ORIGINAL ARTICLE

Veterinary Research Forum. 2022; 13 (4) 513 - 520
doi: 10.30466/vrf.2021.530203.3179
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Effect of chlorogenic acid on follicular development, hormonal status and biomarkers of oxidative stress in rats with polycystic ovary syndrome

Neda Abedpour[1]*, Masoumeh Zirak Javanmard[1], Mojtaba Karimipour[1], Amirreza Pourmajed Liqvan[2]

[1] Department of Anatomy, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran; [2] Student Research Committee, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.

Article Info

Article history:

Received: 17 May 2021
Accepted: 13 September 2021
Available online: 15 December 2022

Keywords:

Chlorogenic acid
Gonadal steroid hormones
Ovarian follicle
Oxidative stress
Polycystic ovarian syndrome

Abstract

Polycystic ovarian syndrome (PCOS) is a complex endocrine and metabolic disorder. Chlorogenic acid (CGA) bears antioxidant properties with protective effects on different tissues. This study was conducted to evaluate the effect of CGA on follicular development, hormonal status and biomarkers of oxidative stress in a rat model of PCOS. In this experimental study, 18 rats were divided into three equal groups including: control, non-treated PCOS [(estradiol valerate (EV): 40.00 mg kg^{-1} intramuscularly)], and PCOS-CGA (EV: 40.00 mg kg^{-1} intramuscularly and CGA: 100 mg kg^{-1} intraperitoneally once a week for eight consecutive weeks). At the end of treatment period, all rats were anesthetized. Then 5.00 mL blood samples of rats in the three groups were taken and prepared for hormonal analyses and their ovaries were isolated and dissected mechanically free of fat and mesentery. The ovaries underwent the following analyses: Morphological study with Hematoxylin and Eosin staining and biochemical study using the malondialdehyde (MDA) level and total antioxidant activity. Data were analyzed using one-way ANOVA and post hoc Tukey's test. The serum level of luteinizing hormone, estrogen, testosterone, antioxidant capacity, glutathione and the number of cystic follicles in the PCOS group treated with 100 mg kg^{-1} Chlorogenic acid compared to the non-treated PCOS group were significantly decreased, however, the serum level of follicle stimulating hormone, progesterone, MDA and the number of secondary, graafian follicles and corpus luteum were significantly increased. Chlorogenic acid could be effective in ameliorating follicular development as well as hormonal and biochemical disorders in rats with PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy disorder that affects 15.00 - 20.00% of women worldwide in reproductive age (between the ages of 18 and 44 years)[1] and can affect their health-related quality of life even after menopause. It has been reported that between 7.10 and 14.60% of Iranian women are suffering from this syndrome.[2] The clinical manifestations of PCOS include menstrual irregularity, impaired fertility, high circulating levels of testosterone, multiple small cysts in ovaries and anovulation.[3] The histological appearance of PCOS under the light microscope is characterized by multiple small antral follicles arrested in their development.[4] Additionally, the pathogenesis of PCOS is frequently associated with abnormal concentrations of hormones. Progesterone concentration is higher, but the estrogen concentration is lower than normal concentration and luteinizing hormone (LH)/follicle stimulating hormone (FSH) is three times more than the normal concentration.[1,4-6] It is mentioned that, a systemic low-grade inflammation in women diagnosed with PCOS is closely linked with the oxidative stress.[7] Oxidative stress, one of the most common forms of metal toxicity, is defined as an imbalance between oxidants and antioxidants in the living organisms. Oxidative stress causes increase in the amount of lipid peroxidation products and other highly toxic products such as malondialdehyde (MDA).[8] Research indicates that oxidative stress is generally present in the women with PCOS and may play an important role in infertility.[9-11] Many researchers have also been committed to trigger the alternative treatment strategies that help reduce oxidative stress and improve ovarian reserve and developmental

***Correspondence:**

Neda Abedpour. PhD
Department of Anatomy, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
E-mail: abedpour.n@umsu.ac.ir

competence of PCOS patients.[11-13] Antioxidant defense systems can suppress oxidative stress and scavenge free radicals or disaccord their functions and relieve the toxic effects.[11,14] Chlorogenic acid (CGA) is one of the most widely available phenolic acids which has a wide range of sources (e.g. different foods and coffee), easy to extract and of high content. In addition, it has important physiological and biological (anti-oxidative and anti-inflammatory) effects. Different scientific pieces of evidences clarify several important biological and therapeutic functions of CGA including antioxidant activity, antibacterial, hepato protective, cardio protective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, antimicrobial, anti-hypertension and a central nervous system stimulator.[15] Some studies have also confirmed the significant antioxidant potential of CGA. Moreover, it is speculated that CGA can scavenge the free radicals.[16,17]

The PCOS is a reproductive hormonal abnormality and causes infertility among women, accordingly, highlighting the potential clinical application of CGA in PCOS patients is thought to be useful for public health. In addition, in view of the critical role of oxidative stress in PCOS, we sought to evaluate, for the first time, the potential therapeutic benefit of CGA on follicular development, hormonal status and biomarkers of oxidative stress by employing a rat model of Estradiol Valerate-induced disease.

