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THE EFFECT OF GALLIC ACID ON HISTOPATHOLOGICAL AND STEREOLOGICAL OVARIAN INDEX IN ANIMAL MODEL OF POLY CYSTIC OVARIES SYNDROME

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Abstract

Polycystic ovary syndrome (PCOs) is a major cause of infertility due to non-ovulation. Studies have shown effects of antioxidant gallic acid. Therefore, the aim of the present study effect of Gallic acid on histopathological and stereological ovarian index in animal model of poly cystic ovaries syndrome. In this study, 40 females Wistar rats were randomly divided into five control groups (without treatment), pco group (receiving Letrozole 1mg/kg for 28 days) and PCOS groups, (receiving 50, 100, and 150 mg/kg doses of gallic acid, respectively). The gallic acid was administered per orally for 14 days. After dissection, the rats' ovaries were removed and put in 10% formalin for fixation and slides preparation processes were carried out. Finally, the study data were entered into the SPSS statistical software (v. 22) and analyzed using the Tukey test. The present study results indicated a significant decrease in the corpus luteum diameter and number of unilamminar follicles, Antral, graffian and corpus luteum and increase number of atretic and cystic follicles in the pcos group comparison to the control group ($p \leq 0.05$) which showed improvement process in high dosage treated groups. However, no significant differences were found regarding the number of primordial follicles and oocyte diameter ($p \leq 0.05$). It seems that Gallic acid affects the returning of follicogenesis in rats with PCOS and non-ovulation, in a way it causes ovulation increment.

Keywords: *Gallic acid, Poly cystic ovarian syndrome, Histopathology, Rat*

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INTRODUCTION:

Poly cystic ovary syndrome (PCOS) is a common endocrine disorder affecting about 10-5% of women with peri-menopausal women [1]. In 1935, Stein and Leontal first described this collection with non-ovulation[2,3]. They reported seven patients (four of whom were obese), with amenorrhea, hemocytosis, and large polycystic ovaries, and they made two-sided shear cutting, of which two of them were pregnant and believed that the layer The surface of the ovary prevents the release of follicles that reach the surface of the ovary [2]. Endocrine disorders in these women include non-ovulation or oligo-ovulation, infertility, hyssostysm and acne, which increases the levels of testosterone biology due to increased production of androgens from the ovaries [3-5]. The presence of multiple follicles (equal to or greater than eight follicles with a diameter of less than 10 mm) on the ovary is independent of the polycystic ovary syndrome and is not related to internal metabolic disorders. As women with polycystic ovaries have reported normal androgen levels and regular ovulation cycles[5,6].

Gallic acid

Trihydroxybenzoic acid, known as Gallic acid, is a phenolic acid having the chemical formula $C_7H_6O_5$

And with a molar mass of 170.12 g / mol. Appearance of this compound is red crystals. Which is found in various plants such as oak, tea, gravel, grape seed and apple [7]? Salts and gallic acid esters are known as Galatea. Gallic acid is commonly used in pharmacy. It is also used as the standard for determining the phenolic content of various compounds takes. Gallic acid has antioxidant, antifungal and anti-viral properties, and gallic acid is an important part of traditional medicine in some countries. Glycolic acid ester inhibits cellular damage by reducing oxidative stress [8]. Its antioxidant properties protect cells from oxidative damage and prevent their cellular cancer by its antiproliferative effect. The Limenez, 2009[7] studies have shown that gallic acid has antibacterial and anti-inflammatory effects. It has also been investigated its antioxidant effects by inhibiting tyrosinase activity [8]. In some other studies, it has been found that gallic acid prevents dyslipidemia from rising fat. Glycogen acid induces apoptosis in cancer cells and increases glutathione peroxidase enzymes, which indicates its antioxidant effect [9]. Polycystic ovary syndrome is a complex and abnormal disease that can include one or more types of phenotypes. One of the complications is metabolic changes, so patients with pcos are usually obese and have insulin resistance and hyperinsulinemia[10,11]. Recent studies have shown that polyphenols such as ethyl halothane in grapes[12], as well as polyphenols, such as acid glycols in the Leaf leaf extract [13], inhibit the migration of smooth muscle cells Veins and possibly in the prevention of atherosclerosis

Roman-Garcia et al., 2011[14]. Babamaleki et al.,[15] In 2015, showed that green tea, due to antioxidant compounds such as gallic acid, increased the concentration of LH and FSH hormones in rats treated with cadmium chloride[15]. A study by Oyagbem et al.[16] In 2015 found that gallic acid administration significantly increased the concentration of superoxide dismutase and glutathione S transferase, and significantly increased levels of LH, testosterone and increased sperm count Live, prevent testicular atrophy and epithelium in mice receiving cyclophosphamide [16]. In a study by Punithavathi et al. In 2015[17] on the protective effects of gallic acid in diabetic rats, it was found that gallic acid significantly reduced blood glucose, lipid peroxidation, and increased insulin levels [17]. According to Jahnifar et al., The high prevalence of PCOS among twins can be explained by perinatal period factors[18], based on the high degree of heterogeneity in ovarian ultrasonography images among 34 twin sisters. The culture of isolated cells from the same size follicles from the ovaries of healthy individuals and PCOS showed that high levels of progesterone and 17α-hydroxyprogesterone and testosterone in PCOS have a resistant biochemical phenotype in these cells [19]. Some people thought that the cause of this increase in androgen production in PCOS was due to an adenyl cyclase enzyme disorder. Therefore, these patients were assigned to the control group for treatment of forcollin (adenosine cyclase activator). The concentration of Forscholine required for maximum steady state stimulation was similar in both groups. Therefore, it is shown that steroid production differences are not dependent on adrenal cyclase dysfunction in the presence of forscholine test [2]. Another theory is that increasing the activity of the steroidogenic cells of a single cell in PCOS patients than normal individuals is due to an increase in LH levels in these individuals. Which disrupts the order in the biosynthesis of androgens from the cells of these individuals [2]? Several genes are essential for the production of androgens, which include the steroidogenic genes regulating protein (STAR), P450C17a gene, the side chain gene division ($CYP11_a$) and 17 beta-hydroxydehydrogenase[19]. Various studies have shown that 3-beta-hydroxydehydrogenase enzymes are involved in polycystic ovary syndrome (Nelson et al., 1999). Some studies have shown that the messenger RNAs of CPY17 and $CYP11_a$ in PCOS individuals are more abundant than normal human cells, but the STAR messenger RNA was similar in normal and PCOS cells [19].

