

Original Research Article

Effect of quercetin on biochemical parameters in letrozole-induced polycystic ovary syndrome in rats

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Abstract

Purpose: To investigate the effect of quercetin, a natural flavonoid, on biochemical parameters in letrozole-induced polycystic ovary syndrome (PCOS) in rats

Methods: Oral glucose test was performed with the aid of a glucometer. Estradiol, testosterone and steroidogenic enzyme activities were determined using standard protocols. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities were determined by standard methods. Histological analysis was carried out with haematoxylin and eosin (H&E) staining.

Results: Quercetin exerted protective effects against PCOS in the rat model by enhancing the levels of antioxidant enzymes, viz, CAT, SOD and GPX. Quercetin also prevented weight gain, and caused significant decline in serum glucose levels in PCOS rats. Furthermore, quercetin normalised estradiol and testosterone levels, as well as steroidogenic enzyme activities, but blocked letrozole-induced abnormalities in PCOS. It also exerted protective effects on the anatomy of the ovaries.

Conclusion: These results indicate that quercetin exerts protective effects on letrozole-induced PCOS in rats. Thus, quercetin may be an important lead molecule for the treatment of PCOS

Keywords: Letrozole, Quercetin, Polycystic ovary syndrome, Oxidative stress, Steroidogenic enzymes

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the prevalent types of endocrine abnormalities that affect 4 to 10 % of women of reproductive age [1,2]. Generally, PCOS is characterised by hyper-androgenism, insulin insensitivity and oligo-ovulation [3]. Studies over the last few decades have confirmed that PCOS is often associated with enhanced risk of type 2 diabetes [4]. It has been reported that women with mild form of PCOS which is associated with slight

hyper-androgenism and normal ovulation, are prone to PCOS later in life [5]. In PCOS, enhanced androgen activity often affects the gonadotropin-triggered estrogen and progesterone biosynthesis in follicles [6].

Currently available treatment strategies for PCOS are based on the use of metformin and other insulin sensitizers [7]. However, these drugs are associated with several side effects which worsen the quality of lives of patients [8]. Therefore, there is a need to develop alternative

treatment strategies for PCOS. Against this background, many studies have focused on the screening of natural products for their potential in the management of PCOS, based on the fact that they have much lower side effects than synthetic drugs. Flavonoids comprise a large group of plant secondary metabolites with tremendous pharmacological potential [9]. Quercetin is an important flavonoid with antidiabetic activity. Studies have shown that quercetin, in combination with other flavonoids, exhibited hypoglycaemic activity in a type 2 diabetes rat model [10]. The present study was carried out to investigate the effect of quercetin on some biochemical parameters in letrozole-induced PCOS in rats.

EXPERIMENTAL

Animals

Virgin female Sprague–Dawley rats (mean weight = 90 ± 5 g) were used in this study. Vaginal cytological analysis was carried out daily to monitor the four day ovarian cycle. The animals were maintained under standard conditions with appropriate light and temperature, and were given free access to drinking water and feed. They were randomly divided into four groups: group I received 1 % CMC only, while group II received 1 % CMC plus 25 mg quercetin/kg body weight. Rats in group III received 1 mg letrozole/kg only, while group IV rats were given the same dose of letrozole along with 25 mg quercetin/kg body weight. The PCOS was induced by administration of letrozole (1 mg/kg body weight) once daily for 21 days.

At the end of the study, the animals were sacrificed under deep isoflurane anaesthesia, and the ovaries were excised for further studies. For histological studies, one of the ovaries was fixed in Bouins fluid and the second ovary was used for the assays of steroidogenic enzyme activities. The study was approved by the Animal Ethics Committee of Nanjing University of Chinese Medicine (Approval No. NUCM/EA/7065 of 2017). The international guidelines for animal studies were strictly complied with all through the investigations [11].

Oral glucose tolerance test (OGT) and determination of steroidal hormone concentration

The rats were fasted continuously for 12 h prior to the end of treatments. They were then fed with glucose (300 mg/kg/body weight), and then oral glucose test (OGT) was carried out as described previously [12]. Blood was collected at 30 min

intervals for 120 min from the tail vein of each rat. The blood samples were subjected to blood glucose determination using a glucometer. Blood levels of testosterone and estradiol were determined using methods described previously [13].

