in patients with PCOS, although the underling mechanism on how puerarin affects SHBG remains unclear.

Insufficient antioxidative activities are accompanied with increased levels of oxidative stress,[39] and are associated with various diseases.[40,41] Studies have revealed the increase in circulating markers of oxidative stress (e.g., reactive oxygen species, ROS) in PCOS patients.[41,42] Oxidative stress has been reported to play an important role in the pathogenesis of PCOS.[43] SOD is an important antioxidant enzyme. A significant decrease in SOD activity was observed in PCOS patients, when compared with the control group.[44] In the present study, it was found that there was a markedly increased level of SOD in patients after treatment with the addition of puerarin, regardless of the BMI status, when compared with patients who were not treated with puerarin. These present results indicate that puerarin has an antioxidant activity, which can be beneficial to PCOS patients. In a rat model, the pretreatment with puerarin was shown to block the production of free radicals induced by H_2O_2, and stimulate SOD activity in isolated pancreatic islets.[45] It has also been reported that puerarin can increase the activities of other antioxidative enzymes (e.g., GPX and glutathione S-transferase, GST) in lead-treated rats.[46] These studies further support the present findings on the antioxidant property of puerarin in patients with PCOS.

Puerarin has been shown to cause changes in lipid profiles. For example, puerarin interferes with copper and induces LDL oxidation in vitro,[47] enhances the production of HDL, and decreases the production of LDL and total cholesterol.[12] The effect of puerarin on lipid metabolism can be partially due to its ability to elevate the cholesterol 7α-hydroxylase mRNA expression,[48] which can reduce the serum cholesterol content.[11] In the present study, it was found that all patients with PCOS had increased levels of high density of lipoprotein cholesterol (HDLC)

after treatment, with or without the addition of puerarin, when compared with before treatment. However, the level of TG significantly decreased in obese patients treated with the addition of puerarin, when compared with obese patients without puerarin. It was also found that the level of TC significantly deceased in obese patients after treatment with puerarin, when compared with before treatment. On the contrary, non-obese patients had increased levels of TC after treatment with the addition of puerarin, when compared with before treatment. These present findings suggest that puerarin has different effects on lipid profiles in obese and non-obese patients with PCOS. Hence, these should be considered when prescribing puerarin for these patients. On the other hand, these present findings further confirm the essence to consider obese patients differently from non-obese patients with PCOS in the clinical setting.

There were some limitations in the present study. First, the relatively small sample size may have limited the present findings from being applied to populations with PCOS. Further studies with more patients from different areas should be conducted in the future. Second, the interaction between puerarin and Diane-35 plus metformin could not be excluded, which could affect the present findings on the treatment effects of puerarin in PCOS patients. A group of patients with PCOS treated with prerarin would be the only solution. However, there would be an ethical issue to treat patients without initially using first-line drugs.

In conclusion, the present study revealed that the addition of puerarin to the present treatment protocol in Chinese patients with PCOS mainly improved the blood levels of SHBG and SOD, and lowered the systolic blood pressure, total cholesterol, and testosterone blood levels of obese patients with PCOS. However, the possibility of the synergistic effect of puerarin with other drugs cannot excluded. In order to solve the problem, a group of patients with PCOS can be treated with puerarin only after careful ethic consideration. Further studies with larger and diverse populations of patients with PCOS are needed.

Author contributions

Conceptualization: Wenjing Li.
Data curation: Wenjing Li, Guofang Zou.
Formal analysis: Zhanzhong Ma, Jing Liu.
Investigation: Fanxiang Li.
Methodology: Wenjing Li.
Supervision: Hongbo Hu.
Validation: Jing Liu.
Writing – original draft: Wenjing Li.
Writing – review & editing: Hongbo Hu.