RESEARCH METHODOLOGY:

How to select and maintain the animals tested

The animals tested in this study included 40 Wistar female rats, which were completely randomized from Shiraz University of Medical Sciences laboratory for animal breeding and maintenance.
Experiments were conducted.
Grouped animals tested
In this research, a total of 40 adult female rats were used. In groups of 8were grouped. Mice were weighed before and after the test.
Animals were categorized completely randomly in the following groups:
Control group: There were no animal treatments in this group, and animals only use ordinary water and food.
In this group, the mice were infected with PCOS. The mice in this group were polycystic ovary syndrome. In this group, the mice received the volume of the drug in the experimental groups of the solvent, namely, normal saline (for induction of polycystic ovary syndrome for 28 days, letrozole (1 mg / kg) in the oral form).
Experimental group 1: The rats received oral glycoprotein (glycine) at 50 mg / kg for 14 days after induction of polycystic ovary syndrome.
2 experimental groups: rats after induction of polycystic ovary syndrome, gallic acid for 14 days with a dose 100 mg / kg received orally.
Experimental group 3: The rats received oral gallic acid at 150 mg / kg for 14 days after induction of polycystic ovary syndrome.

How to prepare and prescribe gallic acid

The gallic acid used in this study was purchased as an acros vial of 100 grams. In order to prescribe gallic acid, the recommended dose (50, 100 and 150 mg / kg body weight) was dissolved according to the administered dose (50, 100 and 150 mg / kg body weight), then the weight of the mice was dosed for 2 weeks according to the weight of the mice.

Polycystic ovary syndrome induction

In order to induce PCOS in this study, the aromatase inhibitor, letrozole, which is considered the best in animal models, was used [20]. Treatments of rats with letrozole tablets at a dose of 1mg / kg were performed by oral gavage for 28 days.

Method of study of tissue sections

Slides from different parts of the ovary allow counting the entire follicles in each ovary. Basal follicles, single layer, multi-layers, secondary (antral), graph and yellow body follicles were counted using a microscope with cellcanus, respectively. After the microscope was attached to the computer, a few photos of follicles were also prepared. Also, the diameter of the corpus luteum and oocyte in different follicles was measured by the sterilization software.

Methods of analysis and statistical calculation

The numbers obtained from counting the number of primiparous follicles, pretetral, antral, graph, atretic, and corpus luteum in different groups were analyzed by SPSS software and analyzed by ANOVA one way and post hoc Tukey tests. And their charts were drawn up using Excel software. The values applied mean ± SD are significant at the significance level ($p \leq 0.05$).

RESULTS:

