

# The Effect of Gallic Acid on Pituitary-Ovary Axis and Oxidative Stress in Rat Model of Polycystic Ovary Syndrome

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## Abstract

**Introduction:** The polycystic ovary syndrome (PCOS) carried along with hormonal– metabolic disorders, oxidative stress and ovulation dysregulation. Several studies have shown the role of Gallic acid (GA) as a potent antioxidant in oxidative stress induced disorders. Considering the antioxidant properties of GA, we investigate the effect of GA on the serum level of pituitary-ovary axis hormones and activity of ovary tissue antioxidant enzymes of PCOS model was studied.

**Methods:** 32 Wistar female rats were divided into four groups: Control, PCOS+Saline, PCOS+GA50, PCOS+GA100. PCOS was induced by single muscular injection of Estradiol valerate (4 mg/kg/BW). Then, GA was prescribed with doses of 50 and 100 mg/kg for 21 days, orally. At the end of treatment period, serum levels of LH, FSH, Estradiol, testosterone and progesterone as well as the tissue level of dismutase superoxide enzymes (SOD), catalase (CAT) and Malondialdehyde (MDA) in the ovaries were measured by ELISA technique. Differences between data were analyzed by one way ANOVA and the  $p < 0.05$  was considered statistically significant.

**Results:** Levels of LH, Estradiol and testosterone as well the MDA in treatment group with GA were increased significantly compared to the PCOS group ( $p < 0.05$ ), while the serum level of FSH and progesterone and tissue level of SOD and CAT enzymes were increased significantly in GA-treated groups than the PCOS group ( $p < 0.05$ ).

**Conclusion:** GA modifies the level of sex hormones in PCOS model by increase of antioxidant enzymes activity.

**Keywords:** Polycystic Ovary Syndrome, Gallic Acid, Sex Hormones, Oxidative Stress, Rat

## Introduction

The polycystic ovary S=syndrome (PCOS) is one of the most prevalent endocrine disorders in females during the fertility age affecting 4%-10% of females (1). Biochemical abnormalities of PCOS include the increased serum concentration of androgenic hormones such as testosterone and androstenedione, 17-hydroxy progesterone and enhanced LH secretion in comparison to FSH (2). Furthermore, this disease has consequence with insulin resistance and increase in blood insulin. Any increase in LH and insulin level mostly leads to disturbance in the process of steroids production (3). In other words,

testosterone and androstenedione are transformed into the estradiol and strone by the P450 aromatase which play critical role in the ovary hormone balance, respectively (4). Among the patients with PCOS, activity of such enzyme is disrupted. As a result, production of estrogen and progesterone from the pituitary gonadotropin are changed in ovarian follicles resulting in the increased production of ovary endogens and disease progress (5). PCOS is created due to the various environmental and genetic factors and among the environmental factors, the oxidative stress and various types of free radicals can be mentioned (6). Specific levels

of free radicals are needed for developed performance of natural cells. However, excessive production of reactive oxygen species (ROS) may affect the body natural antioxidant defence system which in turn can lead to a number of diseases including the endometriosis, polycystic ovary syndrome and infertility. In pathological conditions, the excessive oxidative stress likely helps the mesenchymal ovarian hyperplasia. Free radicals damage DNA and ovarian epithelium; also it causes cell apoptosis in ovaries and in consequence PCOS progress (7). Reviewing the oxidative stress parameters among the PCOS patients showed the increased oxidative stress indices like ROS, decreased activity of antioxidant enzymes and total capacity of blood antioxidant. Based on this, the oxidative stress may play a key role in pathophysiology of this disorder and trend of PCOS prevention and treatment has been already created using the antioxidants (8). Drug therapies in PCOS cover various medicines including the Metformin, Letrozole and Clomiphene. But these drugs have various side effects after a long-term use (9). The existence of natural antioxidants and tendency of the global approach for using these compounds in disease treatment, persuade researchers to use these products for PCOS treatment studies (10). Useful effects of some natural products including Resveratrol and Catechin were proved to improve the PCOS symptoms (11, 12). Gallic acid (GA, 3,4,5-trihydroxybenzoic acid) is one of the most important products of polyphenol in plants (13, 14). The natural products includes different biological activities such as apoptosis induction, anti-inflammatory and antioxidant activity, anticoagulants, antibiotics, anticancer and improvement of heart, liver and kidney damages (15). Concerning the antioxidant properties of GA, its impact on the pituitary-ovary axis hormones and oxidative stress parameters in PCOS-induced rats were investigated.