Preparation of ovarian homogenate and assay of steroidogenic enzyme activities

Tris-HCl buffer (0.1 M, pH 7.8) was used to prepare 10 % ovarian homogenate. The homogenate was then centrifuged at 8000 g for 1 h, and the supernatant was used for estimation of protein content and steroidogenic enzymes. The activity of 3β -hydroxy steroid dehydrogenase (3β -HSD) was determined spectrophotometrically in terms of nanomoles of NADH produced/min/mg protein [14]. The activity of 17β -HSD was estimated as described previously, and the activity was expressed as nanomoles of NADPH oxidized/min/mg protein [15].

Determination of enzymatic antioxidants

Superoxide dismutase (SOD) was determined with the method of Kakkar *et al* [16]. Catalase activity was determined as described by Whanger *et al* [17], while glutathione peroxidase (GPX) activity was assayed according to the method outlined by Paglia and Valentine [18].

Histological examination of ovaries

The ovary samples were fixed in Bouins fixative and used for histological analysis in accordance with standard procedures. Sections (5- μ m thick) were cut from paraffin-embedded tissue and stained with haematoxylin and eosin (H & E) prior to examination under the microscope.

Statistical analysis

All experiments were carried out in triplicate and data are presented as mean \pm SD. Statistical analysis was done using one-way analysis of variance (ANOVA), followed Tukey's test. All analyses were done with GraphPad 7 software. Values of $p < 0.01$ and < 0.05 were considered significant.

RESULTS

Effect of quercetin on body weight and oestrous cycle of PCOS rats

Administration of letrozole continuously for 21 days caused significant increases in body weights of the rats, relative to rats in the control

(Figure 1). However, quercetin administration caused significant decreases in the body weights of the PCOS rats (group IV), when compared to rats given letrozole alone (group III). PCOS rats (Group III) showed prolonged dioestrous stage, but this was reversed by exposure to quercetin (25 mg/kg), making it less prolonged compared to PCOS rats. However, there was no difference between group I and group II with respect to the time of dioestrous stage.

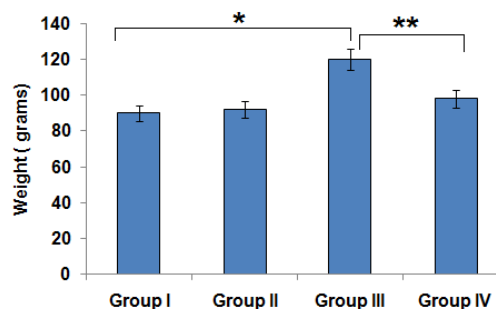


Figure 1: Effect of quercetin on the weight of rats in the different groups. The experiments were carried out in triplicate and values are presented as mean \pm SD (* $p < 0.01$, ** $p < 0.05$)

Effect of quercetin on plasma glucose levels

There was no significant difference in plasma glucose levels between group I and group II rats. However, administration of quercetin (25 mg/kg) to the letrozole-treated rats (group IV) resulted in reduction in plasma glucose levels, when compared to the rats administered letrozole only (group III; Figure 2).

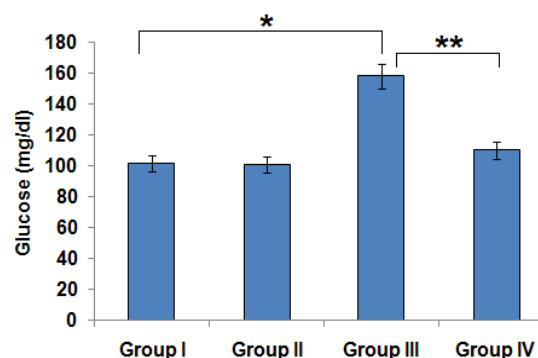


Figure 2: Effect of quercetin on the plasma glucose levels of mice in the different groups. The experiments were carried out in triplicates, and the values are presented as mean \pm SD (* $p < 0.01$, ** $p < 0.05$)

Effect of quercetin on testosterone and estradiol levels

The testosterone levels of rats given letrozole were significantly increased, but were reduced by quercetin administration (25 mg/kg body weight.