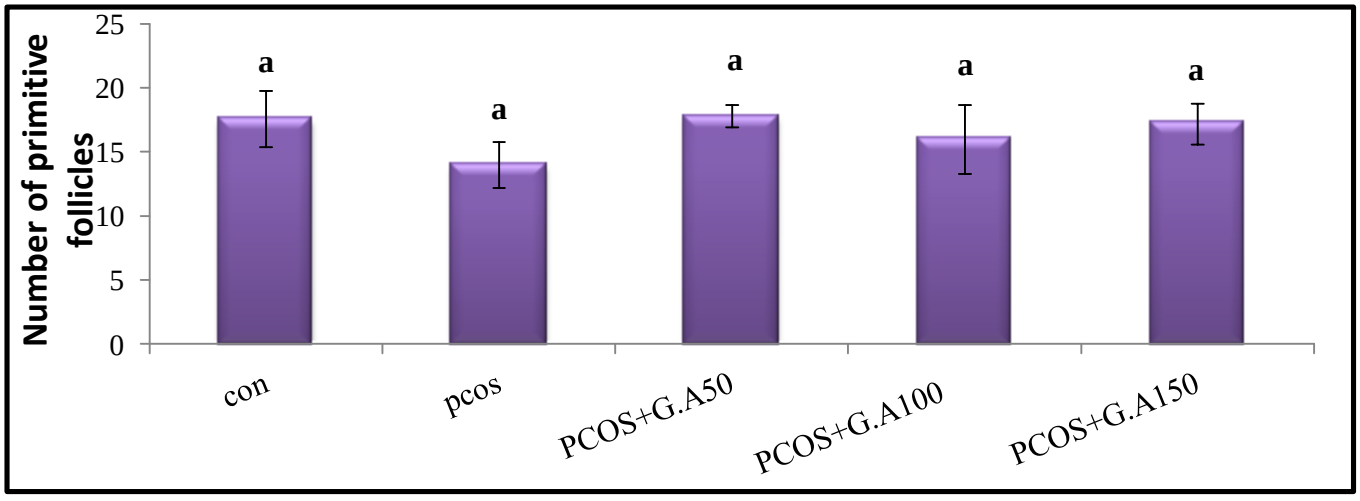

Fig 1: Results of changes in the number of primiparous follicles

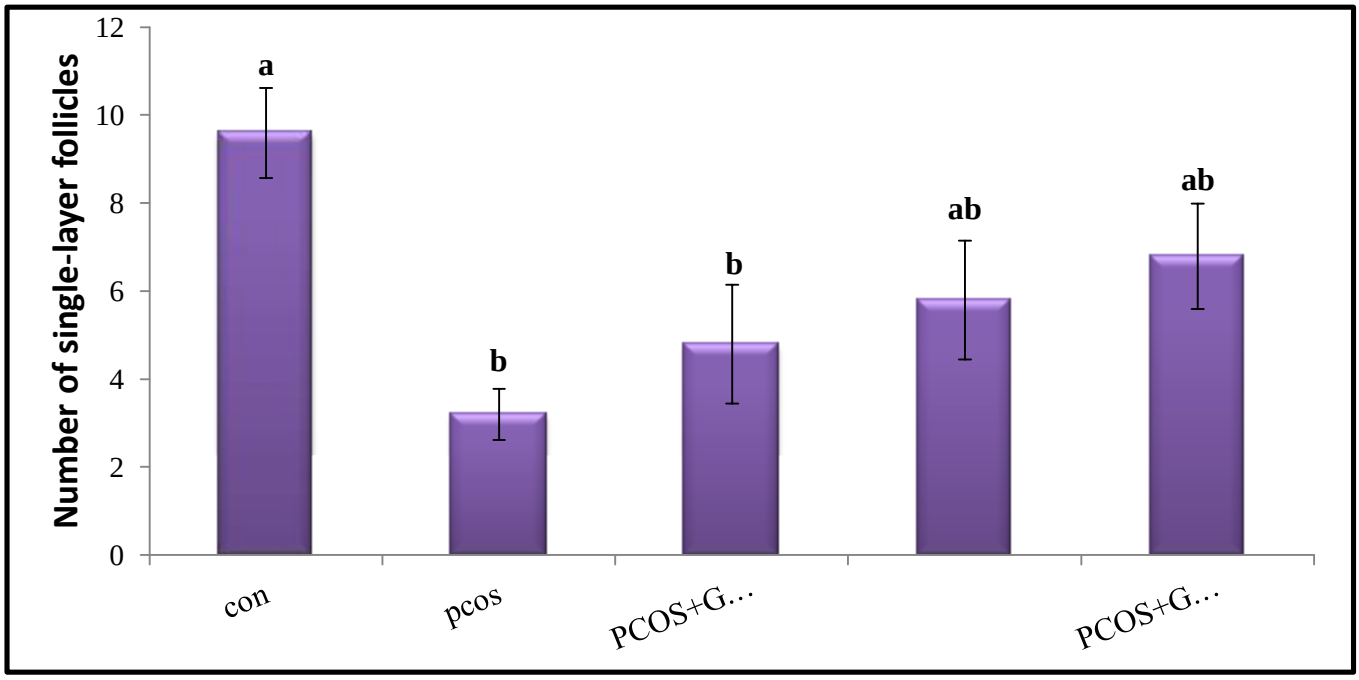

Fig 2:shows the results of changes in the number of single-layer follicles