Materials and Methods

Animals. In this original study, 8-week-old albino female rats (250 ± 10.00 g) were used. The rats were bred in standard conditions and subjected to controlled room temperature (23.00 ± 2.00 ˚C) with constant humidity (40.00 - 50.00%) under a 12-hr light/dark cycle (with lights on at 7:00 a.m.). Animal procedures and protocols were conducted in accordance with the Principles of Laboratory Animal Care - ethically approved by the Animal Care and Use Committee in Urmia University of Medical Sciences (Ethical code number: IR.UMSU.REC.1398.117, Urmia, Iran).

Experimental design. The female rats were randomly divided into three groups (n = 18): I: Control, II: PCOS-Control [40.00 mg kg^{-1} estradiol valerate (EV; Aboureihan Pharmaceutical Co., Tehran, Iran), intramuscularly], and III: PCOS-CGA [(40.00 mg kg^{-1} EV, intramuscularly and 100 mg kg^{-1} CGA (Sigma-Aldrich, St. Louis, USA), intraperitoneally for eight consecutive weeks]. At the end of the 8^{th} week of the study, the rats (n = 45) were anesthetized with a combination of 100 mg kg^{-1} ketamine (Bremer Pharma GmbH, Warburg, Germany) and 10.00 mg kg^{-1} xylazine (Serva Feinbiochemica, Heidelberg, USA) intraperitoneally injected to the rats.[18] The ovaries were isolated and dissected mechanically free of fat and mesentery after blood was taken from the heart. The ovaries underwent the following analyses: Morphological study with Hematoxylin and Eosin staining (H&E) and biochemical study using the MDA level and total antioxidant activity. In addition, 5.00 mL blood samples of rats in all the three groups were obtained and prepared for hormonal analyses.

Administration of estradiol valerate and chlorogenic acid. The PCOS was induced by a single intramuscular injection of EV as described previously with minor modification.[19] Next day after the single EV injection, rats were intraperitoneally injected with 100 mg kg^{-1} of CGA in 0.10 mL of ethanol solution (Sigma-Aldrich) weekly for eight weeks. The dose of CGA was chosen following the previous studies.[16,17] Rats injected with only ethanol (0.10 mL) were served as control.

Measurement of body weight. The weights of rats were measured and Lee's index (= body weight 1/3) was estimated every week to calculate drug doses.[20] After each weighting, the CGA was injected intraperitoneally according to the amount of gained weight. Measurements lasted until the rats were humanely sacrificed.

Tissue sample collection. Blood samples were obtained transcardially and placed into tubes and centrifuged at 1,000 *g* for 10 min. The obtained serum of each rat was kept at – 20.00 ˚C for subsequent analyses. Following the collection of blood samples, both ovaries of rats were quickly isolated and dissected mechanically free of fat and mesentery. Right ovarian tissues were fixed in 10.00% neutral buffered formalin solution for light microscopic histological evaluations, whereas, the left ovarian tissues were thoroughly homogenized (with a tissue homogenizer in 1/10; w/v phosphate-buffered saline (Gibco, Grand Island, USA) and centrifuged for 10 min at 3000 rpm. Following centrifugation, the supernatants were collected carefully and kept at – 20.00 ˚C for biochemical analyses.

Histological evaluation. Whole ovarian tissues (n = 5 from each group) were randomly fixed in 10.00% neutral buffered formalin solution (pH = 7.40) for 48 hr at room temperature and dehydrated through ascending grades of ethanol (30.00%, 50.00%, 70.00%, 90.00% and 100%) for 1 hr each, and subsequently immersed in xylene. For embedding, the dehydrated samples were processed in paraffin blocks and manually sectioned at 5.00 µm. Then, sections were mounted on glass slide with five intervals. Then, deparaffination, rehydration (in descending series of ethanol), and the H&E staining were done and slides were assayed. Finally, we evaluated tissue morphology including hyperemia, percentage of cysts and number of large antral follicle under a light microscope (Nikon, Tokyo, Japan). In each section to prevent the repetition of a follicle count only follicles with distinct nucleoli were counted.

Serum hormone levels. The levels of Anti-Mullerian hormone (AMH), LH, FSH, 17 beta-estradiol, progesterone and testosterone were established in the collected serum

samples obtained from each group (each experimental data point consisted of 15 samples for three repeats) using an ELISA kit according to the provider's instruction. The analytical sensitivity of the assay was expressed as ng mL^{-1} for 17 beta-estradiol (MyBioSource Inc., San Diego, USA), testosterone (IBL International GmbH, Hamburg, Germany), progesterone (Antibodies-online, Aachen, Germany) and AMH (Antibodies-online), and as mIU mL^{-1} for FSH (Antibodies-online) and LH (Antibodies-online), respectively.

