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Therapeutic Effect of Quercetin in Letrozole Induced Polycystic Ovary Syndrome in Rat Model

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Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders diagnosed in women. The prevalence of polycystic ovary syndrome is estimated to be between 2.2% and 26% among women. Inflammation is thought to play a role in the pathogenesis of PCOS. Quercetin is a flavonoid that has been shown to be anti-inflammatory and antioxidant so, the aim of this study was to differentiate between the effects of two doses of quercetin & to provide natural, safe, and effective treatment of polycystic ovary syndrome.

Aim of Study: The aim of the present study was to determine the role of quercetin in treatment of Letrozole Induced polycystic ovary syndrome in rat model and determine the effects of two different doses of quercetin.

Material and Methods: Adult nonobese female rats were induced to polycystic ovary syndrome by using 1mg/kg of letrozole and confirmed to be diseased by vaginal smears. A group of the experimental rats received 50mg/kg of quercetin; another group received 100mg/kg of quercetin & another one received 100mg/kg of metformin. LH, FSH, (LH/FSH) ratio, hormonal profile, catalase, TNF-α, serum glucose level, insulin sensitivity and ovarian morphology by hematoxylin & eosin (H&E), Masson trichrome (MTC), periodic acid-Schiff (PAS) & anti transforming growth factor beta (TGF-β) stains were then assessed in addition to the weight of the rats and their temperature after induction and after treatment.

Results: Treatment with quercetin significantly decreased testosterone & TNF-α and increased FSH, LH, estrogen, progesterone & catalase. It decreased body weight & body hair distribution. It also improved insulin sensitivity & improved histological structure of the ovary. 100mg/kg of quercetin had better results than the dose of 50mg/kg of quercetin & metformin in the parameters we measured. 50mg/kg of quercetin & metformin were comparable in their results.

Key Words: *Polycystic ovary syndrome – Quercetin – Letrozole.*

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Introduction

POLYCYSTIC ovary syndrome is one of the most prevalent endocrine disorders affecting women of childbearing period. Polycystic ovary syndrome (PCOS) affects 2.2-26% of women of reproductive age around the world [1]. It is a major health problem that leads to subfertility, metabolic disorder, glucose intolerance, psychological manifestations such as depression and anxiety, cardiovascular and cerebrovascular complications [2]. The National Institute of Health (NIH) described polycystic ovary syndrome as having hyperandrogenism and/or hyperandrogenemia, oligo-ovulation, absence of established disorders such as Cushing's syndrome, hyperprolactinemia, and congenital adrenal hyperplasia (CAH), and polycystic ovaries on ultrasound. Alternatively, according to the Rotterdam 2003 criterion, two of the following three characteristics must be present: On ultrasound, polycystic ovaries, oligo- or anovulation, physiological and/or biochemical symptoms of hyperandrogenism [3].

Treatment options of polycystic ovary syndrome including weight reduction by diet, exercise or bariatric surgery. Other options are ovulation induction by clomiphene citrate, aromatase inhibitors, glucocorticoids, gonadotropins. Laparoscopic ovarian diathermy, in vitro fertilization techniques. Metformin is also used to treat the hyperglycemia associated with PCOS [4]. Letrozole (LET), a non-steroidal aromatase inhibitor, produces an animal model to study PCOS. Letrozole blocks aromatase enzyme functions. So, ovarian cells can't produce estrogen, and a polycystic ovary with an abnormal follicular cycle develops due to an increased circulating androgen level [5].

Many researches have been done to discover treatment for polycystic ovary syndrome with

natural substances including; Liquorice, Ginseng saponin, Flaxseed, Aloe-vera, Chaste berry, White peony, Cinnamon, Milk thistle & Chamomile. Natural substances is better than synthetic drugs to avoid their side effects [6]. Quercetin is a flavonoid present in many vegetables and fruits has anti-inflammatory effect and immunosuppressive role on the activity of dendritic cells function [7]. Quercetin, as a natural antioxidant, was utilized to protect rats and mice's ovaries from the gonadotoxic effects of cadmium by scavenging free radicals and boosting the activities of other antioxidants including catalase, SOD, and GPx. As a result, it increased the health of the ovaries by raising estradiol, FSH, and LH output [8].