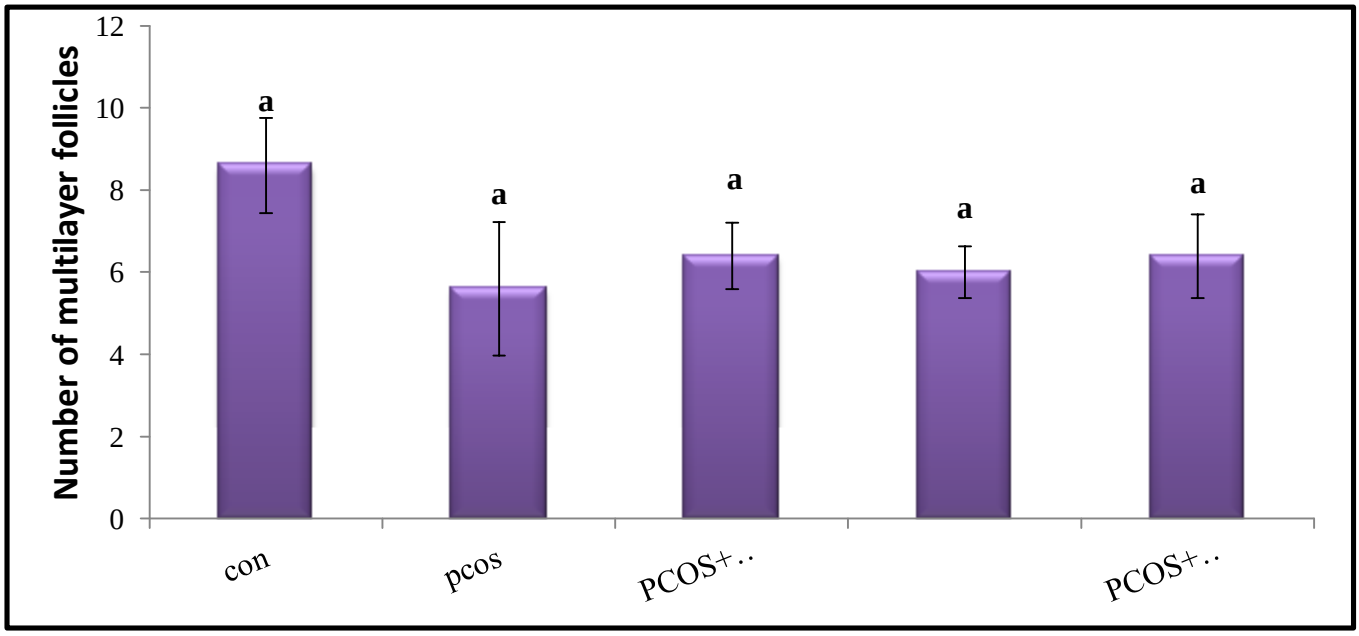

Fig 3: shows the results of changes in the number of multilayer follicles

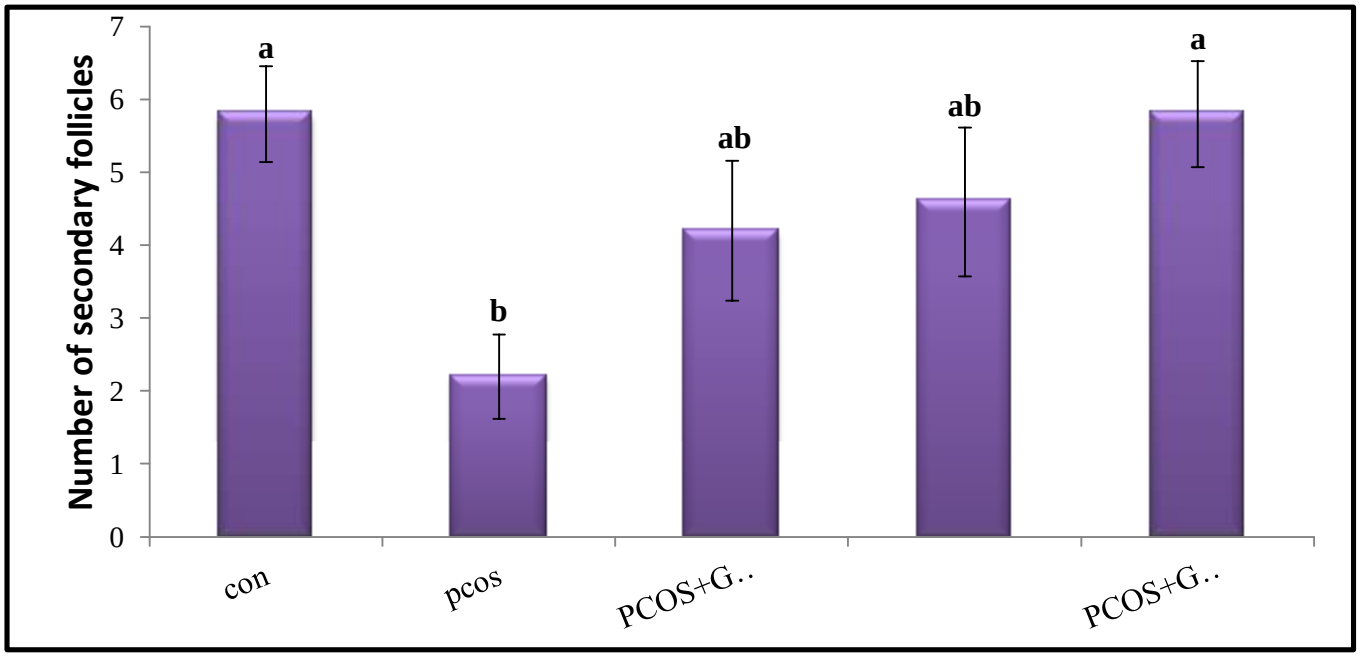

Fig 4: Results of changes in the number of secondary follicles