Assays for oxidative stress markers. Biochemical assays were carried out for evaluating oxidative stress markers of MDA, glutathione (GSH) and Total Antioxidant Capacity (TAC) in the experimental groups by ELISA kit according to the provider's instruction (ZellBio GmbH, Lonsee, Germany). For this purpose, ovarian tissue was homogenized in phosphate buffer (pH = 7.40) or 1.15% of 1:9, w/v KCl buffer (Sigma-Aldrich, Chemie GmbH, Eschenstrasse, Germany) using a manual glass homogenizer with a tissue homogenizer in 1/10; w/v phosphate-buffered saline and centrifuged for 10 min at 3,000 rpm. Then, the supernatant was separated and stored at – 80.00 ˚C until analysis.

Statistical analysis. Statistical Package for Social Sciences (version 16.0; SPSS Inc. Chicago, USA) software was applied for data analysis. One-way analysis of variance (ANOVA) was used to test the hypotheses. Furthermore, follow up Tukey's test analysis was used for pairwise comparisons across the groups to locate the possible differences between pairs. The results of all stages of this study were expressed by mean ± standard deviation.[20] Differences with a *p*-values of < 0.05 were considered as significant at the 95.00% confidence level.

Results

Assessment of animal body weight. Body weights were monitored and measured within 8 weeks of PCOS model induction in all experimental groups. As shown in Figure 1, in the PCOS-control group, body-weight mean was significantly higher ($p < 0.05$) which indicated the successful induction of PCOS. However, no significant difference was observed between PCOS-CAG and control group ($p > 0.05$). Intraperitoneal injection of CGA inhibited the weight gain in the PCOS rats and significantly decreased the body weights compared to the PCOS-Control group ($p < 0.05$).

Light microscopy observation. The histological morphology of the ovarian tissues stained with H&E is presented in Figure 2. Ovarian sections of the control group exhibited a normal morphology with a central medulla composed of fibromuscular stroma. Besides, a number of blood vessels and a cortical part containing abundant follicles at different stages of maturation were observed. Also, the outcome showed corpus luteum which is an indicator of ovulation. Regular organization was seen between the granulosa and theca cells in follicles at different developmental stages. Oocyte and surrounding zona pellucida turned out intact (Fig. 2A). In sharp contrast, general morphology of the PCOS had a swollen appearance, with lots of cystic and atretic follicles a few corpus luteum, and well-developed theca interna cells, supporting fully developed PCOS. Granulosa cell layers were decreased and loosely arranged. Moreover, many granulosa cells in cystic follicles full-filled antrum (Fig. 2B) were especially seen. The damaged follicles with pyknotic nuclei oocytes and irregularly shaped granulosa cells, indicating atresia of follicle, were prominent in this group. In the PCOS-CGA group, oocyte and surrounded zona pellucida of follicles were intact and ovarian architecture was ameliorated to comparable levels with the control group (Fig. 2C).

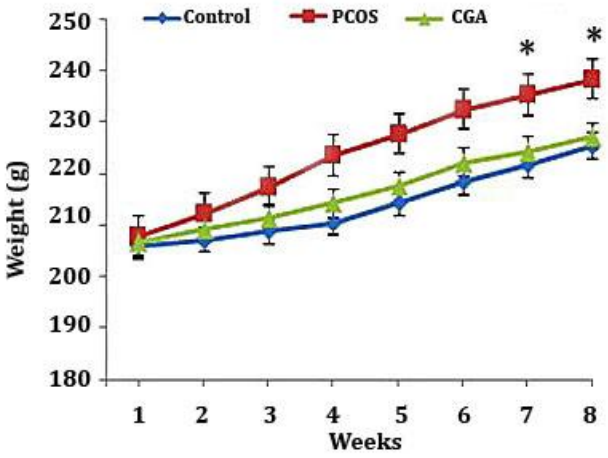

Fig. 1. The mean changes in body weight of rats weekly for eight weeks. Data are expressed as means ± SD.
* indicates significant difference compared to control group.

The numbers of follicles at different developmental stages. In addition to morphological findings, the numbers of normal follicles at different developmental stages were analyzed in the study groups (Fig. 3). The percentage of unilaminar primary, multilaminar primary, secondary and graafian follicles significantly ($p < 0.05$) was decreased in the PCOS-control group (18.60 ± 2.00%, 3.75 ± 2.20%, 9.87 ± 2.00%, and 2.63 ± 2.20%, respectively) compared to those of the control group (24.65 ± 2.10%, 9.26 ± 2.40%, 16.20% ± 1.98, and 7.64 ± 2.00%, respectively), however, these numbers in the PCOS-CGA group (18.18 ± 2.20%, 9.35 ± 1.87%, 13.78 ± 2.00%, and 5.75 ± 2.20%) were significantly ($p < 0.05$) higher than those of the PCOS-control group. However, no significant difference was observed between CGA-PCOS and the control group. Further analysis indicated that the number of primary follicles was statistically ($p < 0.001$) diminished in all groups in comparison with the control group. No significant difference was seen between PCOS-CGA and PCOS-control groups. Results showed that the accumulation