References

[1] Azziz R. PCOS in 2015: new insights into the genetics of polycystic ovary syndrome. Nat Rev Endocrinol 2016;12:74–5.
[2] Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2013;98:4565–92.
[3] Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril 2018;110: 364–79.
[4] Martin KA, Anderson RR, Chang RJ, et al. Evaluation and treatment of Hirsutism in premenopausal women: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018;103:1233–57.
[5] Ruan X, Song J, Gu M, Wang L, Wang H, Mueck AO. Effect of Diane-35, alone or in combination with orlistat or metformin in Chinese polycystic ovary syndrome patients. Arch Gynecol Obstet 2018;297: 1557–63.
[6] Wei SY, Chen Y, Xu XY. Progress on the pharmacological research of puerarin: a review. Chin J Nat Med 2014;12:407–14.
[7] Niafar M, Hai F, Porhomayon J, Nader ND. The role of metformin on vitamin B12 deficiency: a meta-analysis review. Intern Emerg Med 2015;10:93–102.
[8] Arentz S, Abbott JA, Smith CA, Bensoussan A. Herbal medicine for the management of polycystic ovary syndrome (PCOS) and associated oligo/amenorrhoea and hyperandrogenism; a review of the laboratory evidence for effects with corroborative clinical findings. BMC Complement Altern Med 2014;14:511.
[9] Raja-Khan N, Stener-Victorin E, Wu X, Legro RS. The physiological basis of complementary and alternative medicines for polycystic ovary syndrome. Am J Physiol Endocrinol Metab 2011;301:E1–0.
[10] Stefanaki C, Bacopoulou F, Livadas S, et al. Impact of a mindfulness stress management program on stress, anxiety, depression and quality of life in women with polycystic ovary syndrome: a randomized controlled trial. Stress 2015;18:57–66.
[11] Hsu FL, Liu IM, Kuo DH, Chen WC, Su HC, Cheng JT. Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. J Nat Prod 2003;66:788–92.
[12] Zhou YX, Zhang H, Peng C. Puerarin: a review of pharmacological effects. Phytother Res 2014;28:961–75.
[13] Teng Y, Cui H, Yang M, et al. Protective effect of puerarin on diabetic retinopathy in rats. Mol Biol Rep 2009;36:1129–33.
[14] Xie W, Du L. Diabetes is an inflammatory disease: evidence from traditional Chinese medicines. Diabetes Obes Metab 2011;13:289–301.
[15] Koutlaki N, Dimitraki M, Zervoudis S, et al. The relationship between Anti-Müllerian hormone and other reproductive parameters in normal women and in women with polycystic ovary syndrome. J Med Life 2013;6:146–50.
[16] Stepto NK, Cassar S, Joham AE, et al. Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp. Hum Reprod 2013;28:777–84.
[17] Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2010;16: 347–63.
[18] Hudecova M, Jan H, Christian B, Poromaa Inger S. Long-term reproductive and metabolic consequences of PCOS. Curr Diabetes Rev 2012;8:444–51.
[19] Teede HJ, Hutchison SK, Zoungas S. The management of insulin resistance in polycystic ovary syndrome. Trends Endocrinol Metab 2007;18:273–9.
[20] Palacio JR, Iborra A, Ulcova-Gallova Z, Badia R, Martínez P. The presence of antibodies to oxidative modified proteins in serum from polycystic ovary syndrome patients. Clin Exp Immunol 2006;144: 217–22.
[21] Jeelani H, Ganie M, Parvez T, Fatima Q, Kawa I, Rashid F. Oxidative stress biomarkers in polycystic ovary syndrome (PCOS). PMJ 2017;2:30–8.
[22] Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update 2013;19:268–88.
[23] Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev 2012;33:981–1030.
[24] Doh E, Mbanya A, Kemfang-Ngowa JD, et al. The relationship between adiposity and insulin sensitivity in African women living with the polycystic ovarian syndrome: a clamp study. Int J Endocrinol 2016;2016: 9201701.
[25] Saxena P, Prakash A, Nigam A, Mishra A. Polycystic ovary syndrome: is obesity a sine qua non? A clinical, hormonal, and metabolic assessment in relation to body mass index. Indian J Endocrinol Metab 2012;16:996–9.
[26] Nestler JE, Jakubowicz DJ. Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. J Clin Endocrinol Metab 1997;82: 4075–9.
[27] Williams RM, Ong KK, Dunger DB. Polycystic ovarian syndrome during puberty and adolescence. Mol Cell Endocrinol 2013;373:61–7.
[28] Rotterdam EA-SPcwgRevised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41–7.