Letrozole is used to produce model of PCOS as it produces animal model that is in several ways similar to the human polycystic ovary syndrome [9]. Metformin, an insulin sensitizer, is frequently prescribed for PCOS and anovulatory infertility in order to decrease free testosterone, estrogen and dehydroepiandrosterone sulphate (DHEAS). It is used in anovulatory PCOS to improve cyclicity and reproductive results [10]. The PCOS pathogenic process can be affected by quercetin through a variety of targets and pathways, with few side effects. It was noted that quercetin can improve ovulation dysfunction, alleviate insulin resistance (IR), lessen androgen, control lipid metabolism, manage gut microbiota, and enhance vascular endothelial function, all of which are crucial for the treatment of PCOS [11].

Jahan et al., [12] studied the effect of one dose (100mg/kg) of quercetin on PCOS, and their study showed that it had improved the physiological and histological parameters of PCOS and recommended to study different doses of quercetin on PCOS from physiologic point of view.

The aim of our work was to compare the effects of two different doses of quercetin on the physiological and histological parameters of PCOS to determine the least effective dose of quercetin in treatment of PCOS.

Material and Methods

Our study is an experimental animal study. The experiments were held in the Physiology Department lab, Faculty of Medicine, Suez Canal University from September 2021 to December 2021.

Animals:

Forty Adult (60-70 days old) nonobese (160-200gm) female wistar rats brought from Faculty of Veterinary Medicine, Zagazig University were used as the animal model in this study. Animals were retained in stainless steel cages (6-8 rats/cage), at room temperature 25 ±5 °C. Ethical approval was admitted from faculty of medicine, Suez Canal University Committee code 3615 dated 5-7-2021.

All the rats were kept under 10/14h dark/light cycle and fed with standard laboratory food pellet and tap water [12]. Vaginal cytological analysis was carried out daily for about 6 days to monitor the four-day ovarian cycle.

The rats were randomly divided into 5 groups (8 rats in each group); Group I: (Control group) was not induced to PCOS. It received 0.5% carboxymethylcellulose (CMC) (Sigma-Aldrich, USA Catalog No: 4193 11) given in a dose (2mg/kg) dissolved in distilled water orally by gavage 4 times /day for 28 days [12]. Group II: (PCOS group) PCOS induction by letrozole (Femara @novartis) (1mg/kg) dissolved in 0.5%CMC diluted by distilled water orally by gavage once daily for 21 days [12]. Group III: (50 mg/day of quercetin treated group) PCOS induced group + 50mg/kg of body weight of quercetin (Sigma-Aldrich, USA Catalog No: Q4951) dissolved in dimethyl sulphoxide (DMSO) (Sigma-Aldrich, USA catalog No: 34869) then was diluted by distilled water to reach final concentration 0.5% of DMSO after induction orally by gavage divided into 4 times/day (12.5mg/kg per time) for 28 days [13]. Group IV: (100mg/day of quercetin treated group) PCOS induced group + 100mg/kg of body weight of quercetin dissolved in DMSO then was diluted by distilled water to reach final concentration 0.5% of DMSO after induction orally by gavage divided into 4 times /day (25mg/kg per time) for 28 days. Group V: (Metformin treated group) PCOS induced group + 100mg/kg of body weight of metformin (Glucophage @merck) dissolved in distilled water after induction and used as the standard treatment orally by gavage divided into 4 times/day (25mg/kg) for 28 days [13].

Baseline assessment: Weight of the animals was measured before the experiment to be able to identify any weight change during the experiment.

Vaginal smears were done to ensure that the rats had a normal estrous cycle.

A vaginal swab was made by wetting a cotton-tipped swab with physiological saline at room temperature and inserting it into the vagina of a confined rat. After gently turning and rolling the swab against the vaginal wall, it should be removed. By sliding the brush across the surface, the cells are transferred to a dry glass slide. The slide is

dried by air, stained, and examined under a microscope [14].

Body weight: Baseline weight was measured to ensure normality of rat weight then it was measured every 5th day during induction and treatment periods by using electronic animal weight scale [12].