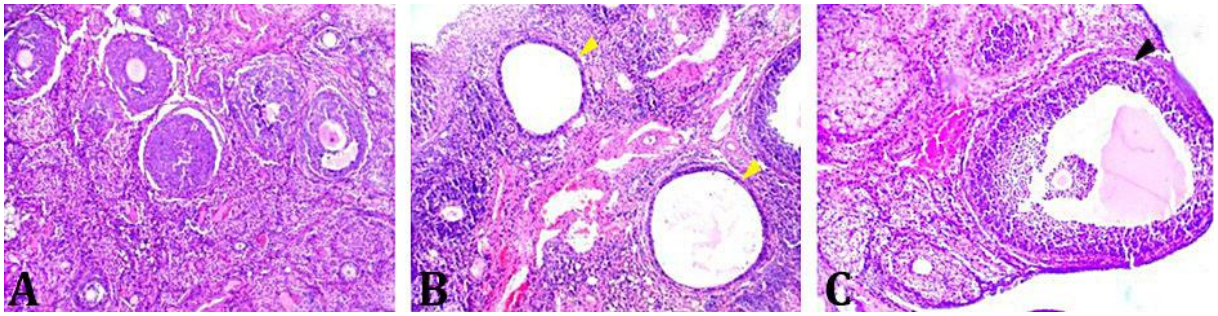

Fig. 2. Histological analysis of ovarian tissue in **A)** control, **B)** PCOS and **C)** PCO-CGA groups. A lot of cysts were seen in the tissue of the ovary in PCOS group compared to the other groups (yellow arrowheads). Many granulosa cells in cystic follicles full-filled antrum were especially seen. In the PCOS-CGA group, oocyte and surrounded zona pellucida of follicles were intact and ovarian architecture was ameliorated to comparable levels with the control group (black arrowhead), (H&E staining, 40×).

of cystic follicles in PCOS-control group (59.75 ± 2.20%) was significantly higher compared to the control group (22.15 ± 2.00%). CGA treatment decreased pre-antral and atretic follicles counts ($p < 0.05$) and remarkably elevated the number of graafian follicles and corpus luteum (18.43 ± 2.20%), ($p < 0.05$). Administration of CGA significantly decreased the size and number of follicular cysts (36.52 ± 2.10%) indicating the inhibitory effect of CGA on the development of follicular cysts (Fig. 3). The highest percentage of corpus luteum was observed in the control group (20.18 ± 2.20%) that indicated the normal sexual cycle (Fig. 3).

Evaluation of the biochemical parameters. The data of the biochemical parameters are presented in Table 1. The highest and the lowest level of concentration of antioxidant were observed in the control group and PCOS-control groups, respectively. In this test, administration of CGA significantly increased the concentration of antioxidant. The tissue concentration of MDA (lipid peroxidation marker) was significantly ($p < 0.05$) higher in the PCOS-control group compare to the other groups which in turn contributes to the incidence of PCOS. However, the same parameter concentrations were significantly ($p < 0.001$) decreased in CGA treatment group.

Hormonal assay. The serum concentration of AMH, LH, FSH, estrogen (E2), progesterone and testosterone were compared between the groups (Table 1). Our results indicated that serum concentration of AMH, LH, E2 was significantly ($p < 0.05$) higher in the PCOS-control group. Furthermore, because of the excess in AMH accumulation, testosterone concentration was significantly ($p < 0.05$) higher in this group compared to the control group. These parameters were significantly reduced by CGA treatments when compared to the PCOS-control group ($p < 0.05$). In addition, the concentration of FSH and progesterone production was significantly ($p < 0.05$) increased in CGA-supplemented group in comparison with non-supplemented groups.