## Methods

In this experimental study, 32 Wistar virgin female rats with the average weight  $200\pm 10$  g and the approximate age of  $85\pm 5$  days were used. In all working stages, animals were kept under the standard conditions of temperature ( $25\pm 2^{\circ}\text{C}$ ) and humidity ( $50\pm 10\%$ ) and 12-hour light and darkness cycle (06:00 a.m. to 06:00 p.m.). Rats were fed ad libitum with special food with standard formulation together with the tap water. All surgery operations and samplings were carried out under the full anaesthesia and it was tried to use the least acceptable samples. Animals were divided into 4 groups: 1. Healthy control group: animals of this group received no treatment and they were used to compare the biochemical properties with others. 2. PCO group: Polycystic ovary syndrome (PCOS) was induced by muscular injection of Estradiol valerate (4 mg/kg). Then, they received the normal saline by gavage for 14 days. 3. PCO +GA50: in this group, after inducing the PCOS, the animals were fed with Gallic acid (Sigma-Aldrich, Germany) with 50 mg/kg for 14 days. 4. PCO+GA100: this group underwent the gavage with 100 mg/kg Gallic acid after the syndrome induction. To conduct this study, animals were initially selected which had 2-3 periods of regular oestrus cycle within 12-14 days of smear vaginal observation. Intraperitoneal administration of Estradiol valerate induces the polycystic ovary syndrome among the rats (16). Irregularity of oestrous cycles and Persistent Vaginal Cornification (PVC) is the symptoms of follicle cysts in ovary (17). At the end of treatment, rats were anesthetized by diethyl ether. Then blood sampling was done directly from the left ventricle. Blood sample was put in incubator (Memmert, Germany) for 12 minutes in  $37^{\circ}\text{C}$ . When the coagulation occurred, tubes were put in centrifuge (Hettich, Germany) for 12 minutes with 5000 rpm. The obtained serum was kept in  $-70^{\circ}\text{C}$  (20). By ELISA Reader (Stat Fax, USA) and ELISA toolkits (Abcam, China), the serum

level of sex hormones LH, FSH,  $\beta$ -estradiol, testosterone and progesterone was measured. Then, ovary was taken out of the rat bodies. After washing with saline solution together with Tris buffer (Sigma-Aldrich, Germany), it was homogenised for 5 minutes by homogeniser (IKA, Germany) with 5000 rpm. The homogenised solution was centrifuged by refrigerated centrifuge (Hermle, Germany). To avoid the damages to enzymes and proteins, all stages were carried out in 4°C (refrigerated centrifuge) and 0.5 mM solution of Phenyl methyl sulphonyl fluoride (Sigma-Aldrich, Germany) as the inhibitor of proteases (16). After the centrifuging operations, supernatant was used in ELISA. By ELISA and FineTest<sup>®</sup> Company toolkits (China), activity of superoxide dismutase (SOD) with sensitivity >9.375 Pg/ml (within range of 15.6-1000 Pg/ml), catalase (CAT) with sensitivity >18.75 mIU/ml (within range of 31.2-2000 mIU/ml) and malondialdehyde (MDA) with sensitivity >4.688 ng/ml (within range of 7.813-500 ng/ml) in ovaries were measured. Statistical analysis among the different groups was done by SPSS V.22. Data were reported in mean $\pm$ SEM. To determine the significant difference among the given groups, one way ANOVA and Tukey post-hoc were used. Differences were considered statistically significant at  $p < 0.05$ .

## Results

Results of one way ANOVA showed that the activity of SOD and CAT of ovarian tissue in PCOS+Saline decreased significantly compared to the control group ( $p < 0.01$ ) and MDA increased significantly ( $p < 0.05$ ). In comparison to the PCOS+Saline group, activity of SOD and CAT in ovarian tissue increased significantly in PCOS+GA50 and PCOS+GA100 in dose manner ( $p < 0.01$ ) and MDA was also reduced significantly ( $p < 0.05$ ). Among the GA-treated groups, the activity of both antioxidant enzymes (SOD and CAT) increased significantly compared to

PCOS+GA50 group and MDA was also reduced significantly ( $p < 0.05$ , Table 1). In reviewing the serum level of pituitary-ovary axis hormones, LH ( $p < 0.01$ ), Estradiol ( $p < 0.001$ ) and testosterone ( $p < 0.05$ ) in PCOS+Saline group increased significantly than the control group, while the FSH serum level and progesterone decreased significantly ( $p < 0.001$ ). Treatment with Gallic acid modifies the serum level of the aforementioned hormones such that in PCOS+GA50 and PCOS+GA100, a significant decrease was seen in LH ( $p < 0.01$ ), Estradiol ( $p < 0.001$ ) and testosterone than the PCOS+Saline group ( $p < 0.05$ ). While the level of FSH and progesterone in GA-treated groups significantly increased than the PCOS+Saline group ( $p < 0.05$ ). No significant differences were observed between the PCOS+GA50 and PCOS+GA100 groups among the sex hormones (Table 2).

## Discussion

The present study investigates the antioxidant effect of Gallic acid on the pituitary-ovary axis, sexual hormones and the activity of antioxidant enzymes in Estradiol valerate-PCOS rat model. Estradiol valerate induces the PCOS though disturbing the pituitary gonadotropins (18). By injecting this substance, the serum level of LH, Estradiol and testosterone was increased, while the serum level of FSH and progesterone decreased than the control group. Doldi *et al.* showed that production of progesterone and Estradiol in granulosa cells among the patients with PCOS would not be normal (19). This indicates that the patients with PCOS give a different response to gonadotropins than the health people. In fact, serum concentrations of Estradiol and Progesterone show significant increase and decrease in PCOS, respectively (19).