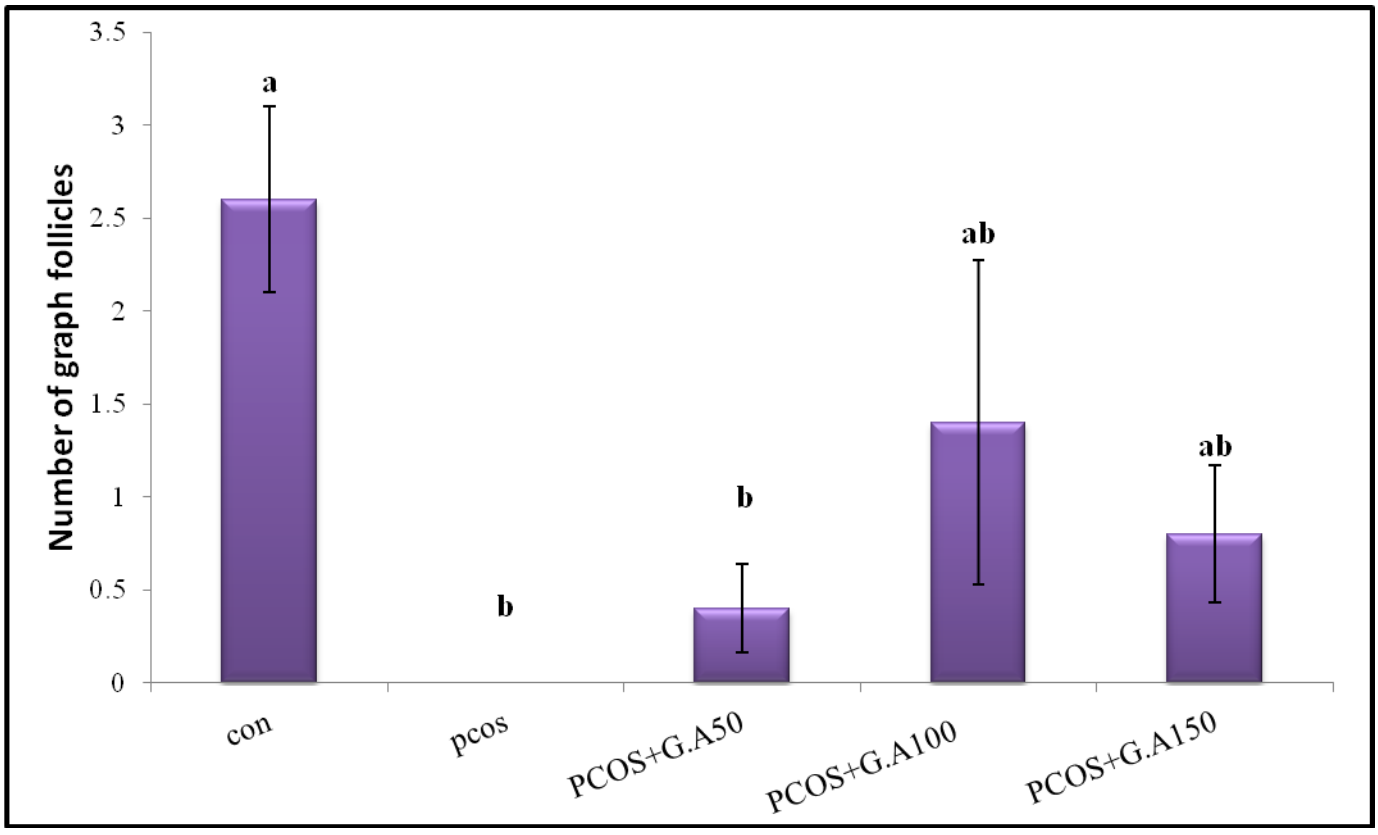

Fig 5: shows the results of changes in the number of graph follicles

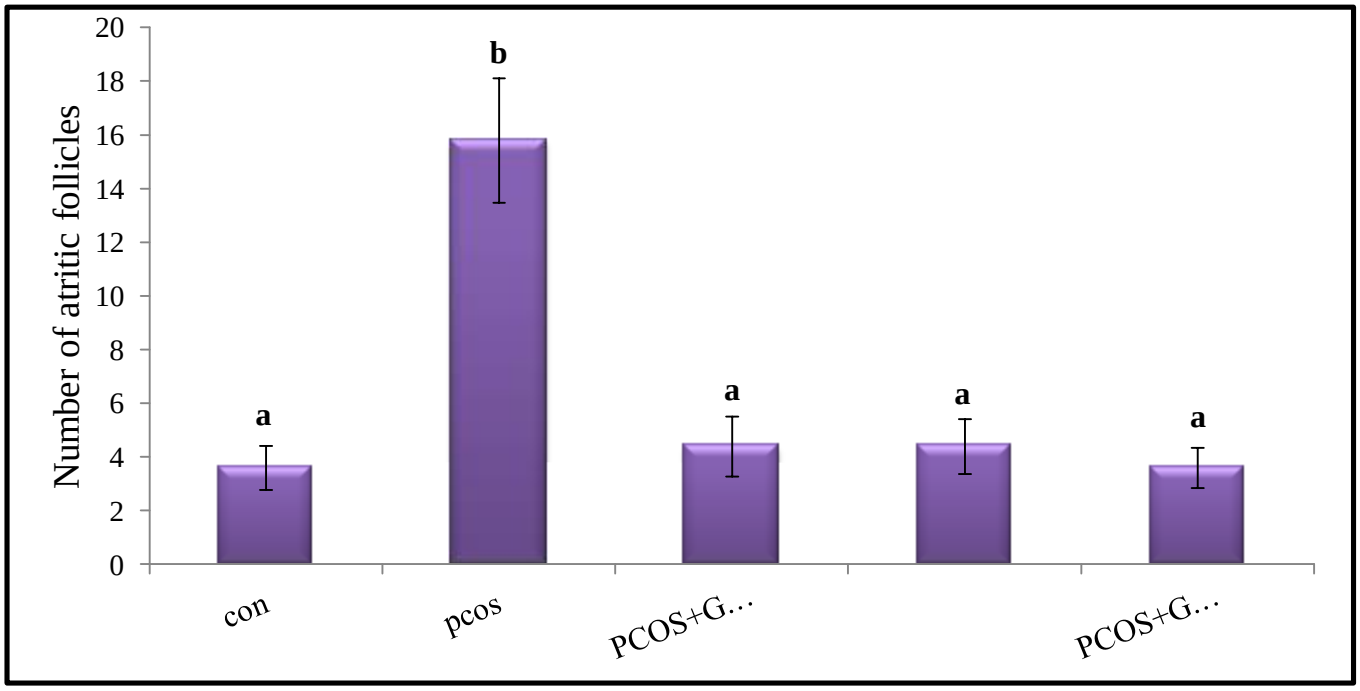

Fig 6:Results of variations in the number of atretic follicles in the studied groups