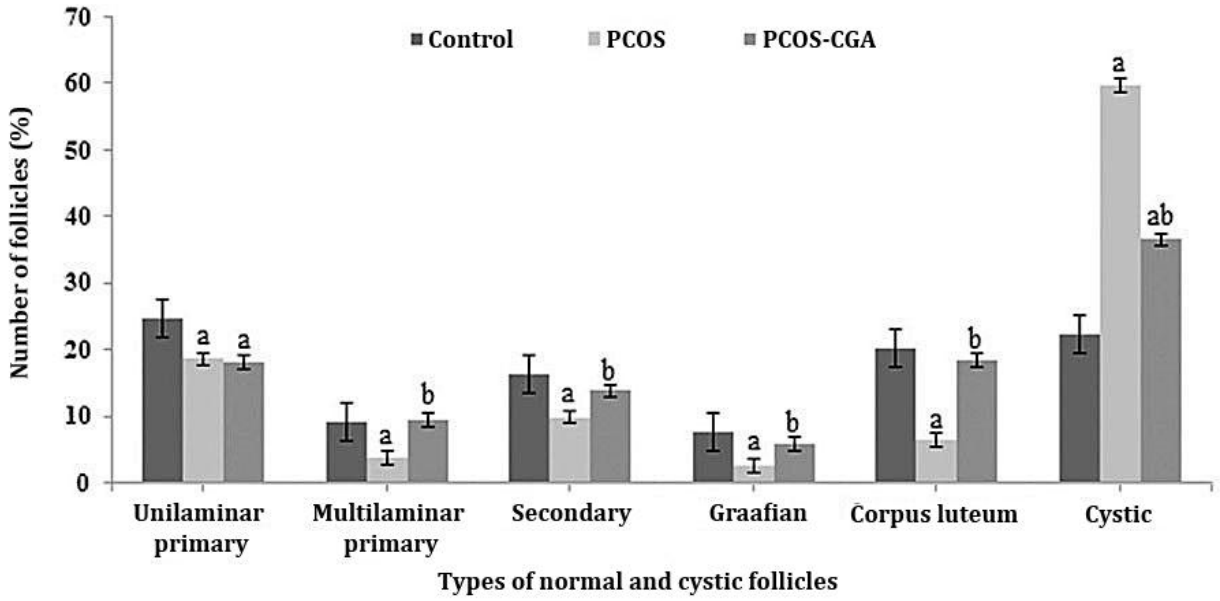

Fig. 3. The number of ovarian follicles in control, PCOS and PCOS-CGA groups. Values are expressed as mean ± SD.
[ab] indicate significant differences versus control and PCOS groups, respectively at $p < 0.05$.

Table 1. The comparison of the biochemical markers and hormonal assay values among the groups. Values are expressed as mean ± SD.

Parameters	Control	Polycystic ovarian syndrome	Chlorogenic acid
Luteinizing hormone (IU L^{-1})	0.47 ± 0.02	1.15 ± 0.01[a]	0.52 ± 0.01[b]
Follicle stimulating hormone (IU L^{-1})	4.80 ± 0.02	2.47 ± 0.04 [a]	5.12 ± 0.08[b]
Anti-mullerian hormone (ng mL^{-1})	3.45 ± 0.03	5.74 ± 0.04 [a]	3.52 ± 0.02[b]
Estrogen (ng mL^{-1})	2.85 ± 0.01	4.73 ± 0.01[a]	3.12 ± 0.02[b]
Progesterone (ng mL^{-1})	10.56 ± 0.02	3.75 ± 0.02[a]	10.15 ± 0.02[b]
Testosterone (ng mL^{-1})	3.75 ± 0.01	9.05 ± 0.03[a]	2.01 ± 0.01[b]
Malondialdehyde (nmol mg^{-1})	0.33 ± 0.04	0.85 ± 0.05[a]	0.38 ± 0.05[b]
Glutathione (mIU mg^{-1})	2.63 ± 0.03	1.23 ± 0.05[a]	2.35 ± 0.04[b]
Total antioxidant capacity (µmol L^{-1})	0.67 ± 0.02	0.32 ± 0.05[a]	0.55 ± 0.03[b]

[ab] indicate significant differences versus control and polycystic ovarian syndrome groups, respectively at $p < 0.05$.

Discussion

In the current study, we systematically investigated the potential benefits of CGA on the follicular development, biomarkers of oxidative stress and hormonal status in a rat model of PCOS. At the first stage, the continuous monitoring of body weight during 8 weeks revealed that the average weight of body was significantly increased in the PCO-control group, however, CGA could reduce the augmented body weight in the treatment group. This finding was in agreement with the studies that considered obesity and overweight as two symptoms of PCO.[21] Similar studies reported that CGA could significantly decrease the body weight and was associated with its reducing effect on messenger RNA expression of stearoyl-CoA desaturase 1, acetyl-CoA carboxylase 1 (ACC1), and ACC2 involved in oxidative phosphorylation, fatty-acid oxidation and lipolysis and could promote weight loss.[22,23] One of the main objectives of the present investigation was to evaluate the histological morphology of the ovarian tissues. Our morphometric study of ovarian sections indicated significant changes in the PCO-control group in comparison with the control group. Moreover, the results demonstrated that EV single injection caused the polycystic changes in the rat ovary, increased the development of cystic follicles and the number of atretic follicles in the PCO-control group. In addition, abundant secondary follicles were observed in this group, however, primordial follicle pool and Graafian follicles were significantly diminished. No corpus luteum was seen indicating lack of ovulation and attributing to the cessation of folliculogenesis.[24] On the other hand, the evaluation of preventive effects of CGA on PCOS revealed that the intraperitoneal injection of 100 mg/kg CGA improved ovarian architecture and led to (a) increase in the number of primordial follicle pool and graafian follicles,[2] growth of antral follicles from the pre-antral stage,[3] lack of formation of large cysts in cortex, and reduced number of small cysts in the medullary region. Moreover, in the presence of CGA, a small number of corpus luteum was observed. However, the number of corpus luteum was significantly higher in control ovaries in comparison with both other groups as a sign of normal sexual cycle. Accordingly, it seems that the injection of 100 mg/kg CGA extract to a rat model of PCO could improve the ovarian follicular cell architecture and reduce the number of cysts. In parallel with our study, many researchers evaluated the beneficial effects of different substances on PCOS.[25,26] Furat Rencber *et al.* colleagues studied the effects of resveratrol and metformin on ovarian reserve in PCOS rat model. They expressed that combined therapy of metformin and resveratrol might improve weight gain, hormone profile and ovarian follicular cell architecture by inducing antioxidant and anti-inflammatory systems.[11] Similarly, Tahmasebi *et al.* investigated the antioxidant effects of calligonum extract on ovarian tissue in rats subjected to PCO induction with EV. In PCO group treated with calligonum extract, similar to our study, the number of ovary cysts was significantly decreased compared to PCO without treatment.[27]