Body temperature: Rectal temperature were measured after induction and after treatment during the sleep cycle three times in each measurement using a mercury thermometer for rats. The average values were used.

Body hair distribution: The difference in distribution of body hair was assessed by observation as was assessed by [12].

Blood sampling:

After 28 days of treatment, the animals were anaesthetized then after the palpebral reflex had disappeared, Blood was collected by inserting an uncoated 20mL microhaematocrit tube (o.d. 1/4 1.3mm) into the medial canthus of the orbit while gently pushing and rotating it forward. The blood was transferred to a centrifuge tube and allowed to coagulate for 30 minutes. To assist separation, the clotted blood was centrifuged for 5 minutes. Serum was isolated and preserved at –20 °C so that biochemical and hormonal analysis could be performed. Then, animals were decapitated at the diestrus stage [12].

Blood glucose level: Before the completion of the treatments, the rats were fasted for a total of 12 hours. Blood was drawn from the medial canthus of the eye of each rat. A glucometer was used to test the blood glucose levels in the samples [15].

Insulin resistance (IR) calculation: After measurement of fasting blood glucose level (FBG) and measurement of fasting insulin (FINS) levels. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula:

IR=FBG (mmol/l) X FINS (m U) / 22.5 [13]. The kit used: Rat INS (Insulin) ELISA Kit, cat no: EH1113, Fine Biotech, Wuhan, Hubei, China. The principle of this test is sandwich enzyme-linked immune-sorbent assay technology.

Hormonal analysis: The concentration of plasma estrogen, progesterone and testosterone were measured via Enzyme Linked Immuno-Sorbent Assay (ELISA) (Rat E2 (Estradiol) ELISA Kit, cat no: 1507, Fine Biotech, Wuhan, Hubei, China, Rat Pg (Progesterone) ELISA Kit, cat no: 1255, Fine Biotech, Wuhan, Hubei, China & Rat T (Testosterone) ELISA Kit, cat no: EH1642, Fine Biotech, Wuhan, Hubei, China). The principle of this kit is Competitive-ELISA detection method.

LH / FSH ratio: Follicular stimulating hormone (FSH) was measured using ELISA kit (Rat FSH (Follicle Stimulating Hormone) ELISA Kit, cat no: ER0960, Fine Biotech, Wuhan, Hubei, China. The principle of these test similar to that of hormonal analysis. Luteinizing hormone (LH) was also measured by using Rat LH (Luteinizing Hormone) ELISA Kit, cat no: ER1123, Fine Biotech, Wuhan, Hubei, China. https://www.fn-test.com/product/er1123/) and its principle is similar to that of the FSH kit. The ratio was calculated.

TNF-α level as an inflammatory marker: Plasma cytokine (TNF-α) levels were calculated using an ELISA kit (Rat TNF-α (Tumor Necrosis Factor Alpha) ELISA Kit, cat no: ELK1396, ELK Biotechnology, Wuhan, Hubei, China) [16]. The test principle applied in this kit is Sandwich enzyme immunoassay.

Measurement of catalase level as an antioxidant marker:

Catalase level was measured using an ELISA kit (Catalase (CAT) Activity colorimetric kit, cat no: E-BC-K031-S, (Elabscience Biotechnology, USA).

Ovarian histological examination: The animals were sacrificed at the end of the experiment after anesthesia with Ketamine (Ketamine 50mg/ml vial @Sigma tec) & Xylazine (Rompun 100mg/ml@ Decra) and their ovaries were excised. The ovaries were fixed in 10% formaldehyde for 24h, then the specimens were processed in graduated ethyl alcohol to paraffin blocks. The blocks were sectioned at 4-5 µm thickness for the histological and immunohistochemical techniques.