However, no significant difference was found in testosterone concentrations between group I and group II rats (Figure 3A). Similar effects were observed with estradiol levels (Figure 3 B).

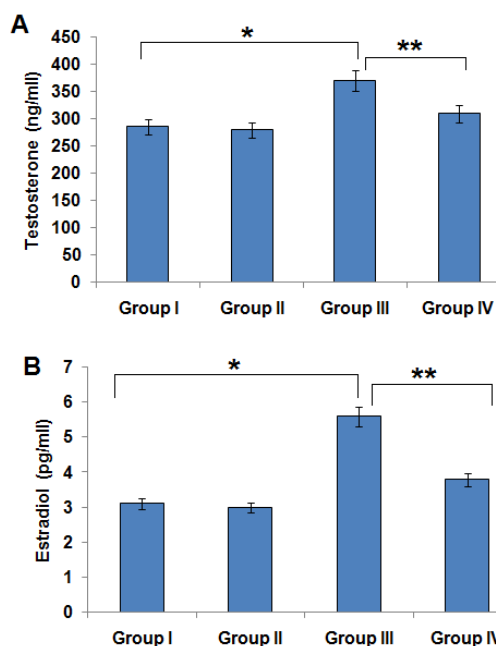


Figure 3: Effect of quercetin on (A) testosterone, and (B) estradiol in the different groups. The experiments were carried out in triplicates. Values are presented as mean \pm SD (* $p < 0.01$, ** $p < 0.05$)

Effect of quercetin on steroidogenic enzymes

In the letrozole-only group (PCOS, Group III), there were significant increases in the activities of 3 β -HSD and 17 β -HSD, relative to the controls (group I and group II). However, administration of quercetin at 25 mg/kg body weight to the PCOS rats (group IV) provoked significant decreases in the levels of 3 β -HSD and 17 β -HSD (Figure 4).

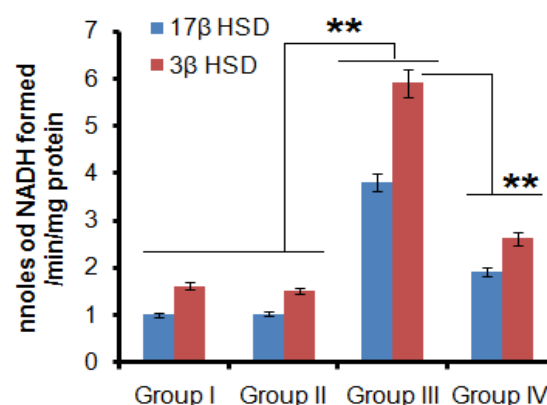


Figure 4: Effect of quercetin on steroidogenic enzyme activities in the different groups. The experiments were carried out in triplicate, and the results are presented as mean \pm SD (* $p < 0.01$, ** $p < 0.05$)

Effect of quercetin on antioxidant enzyme levels

The activities of SOD, CAT and GPX were significantly decreased in PCOS rats, when compared with the control groups (group I and group II). However, quercetin administration reversed the letrozole-induced decreases in the activities of these enzymes (Figure 5 A-C).

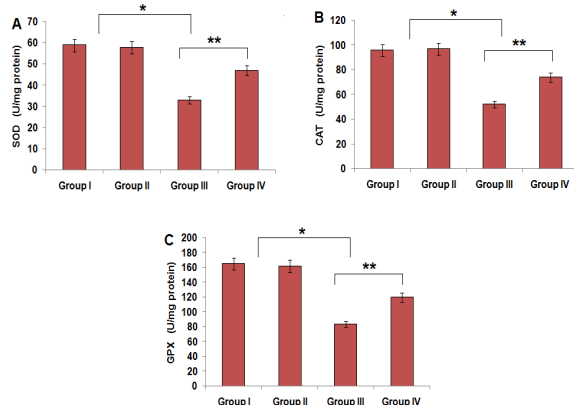


Figure 5: Effect of quercetin on the activities of (A) SOD, (B) CAT, and (C) GPX in rats in the different groups. The experiments were carried out in triplicate, and the results are presented as mean ± SD (**p* < 0.01, ***p* < 0.05)

Histological features

The control rats (groups I and II) exhibited normal ovary architecture, but the PCOS rats (group III) showed follicular cysts, reduced granulosa cell layers, and hyperplasia. However administration of quercetin to the PCOS rats (group IV) restored normal ovary anatomy appreciably (Figure 6).