**Table 1.** Antioxidant enzyme activity and MDA level in ovary tissue (n=10)

Groups	SOD Pg/ml	CAT mIU/ml	MDA ng/ml
Control	71.48±3.17	130.48±10.53	35.24±3.16
PCOS+Saline	25.60±3.71 †	55.37±8.64 †	87.26±9.87 †
PCOS+GA50	41.45±4.24 *	81.61±9.41 *	65.59±7.64 *
PCOS+GA100	59.26±4.55 *#	100.71±6.67 *#	51.41±6.70 *#
Sig. (ANOVA)	0.004	0.003	0.013

Data are shown in mean±SEM and analysed by one way ANOVA with Tukey post hoc:

†:  $p < 0.05$ , control vs. PCOS+Saline

\*:  $p < 0.05$ , PCOS+Saline vs. PCOS+GA50,100

#:  $p < 0.05$ , PCOS+GA50 vs. PCOS+GA100

**Table 2.** Serum level of sex hormones in different groups (n=10)

Groups	LH mIU/ml	FSH mIU/ml	Progesterone ng/ml	Estradiol pg/ml	Testosterone pg/ml
Control	6.96±0.33	11.31±0.4	14.86±0.35	51.51±3.81	5.46±0.32
PCOS+Saline	16.32±0.3 †	3.48±0.42 †	2.56±0.34 †	141.25±12.3 †	14.98±1.23 †
PCOS+GA50	12.9±0.3 *	6.04±0.12 *	7.63±0.26 *	96.87±4.6 *	11.36±0.67 *
PCOS+GA100	10.2±0.52 *	8.21±0.18 *	10.6±0.48 *#	86.09±2.94 *#	9.30±0.38 *
Sig. (ANOVA)	0.005	0.001	0.05	0.001	0.013

Data are shown in mean±SEM and analysed by one way ANOVA with Tukey post hoc:

†:  $p < 0.05$ , control vs. PCOS+Saline

\*:  $p < 0.05$ , PCOS+Saline vs. PCOS+GA50,100

#:  $p < 0.05$ , PCOS+GA50 vs. PCOS+GA100

Activity of antioxidant enzymes in ovaries (i.e. CAT and SOD) in untreated PCOS rats decreased significantly compared to controls. However, the lipid peroxidation and activity of MDA increased significantly. Scientific evidence shows that the activity of the antioxidant enzymes of ovaries in patients with PCOS is decreased and as a result of oxidative stress conditions, the ovarian androgens are increased and the evolution process of follicles is disrupted (20). It was also reported that due to the weakened defence antioxidant system among these patients, body lipids are not protected against the oxidation; hence with regards to the increased concentration of blood lipids and weakened antioxidant defence system, the chance of lipids oxidative injury is enhanced and is

revealed as increased serum level of MDA (21). Results of this study showed that GA-treated PCOS model increases significantly the concentrations of FSH and progesterone compared to the PCOS+Saline group. Also, GA in dose manner decreases the serum level of LH, Estradiol and testosterone in GA-treated rats than the PCOS+Saline group. In other words, GA ameliorates the sex hormones in this model. Numerous studies have been shown antioxidant, and anti-inflammatory properties of GA (22). GA is highly capable to scavenger the free radicals like the superoxide anion, peroxide hydrogen, hydroxyl and hypochlorite radicals and reduces the serum level of MDA by decreasing the lipid peroxidation (23). There are evidences indicating the direct relationship between the

inflammation, oxidative stress and PCOS (24). Systemic and local hyperglycaemia in the PCOS patients increases the reactive oxygen species (ROS) from the single nucleus blood cells. The abnormal level of ROS in the patients leads to induction of oxidative stress in cells and by activating the nuclear factor kappa B (NF-KB) path, it increases the transcription of tumour necrosis factor-alpha (TNF- $\alpha$ ) gene and inhibits the tyrosine kinases and the insulin resistance and created cysts in PCOS patients are resulting from the disturbance in activity of this enzyme (25, 26). Therefore, it can be said that GA with antioxidant property inhibits free radicals induced deficits and ameliorate PCOS symptoms including the hormonal changes. In other studies, the effects of GA on free testosterone and Estradiol in PCOS models have been investigated. In this field, Rafiei *et al.* investigated the neuroprotective effect of GA in cognitive deficits induced PCOS with normalization of testosterone and Estradiol serum levels (27).

### Conclusion

Gallic acid with its antioxidant properties increases the activity of antioxidant enzymes and decreases the lipid peroxidation in ovaries and normalizes the serum level of sex hormones in PCOS rats. Also, concerning the results of this study, it can be said that Gallic acid is helpful to reduce some complications of PCOS.

### Ethical issues

Not applicable.

### Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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