Our study could be considered a pioneer work, so that it evaluated the effect of CGA on improving folliculogenesis. Parallel to the histological results, our hormonal assessment data demonstrated that AMH concentration was significantly increased in the PCOS group. Similar studies confirmed that high serum and intrafollicular concentration of AMH played an important role in the characteristic follicular arrest, and caused increased number of small antral follicles in PCO patient. This excess of AMH inhibited aromatase expression and FSH action that resulted in follicular atresia due to lower conversion of androgen to estrogen.[28] Besides, our results indicated that administration of CGA significantly decreased the concentration of AMH consistent with decrease in the number of secondary follicles which supported the maintenance of folliculogenesis. Some recent studies demonstrated that antioxidants could act through their AMH decreasing effects and might activate aromatase expression which eventually improved folliculogenesis.[29,30] It seems that CGA might stimulate granulosa and theca cells to express cytochrome P450 aromatase genes that convert the androgens to 17-β-estradiol and promote ovarian follicular development.

Further analysis of the findings indicated that FSH, 17-β-estradiol (functional marker) and progesterone concentrations were significantly decreased in the PCO-control. In contrast LH and testosterone concentration were significantly elevated in this group. However, these

parameters were significantly ameliorated by CGA treatments. The low levels of LH in serum of PCOS-CGA group could be attributed to the inhibitory effects of CGA on the activity of nitric oxide synthase.[31] Reducing the level of LH is necessary in order to balance LH/FSH ratio and exert a stimulatory effect of FSH on follicle growth,[32] therefore, it seems that the low level of LH in mice with CGA-treated PCOS has helped to initiate the development of follicular groups and ovulation. The increase in the number of secondary follicles, graafian follicles and corpus luteum is a good explanation for the results. In addition, evidence demonstrated that CGA could decrease cholesterol levels. Low cholesterol levels are associated with low production of gonadal steroids.[33] It seems that CGA reduces the synthesis of gonadal steroids including testosterone by decreasing the level of cholesterol. This finding was in agreement with Zhou *et al*. who postulated that CGA exerted estrogenic activity and prevented estrogen deficiency-induced osteoporosis in ovariectomized rats.[34] Also, Wang *et al*. indicated that CGA exhibited an estrogen-stimulating effect on ovarian granulosa cells. They expressed that CGA might have the therapeutic potential for the treatment of postmenopausal syndrome.[35] In this study, in addition to stimulatory effects of CGA on folliculogenesis and hormonal status, antioxidant properties of CGA supported these improvements. In this regard, some studies proved that OS contributes to the incidence of PCO. It was also demonstrated that the markers of oxidative status such as MDA and glutathione peroxidase (GPx) could be altered in patients with PCOS.[36] Likewise, antioxidant effects of CGA in different tissues (not in ovary) have been reported previously.[37-39] Our findings indicated that CGA could recover the concentrations of both TAC and GSH involved in the reduction of lipid hydro peroxides which in turn induced cell death.

Moreover, the findings indicated that high concentrations of MDA observed in the PCOS group were decreased by CGA. This suggested that the reduction of serum MDA concentrations were due to the antioxidant and ROS production reducing the properties of CGA.[40]

Our results were in accordance with those of Ali *et al*. who confirmed that CGA could significantly increase activities of anti-oxidant armory such as SOD (scavenges the superoxide anion to form H2O2), CAT (convertor of H_2O_2 to H_2O) and GSH content.[38] Koriem *et al*. showed that caffeic acid enhanced GSH concentrations while it reduced MDA and catalyzed concentrations in brain, liver, and kidney tissues.[41] It has been discerned that hydroxyl groups (OH) on phenolic acids act as positive moieties for their antioxidant properties which is associated with the number of hydroxyl groups as follows: Tri-hydroxy phenolic acids > di-hydroxy (catechol) > mono-hydroxy.[15,42] These morphological, hormonal and biochemical findings showed developmental ability and functional potential of CGA in PCOS.

In conclusion, whereas PCOS is the most common endocrine-metabolic disorder and causes infertility among women, focusing on the CGA potential therapeutic effects on ovary will be useful for public health. This was the first study to reveal the effects of CGA in a rat model of PCOS. Based on the biochemical and morphological results of this study, CGA could reduce cysts in PCO model and eliminate oxidative stress in PCOS. Hence, we proposed that CGA might be used to improve ovarian functions in PCOS in human being. Thus, before clinical application, it is necessary to precisely evaluate the effectiveness and molecular mechanism of CGA in PCOS.