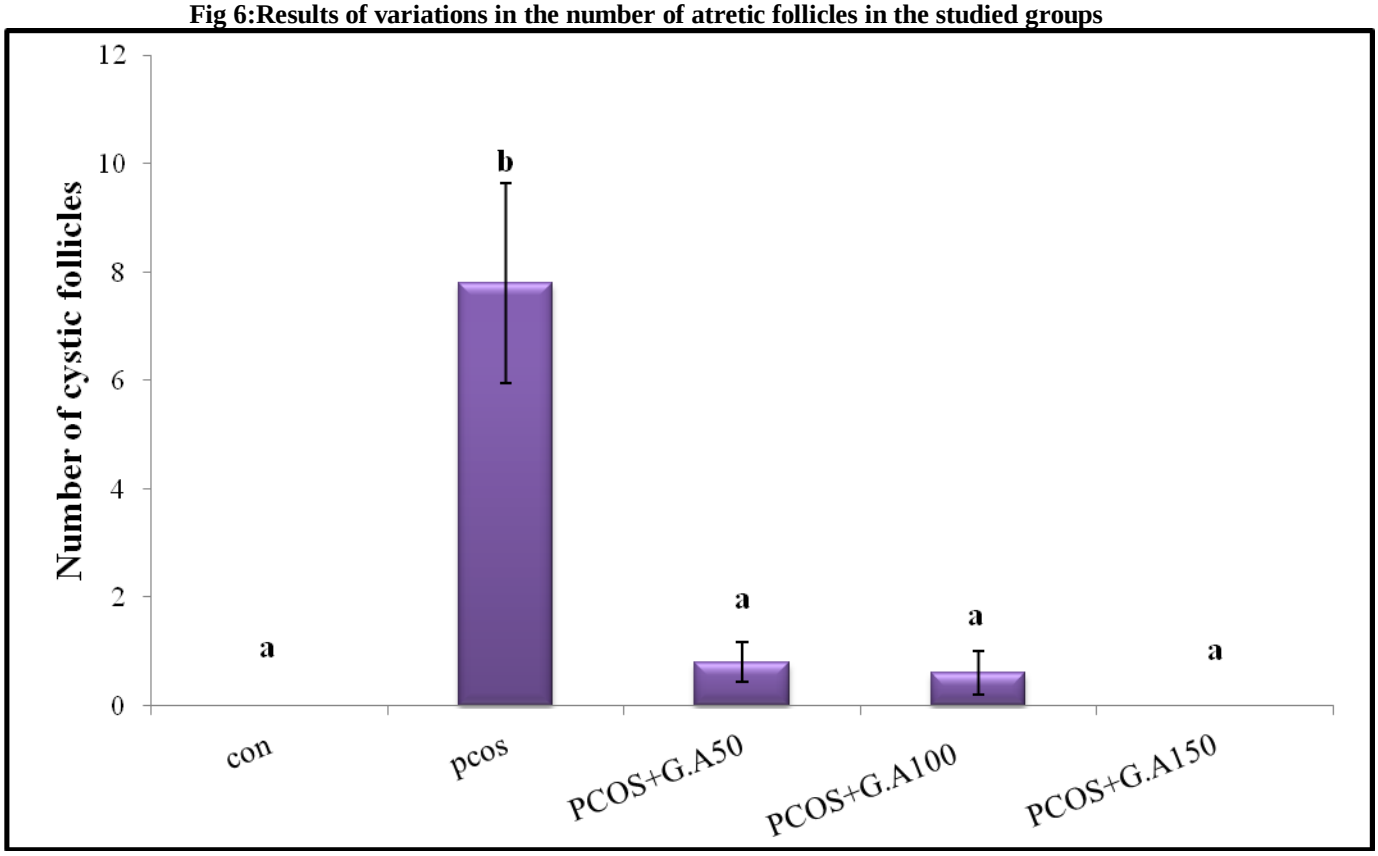

Fig 7: Results of changes in the number of cystic follicles in the studied groups