Histopathological examination: Ovarian sections were stained with hematoxylin and eosin (H&E) and examined at 100x and 400x magnifications using a power light microscope to study the general architecture, histopathological changes, and the number of normal growing and atretic follicles of 15 sections per ovary (in randomly chosen fields) using a quantitative analysis. On the basis of the subsequent definitions, follicles were counted. Primordial follicles have monolayer of follicular epithelial cells. Primary follicles have monolayer or multilayer follicular epithelial cells enclosing the oocyte that are equally or strongly

prismatic. A secondary follicle is one in which the antrum is beginning to develop. A Graafian (tertiary) follicle has a single, big antrum that is filled with follicular fluid, granulosa cells surround the antrum, and granulosa cells surround the oocyte (cumulus oophorus cells). The atretic follicle is characterized by degenerated zona pellucida or an oocyte, granulosa cells that have undergone pyknosis, and cell debris in the antral cavity **[17]**. For morphometric analysis, the diameter of the biggest follicle with a clear oocyte, the thickness of granulosa and theca cells were measured using image J2x software **[12]**.

Ovarian sections were also examined using Masson's trichrome stain (MTS) to study the connective tissue content collagen fibers distribution of the ovaries. The amount of collagen was estimated through assessing the color area percentage of the green stained collagen using image J2x software. Ovarian sections were also examined using periodic acid-Schiff (PAS) for attaining of glucosaminoglycans (GAGs) detection **[18]**.

All histological sections were analyzed using (LEICA DM 1000) microscope & photographed using (LEICA DF425) camera.

Immunohistochemistry staining of anti TGF-β: Formalin-fixed paraffin-embedded blocks of ovarian tissue were sectioned (4- µm thick) and mounted on positively charged slides, then processed for manual immunohistochemical staining using transforming growth factor beta (TGF β 1) rabbit polyclonal antibody (Catalog no: PA 1029020, ThermoFisher Scientific co., USA) according to manufacturer protocol.The color area percentage of the brown stained cytoplasm in 15 random sections per ovary at 400X magnification was measured using image J2x software **[19]**.

Data management:

- The data were collected, coded and entered into the computer via Microsoft Excel 2019 program.
- Statistical Package for windows version 20.0 (SPSS, Chicago, IL, USA) was used for data analysis. Normality test was done by Shapiro-Wilk test. Data which showed normal distribution were proven by applying one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for pair-wise comparisons. Non parametric parameters were analyzed by Kruskal Wallis test followed by post hoc, least significant difference test (LSD). Friedman's test and serial repeated measurements of ANOVA were used for serial measurements among one group non-parametric and parametric respectively. All Values were described by mean ± standard deviation (SD). The *p*-value of 0.05 was used as the limit of statistical significance.

Results

Body hair: PCOS rats showed increased body hair distribution in ventral body compared to normal rats (Fig. 1-A). Quercetin and metformin groups showed decreased body hair distribution compared to PCOS rats (Fig. 1-B, C & D).

Body weight: Serial measurements of body weight every 5th day in all groups were done during the periods of induction and treatment. Weight measurement during the period of induction showed; within control group there was no statistically significant increase in body weight (*p*-value =0.899) for the control group, other groups that were induced to PCOS showed high statistically significant increase in body weight (*p*-value=0.001). The statistical significance began at the 15 th day of induction and continues to the end of induction period at 21 st day compared to control group (Fig. 2).

During treatment period, serial measurements of body weight every 5 th day were taken. Control group showed high statistically significant increase in body weight (*p*-value <0.001) throughout this period. The weight increased from 179.3 ±6.6 gm to 188.5±7.3 gm. Diseased group showed statistically significant increase in body weight from 199.5± 19.1 gm to 213.3± 15.5 g (*p*-value=0.026) as compared to control group. High dose quercetin group showed high statistically significant decrease in body weight (*p*-value=0.001) at the end of treatment period, from 188.1 ±21.4 gm to 210.9± 18.7 gm. Metformin treated group showed also high statistically significant decrease in body weight (*p*-value <0.001) from 197± 14 gm to 178.3± 11.3 gm after treatment. However, the low dose quercetin group showed less but statistically significant decrease in body weight (*p*-value=0.002) from 204.6±22.4 gm to 192.1 ±20.8 gm (Fig. 3).

At the end of treatment all treated groups showed statistically significant decrease in body weight compared to PCOS diseased group (*p*-value =0.002) and there was no significant difference compared to control group indicating near normalization of the body weight of treated rats. High dose quercetin & Metformin groups showed more statistically significant reduction of body weight better than low dose quercetin (Fig. 3).