Acknowledgments

This work was supported by Urmia University of Medical Sciences.

Conflict of interest

The authors report no conflicts of interest.

References

1. Stener-Victorin E, Padmanabhan V, Walters KA, et al. Animal models to understand the etiology and pathophysiology of polycystic ovary syndrome. Endocr Rev 2020; 41(4): bnaa010. doi: 10.1210/ endrev/bnaa010.
2. Tehrani FR, Simbar M, Tohidi M, et al. The prevalence of polycystic ovary syndrome in a community sample of Iranian population: Iranian PCOS prevalence study. Reprod Biol Endocrinol 2011; 9: 39. doi: 10.1186/ 1477-7827-9-39.
3. Setji TL, Brown AJ. Polycystic ovary syndrome: update on diagnosis and treatment. Am J Med 2014; 127(10): 912-919.
4. Lewandowski KC, Cajdler-Łuba A, Salata I, et al. The utility of the gonadotrophin releasing hormone (GnRH) test in the diagnosis of polycystic ovary syndrome (PCOS). Endokrynol Pol 2011; 62(2): 120-128.
5. Shi Y, Kong X, Yin H, et al. Effect of hawthorn leaf flavonoids in dehydroepiandrosterone-induced polycystic ovary syndrome in rats. Pathobiology 2019; 86(2-3): 102-110.
6. Weiss NS, Kostova E, Nahuis M, et al. Gonadotrophins for ovulation induction in women with polycystic ovary syndrome. Cochrane Database Syst Rev 2019; 16: (1). CD010290. doi: 10.1002/ 14651858. CD010290.pub3.
7. Mohammadi M. Oxidative stress and polycystic ovary syndrome: a brief review. Int J Prev Med 2019; 10: 86. doi: 10.4103/ijpvm.IJPVM_576_17.
8. Ekici K, Temelli O, Parlakpinar H, et al. Beneficial effects of aminoguanidine on radiotherapy-induced kidney and testis injury. Andrologia 2016; 48(6): 683-692.

9. Murri M, Luque-Ramírez M, Insenser M, et al. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update 2013; 19(3): 268-288.
10. Blair SA, Kyaw-Tun T, Young IS, et al. Oxidative stress and inflammation in lean and obese subjects with polycystic ovary syndrome. J Reprod Med 2013; 58(3-4): 107-114.
11. Furat Rencber S, Kurnaz Ozbek S, Eraldemır C, et al. Effect of resveratrol and metformin on ovarian reserve and ultrastructure in PCOS: an experimental study. J Ovarian Res 2018; 11(1): 55. doi: 10.1186/s13048-018-0427-7.
12. Chen J, Guo Q, Pei YH, et al. Effect of a short-term vitamin E supplementation on oxidative stress in infertile PCOS women under ovulation induction: a retrospective cohort study. BMC Womens Health 2020; 20(1): 69. doi: 10.1186/s12905-020-00930-w.
13. Wu G, Hu X, Ding J, et al. The effect of glutamine on dehydroepiandrosterone-induced polycystic ovary syndrome rats. J Ovarian Res 2020; 13: 57. doi: 10.1186/s13048-020-00650-7.
14. Surai PF, Kochish II, Fisinin VI, et al. Antioxidant defence systems and oxidative stress in poultry biology: an update. Antioxidants (Basel) 2019; 8(7): 235. doi: 10.3390/antiox8070235.
15. Tajik N, Tajik M, Mack I, et al. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. Eur J Nutr 2017; 56(7): 2215-2244.
16. Nguyen TV, Wittayarat M, Do LTK, et al. Effects of chlorogenic acid (CGA) supplementation during *in vitro* maturation culture on the development and quality of porcine embryos with electroporation treatment after *in vitro* fertilization. Anim Sci J 2018; 89(8): 1207-1213.
17. Nguyen TV, Tanihara F, Do L, et al. Chlorogenic acid supplementation during *in vitro* maturation improves maturation, fertilization and developmental competence of porcine oocytes. Reprod Domest Anim 2017; 52(6): 969-975.
18. Javanmard MZ, Meghrazi K, Ghafori SS, et al. The ameliorating effects of Vitamin E on hepatotoxicity of ecstasy. J Res Med Sci. 2020; 25: 91. doi: 10.4103/jrms.JRMS_496_19.
19. Brawer JR, Munoz M, Farookhi R. Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. Biol Reprod 1986; 35(3): 647-655.
20. Beloosesky R, Gold R, Almog B, et al. Induction of polycystic ovary by testosterone in immature female rats: Modulation of apoptosis and attenuation of glucose/insulin ratio. Int J Mol Med 2004; 14(2): 207-215.
21. Arentz S, Smith CA, Abbott J, et al. Combined lifestyle and herbal medicine in overweight women with polycystic ovary syndrome (PCOS): a randomized controlled trial. Phytother Res 2017; 31(9): 1330-1340.
22. Watanabe T, Kobayashi S, Yamaguchi T, et al. Coffee abundant in chlorogenic acids reduces abdominal fat in overweight adults: a randomized, double-blind, controlled trial. Nutrients 2019; 11(7): 1617. doi: 10.3390/nu11071617.
23. Sun L, Ji C, Jin L, et al. Effects of exenatide on metabolic changes, sexual hormones, inflammatory cytokines, adipokines, and weight change in a DHEA-treated rat model. Reprod Sci 2016; 23(9): 1242-1249.
24. Ghafurniyan H, Azarnia M, Nabiuni M, et al. The effect of green tea extract on reproductive improvement in estradiol valerate-induced polycystic ovarian syndrome in rat. Iran J Pharm Res 2015; 14(4): 1215-1233.
25. Guan Y, Wang D, Bu H, et al. The effect of metformin on polycystic ovary syndrome in overweight women: a systematic review and meta-analysis of randomized controlled trials. Int J Endocrinol 2020; 2020: 5150684. doi: 10.1155/2020/5150684.
26. Naseri L, Khazaei MR, Khazaei M. Potential therapeutic effect of bee pollen and metformin combination on testosterone and estradiol levels, apoptotic markers and total antioxidant capacity in a rat model of polycystic ovary syndrome. Int J Fertil Steril 2021; 15(2): 101-107.
27. Tahmasebi F, Movahedin M, Mazaheri Z. Antioxidant effects of calligonum extract on ovarian tissue of PCO model: An experimental study. Int J Reprod Biomed 2018; 16(10): 641-648.
28. Matsuda F, Inoue N, Manabe N, et al. Follicular growth and atresia in mammalian ovaries: regulation by survival and death of granulosa cells. J Reprod Dev 2012; 58(1): 44-50.
29. Ergenoglu M, Yildirim N, Yildirim AG, et al. Effects of resveratrol on ovarian morphology, plasma anti-mullerian hormone, IGF-1 levels, and oxidative stress parameters in a rat model of polycystic ovary syndrome. Reprod Sci 2015; 22(8): 942-947.
30. Grossman MP, Nakajima ST, Fallat ME, et al. Müllerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. Fertil Steril 2008; 89(5 Suppl): 1364-1370.
31. Chen WP, Wu LD. Chlorogenic acid suppresses interleukin-1β-induced inflammatory mediators in human chondrocytes. Int J Clin Exp Pathol 2014; 7(12): 8797-8801.
32. Grosser PM, McCarthy GF, Robaire B, et al. Plasma patterns of LH, FSH and prolactin in rats with a polycystic ovarian condition induced by oestradiol valerate. J Endocrinol 1987; 114(1): 33-39.
33. Wan CW, Wong CN, Pin WK, et al. Chlorogenic acid exhibits cholesterol lowering and fatty liver attenuating properties by up-regulating the gene