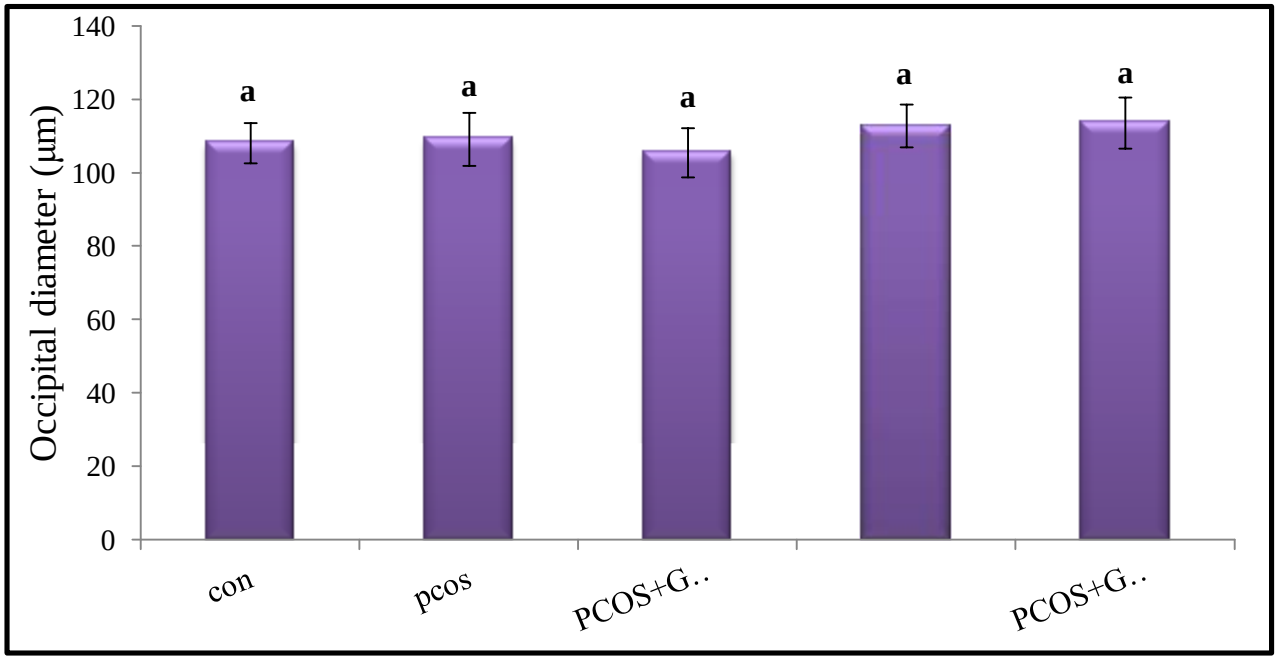

Fig 8: Results of oocyte diameter changes in the studied groups

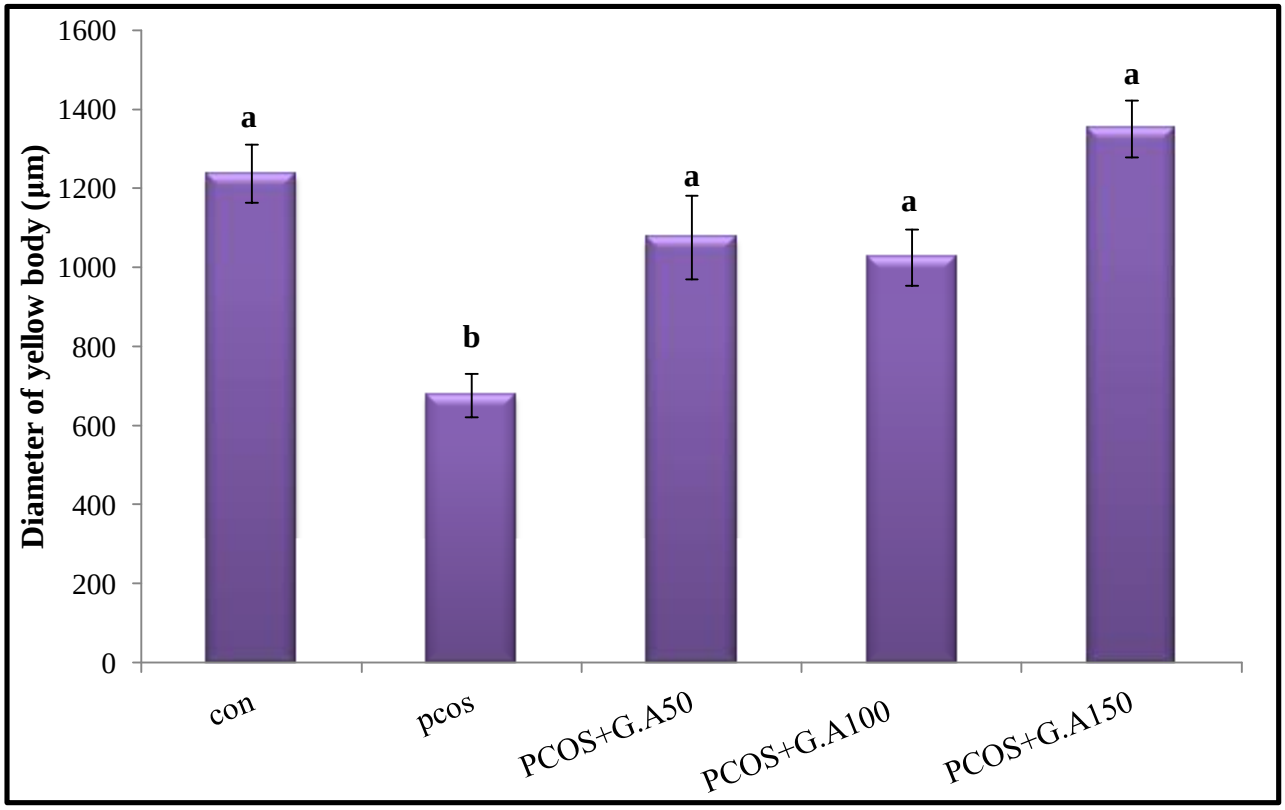

Fig 9: Results of changes in the diameter of the corpus luteum in the studied groups

DISCUSSION AND CONCLUSION:

Polycystic ovary syndrome is the most common endogenous endocrine disorder in women of reproductive age, which is a pathological condition of angiogenesis and ultimately leads to infertility. This syndrome is associated with fast follicular growth, no selection of a dominant follicle from increased follicular storage (follicular growth stops), accumulation of at least 10 cysts around ovarian stroma and ovarian volume, elevated ovarian layers (hyperkinesis), hyperandrogenism, and increased LH ratio Appears to FSH (Kelly et al, 2001). The syndrome is associated with symptoms such as prenusi, menstrual disorders, ovulation failure, diabetes, obesity, high blood pressure, fatty liver, sleep deprivation, unusual uterine bleeding, chronic pelvic pain, androgen and male hormones and acne. [21]. In the present study, after induction of polycystic ovary syndrome in rats, there was a significant decrease in the number of single-layer follicles, graph, secondary, yellow body and a significant increase in the number of atretic and cystic follicles in the letrozole group compared to the group. The receiver has been monitored. However, there was no significant change in the number of primiparous follicles, multilayer, and oocyte diameter in the studied groups. In this study, an aromatase enzyme inhibitor, letrozole, was used to create a polycystic ovary in the animal model. Letrozole is a non-steroidal aromatase inhibitor and has great potential for inhibiting aromatase enzymes. Reducing the activity of the aromatase enzyme disrupts ovarian steroidogenesis, which ultimately induces PCOS. This enzyme catalyzes the estrogen biosynthesis of the androgen, reducing the activity of this enzyme, inhibiting the testosterone flavors to estradiol, resulting in an increase in testosterone levels and decreases in estrogen levels Finds [22]. The decrease in estrogen levels indicates degradation and apoptosis of granulosa cells in ovarian follicles. In the present study, there was a significant increase in the number of atriatic follicles in the ovarian tissue. Which is compatible with the hormonal results of previous studies? In a study by Oyagbemi et al. In 2016[16], it was found that gallic acid increases the level of FSH and thus affects the activities of the hypothalamic-pituitary gonadal axis [16]. Therefore, it is likely that there is a significant increase in estrogen concentration in treated groups, which is probably due to increased activity of the aromatase enzyme and the conversion of testosterone to estrogen, which requires further study in this field, in other words, in Future research should examine this issue in order to determine its exact mechanism. Some studies have shown that serum follicular stimulatory hormones (FSH) concentrations in PCOS-free ovulation women are similar to those of women who were normal in the follicular mid-cycle of menstrual cycle. But in women, who are in the early follicular phase, this difference may be related to the mechanism of ovulation failure and there is little chance that its main cause [23]. A study by Elizabeth in 2009 found that in women with this syndrome, the levels of testosterone, estradiol, and LH increased, and progesterone and also FSH decreased, although sometimes, the level of FSH did not change [24]. Studies show that in polycystic ovary syndrome, follicles progress to the middle of the atherosclerotic stage, and then the process of puberty stops and with apoptosis of the granulosa cells, follicles are atrested, follicles stop growing, and Cystic follicles and Atretic formations [25,26]. Studies have shown that gallic acid has antibacterial and anti-inflammatory effects. It has also been investigated its antioxidant effects by inhibiting tyrosinase activity[8]. Gallic acid with antioxidant properties [8] can possibly have adverse effects from

Free radicals, so that in the present study, there was a significant decrease in the number of atrotic follicles in treated groups than in the PCOS group. It has been found in some other studies that gallic acid induces apoptosis in cancer cells and increases glutathione peroxidase enzymes, which indicates its antioxidant effect [9]. Sarkaki [27]and his colleagues in 2015 showed that gallic acid was significantly increased at the time of injury to the brain can reduce tumor necrosis factor production. It should be acknowledged that this is not just gallic acid function Can play a role in inhibiting cell death, but also in reducing inflammatory responses[27]. Since gallic acid inhibits the production of these inflammatory cytokines (interleukin 1 and tumor necrosis factor), Thus decreasing the inflammatory response and indirectly inhibiting cellular damage. In the results of microscopic studies, the cell death process in the treated groups has significantly shown it [28]. Obviously, gallic acid has an effect on the cellular and vascular events of the inflammation process and inhibition of the expression of the binding molecules and inhibition of the release of inflammatory cells and the reduction of the production of inflammatory mediators, cytokines (IL-1α, IL-1β, IL-6, IL- 8, TNF-α) and growth factors can be effective in reducing and inhibiting cell death in treated groups [6,29,30]. In the present study, there was a significant decrease in the diameter of the corpus luteum in the group receiving letrozole. This is probably due to an increase in apoptosis in lutein cells. In a study conducted by Elizabeth et al. In 2009, it was found that progesterone concentrations in PCOS patients showed a significant decrease compared to healthy subjects (Elizabeth et al., 2009), which was associated with histological findings of the study The present is the same. In a study by Ghafurniyan et al in 2015 on the anti-inflammatory

effects of green tea on levels of interleukin-6 and CRP in rats with PCOS, it was found that inflammatory indices in green tea groups Shows a significant decrease compared to the PCOS group. Also, the number of yellow corpuscles and the thickness of the granulosa layer in the PCOS group decreased and the thickness of the layer increased slightly, which was observed in groups treated with green tea extract containing antioxidant compounds such as gallic acid (Ghafurniyan et al. 2015) . Which is consistent with the results of this study. But in the groups treated with gallic acid, the diameter of the corpus luteum was within the control group and showed a significant increase compared to the group receiving Letrozole (PCOS). Which indicates the beneficial effects of gallic acid in improving the complications of polycystic ovary syndrome?

Total resulting

The results of the present study indicate that gallic acid has inhibitory effects on the formation of cystic and atritic follicles, which ovulate and improve the histology of polycystic ovaries and lead to healthy and active ovaries. To make Therefore, it is hoped that further studies will help treat infertility and improve the complications of polycystic ovary.

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