[29] Zheng QH, Li XL, Mei ZG, et al. Efficacy and safety of puerarin injection in curing acute ischemic stroke: a meta-analysis of randomized controlled trials. Medicine (Madr) 2017;96:e5803.
[30] Sepilian V, Nagamani M. Effects of rosiglitazone in obese women with polycystic ovary syndrome and severe insulin resistance. J Clin Endocrinol Metab 2005;90:60–5.
[31] Deswal R, Yadav A, Dang AS. Sex hormone binding globulin - an important biomarker for predicting PCOS risk: A systematic review and meta-analysis. Syst Biol Reprod Med 2018;64:12–24.
[32] Behboudi-Gandevani S, Ramezani Tehrani F, Rostami Dovom M, et al. Insulin resistance in obesity and polycystic ovary syndrome: systematic review and meta-analysis of observational studies. Gynecol Endocrinol 2016;32:343–53.
[33] Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2012;18:618–37.
[34] Nestler JE. Sex hormone-binding globulin: a marker for hyperinsulinemia and/or insulin resistance? J Clin Endocrinol Metab 1993;76:273–4.
[35] Preziosi P, Barrett-Connor E, Papoz L, et al. Interrelation between plasma sex hormone-binding globulin and plasma insulin in healthy adult women: the telecom study. J Clin Endocrinol Metab 1993;76:283–7.
[36] Goodman-Gruen D, Barrett-Connor E. Sex hormone-binding globulin and glucose tolerance in postmenopausal women. The Rancho Bernardo Study. Diabetes Care 1997;20:645–9.
[37] Chen MJ, Yang WS, Yang JH, et al. Low sex hormone-binding globulin is associated with low high-density lipoprotein cholesterol and metabolic syndrome in women with PCOS. Hum Reprod 2006;21:2266–71.
[38] Fenske B, Kische H, Gross S, et al. Endogenous androgens and sex hormone-binding globulin in women and risk of metabolic syndrome and type 2 diabetes. J Clin Endocrinol Metab 2015;100:4595–603.
[39] Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003;552:335–44.
[40] Insenser M, Montes-Nieto R, Murri M, Escobar-Morreale HF. Proteomic and metabolomic approaches to the study of polycystic ovary syndrome. Mol Cell Endocrinol 2013;370:65–77.
[41] Murri M, Luque-Ramirez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update 2013;19:268–88.
[42] Gonzalez F, Rote NS, Minium J, Kirwan JP. Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:336–40.
[43] Papalou O, Victor VM, Diamanti-Kandarakis E. Oxidative stress in polycystic ovary syndrome. Curr Pharm Des 2016;22:2709–22.
[44] Seleem AK, El Refaeey AA, Shaalan D, et al. Superoxide dismutase in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection. J Assist Reprod Genet 2014;31:499–504.
[45] Xiong FL, Sun XH, Gan L, Yang XL, Xu HB. Puerarin protects rat pancreatic islets from damage by hydrogen peroxide. Eur J Pharmacol 2006;529:1–7.
[46] Liu CM, Ma JQ, Sun YZ. Puerarin protects the rat liver against oxidative stress-mediated DNA damage and apoptosis induced by lead. Exp Toxicol Pathol 2012;64:575–82.
[47] Liu R, Meng F, Bai H, Liu Y, Tang CW, Liu BW. Inhibitory effect of quercetin, rutin and puerarin on LDL oxidation induced by Cu2+. Zhongguo Zhong Yao Za Zhi 2007;32:2058–62.
[48] Chung MJ, Sung NJ, Park CS, et al. Antioxidative and hypocholesterolemic activities of water-soluble puerarin glycosides in HepG2 cells and in C57BL/6J mice. Eur J Pharmacol 2008;578:159–70.