expression of PPAR-α in hypercholesterolemic rats induced with a high-cholesterol diet. Phytother Res 2013; 27(4): 545-551.
34. Zhou RP, Lin SJ, Wan WB, et al. Chlorogenic acid prevents osteoporosis by Shp2/PI3K/Akt pathway in ovariectomized rats. PLoS One 2016; 11(12): e0166751. doi: 10.1371/journal.pone.0166751.
35. Wang X, Wang GC, Rong J, et al. Identification of steroidogenic components derived from *Gardenia jasminoides* Ellis potentially useful for treating postmenopausal syndrome. Front Pharmacol 2018; 9: 390. doi: 10.3389/fphar.2018.00390.
36. Mancini A, Bruno C, Vergani E, et al. Oxidative stress and low-grade inflammation in polycystic ovary syndrome: controversies and new insights. Int J Mol Sci 2021; 22(4): 1667. doi: 10.3390/ijms22041667.
37. Dkhil MA, Abdel Moneim AE, Bauomy AA, et al. Chlorogenic acid prevents hepatotoxicity in arsenic-treated mice: role of oxidative stress and apoptosis. Mol Biol Rep 2020; 47(2): 1161-1171.
38. Ali N, Rashid S, Nafees S, et al. Protective effect of Chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: An experimental approach Chem Biol Interact 2017; 272: 80-91.
39. Santana-Gálvez J, Cisneros-Zevallos L, Jacobo-Velázquez DA. Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. Molecules 2017; 22(3): 358. doi: 10.3390/molecules22030358.
40. Liu Y, He XQ, Huang X, et al. Resveratrol protects mouse oocytes from methylglyoxal-induced oxidative damage. PLoS One 2013; 8(10): e77960. doi: 10.1371/ journal. pone.0077960.
41. Koriem KMM, Arbid MS. Role of caftaric acid in lead-associated nephrotoxicity in rats via antidiuretic, antioxidant and anti-apoptotic activities. J Complement Integr Med 2017; 15(2). doi: 10.1515/ jcim-2017-0024.
42. Naveed M, Hejazi V, Abbas M, et al. Chlorogenic acid (CGA): A pharmacological review and call for further research. Biomed Pharmacother 2018; 97: 67-74.