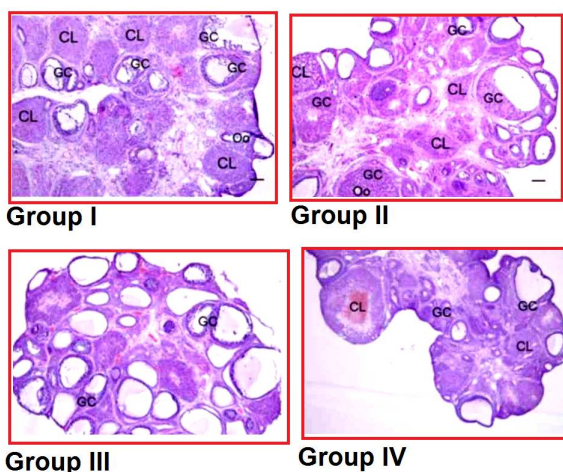


Figure 6: Effect of quercetin on histopathology of rat ovaries in the different groups (H&E). (GC: granulosa cell, Oo: oocyte, CL: corpus luteum)

DISCUSSION

The exact cause of PCOS is still not clear. Nonetheless, it is believed to be a genetic disorder associated with abnormal production of gonadotropin, enhanced production of steroidogenic enzymes, insensitivity to insulin, oligo-ovulation, as well as irregular menstruation which often lead to sub-fertility [2]. Currently, PCOS is treated mainly by administration of insulin sensitizers. However, these treatments have lots of side effects which necessitate the development of alternative treatment options for better management of the disease [3].

Plant products have long been considered as rich sources of drugs. In fact, a number of currently used drugs come from plants. Plants produce diverse metabolites that exhibit capacities to interact with wide array of cellular molecules such as enzymes [19]. Among plant secondary metabolites, flavonoids form a very diverse and large group. They have been reported to exhibit beneficial pharmacological potential [20]. Moreover, flavonoids are prevalently found in edible plants and are generally considered safe for human consumption [21].

Recently, it was reported that soy flavonoids exerted protective effects against letrozole-induced PCOS in rats [22]. Given this background, the effect of quercetin, one of the strong antioxidant flavonoids, in a letrozole-induced PCOS rat model was investigated in the present study. It was observed that quercetin prevented weight gain associated with PCOS, and reduced serum glucose levels in PCOS rats. These results are in agreement with those from previous studies wherein quercetin was reported to exhibit hypoglycaemic effects in diabetic rats [23].

Quercetin decreased the activities of the steroidogenic enzymes (3β-HSD and/or 17β-HSD) in the PCO rat model. This could possibly be due to the presence of the phenolic ring B in quercetin [24]. It has been reported that this flavonoid ring exhibits the capacity to inhibit 3β-HSD and/or 17β-HSD. Quercetin also decreased the levels of testosterone and estradiol in PCOS. Oxidative stress has been reported as the main pathological characteristic of PCOS in women [1]. In the present study, evaluation of the effect of quercetin on the antioxidant enzymes revealed that quercetin significantly increased the activities of SOD, CAT and GPX in PCOS rat model, and also reversed the effects of letrozole on ovarian morphology.

CONCLUSION

These results indicate that quercetin exerts protective effects on letrozole-induced PCOS. Thus, quercetin has a promising potential for use in the management of PCOS in women.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Yanli Hong, Yanyun Yin, Yong Tan performed all of the experiments. Ke Hong and Fengrong Jiang gave assistance during revision. This study was designed and under supervision of Yao Wang.

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