Body temperature: Diseased and low dose quercetin groups showed statistically significant de-

crease in body temperature at the end of experiment period, while high dose quercetin and metformin treated groups showed protection against decrease in body weight (Fig. 4).

Hormonal assay: Diseased group showed significant decrease in FSH & LH levels compared to control group. Low & High dose quercetin groups showed significantly increased FSH & LH compared to diseased & metformin groups but also still significantly decreased from control group. So, high dose quercetin had better effect than low dose quercetin and both were better than metformin in normalization of FSH & LH level. LH/FSH ratio showed no statistically significant change among study groups (p-value=0.224) (Fig. 5).

About Estrogen, progesterone, the diseased group showed significant decrease in their levels compared to control group. High, low dose quercetin & Metformin groups showed significantly increase in them compared to diseased group but also still significantly decreased from control group. High dose quercetin had significant better effect than its low dose & metformin (Fig. 6). Regarding testosterone, PCOS diseased group showed statistically significant increased level of testosterone. High dose quercetin was better than low dose quercetin & both were better than metformin in decreasing testosterone level.

TNF-α & catalase: Diseased group showed statistically significant increase & decrease in TNF-α & catalase respectively compared to control group. Low, high dose quercetin & metformin showed statistically significant improvement in both, but high dose quercetin had better effect on both normalization than both low dose quercetin and metformin (Figs. 7,8).

Insulin sensitivity: Diseased group showed statistically significant increase in FBS, insulin & HOMA-IR as compared to control group. Low, High dose quercetin & metformin showed statistically significant decrease in them comparing to diseased group. But High dose was better than low dose quercetin & both were better than metformin in normalization of them (Table 1).

Histopathological and morphometric results:

Vaginal smear histology:

All rats at the beginning of the study groups' showed normal and regular 4-5 days estrous cycle including the 4 phases of estrous cycle; proestrus consisting of round nucleated epithelial cells (EC) a polygonal anucleated keratinocytes (KC) (Fig. 9-A), estrus phase with polygonal anucleated keratinocytes (KC) (Fig. 9-B), met-estrous where there are the leukocytes (LC) with the anucleated keratinocytes (KC) (Fig. 9-C) & diestrus phase that has predominance of leukocytes (LC) with few keratinocytes (KC) (Fig. 9-D). PCOS induced rats showed persistent estrus phase estrous cycle (Fig. 9-E).

1- Control group:

H&E stained sections of the ovaries in the control group showed normal ovarian structure. It consists of outer cortex & inner medulla. The cortex contains the different stages of follicular development (Fig. 10A,B) including; primordial follicle containing small rounded oocyte surrounded by flat stromal cells (Fig. 11-A), primary follicle containing bigger oocyte surrounded by zona pellucida and few layers of granulosa cells and outer flat stromal cells (Fig. 11-B), secondary follicle consisting of big oocyte surrounded by zona pellucida, layer of corona radiate, multiple layers of granulosa cells and theca layer; theca interna and theca externa and containing fluid cavity liquor folliculi (Fig. 11C) & mature graafian follicle consisting of big oocyte surrounded by zona pellucida, a layer of cells corona radiata, cumulous oophorus connecting it to the outer wall of the follicle which consists of multiple layers of granulosa cells, theca interna and theca externa surrounding large fluid cavity named antrum. The medulla containing the blood vessels (Fig. 11-D). There are also corpus lutei consisting of large acidophilic cells (Fig. 10-C).

2- Diseased group:

Microscopic examination of H&E-stained sections from PCOS induced ovaries showed the cysts that have thin granulosa layer (14 ±4.7 μm) and thick theca layer (33.9 ±3.5 μm) (Fig. 20) with separation of cells and presence of cell remnants, the medulla shows congested blood vessels (Fig. 12). There is inflammatory infiltration of multinucleated polymorphs (Fig. 13A,B). It shows a significant increased number of cysts (6.5 ±2.6 cyst) & atretic follicles (7 ±2.6 atretic follicle). It also showed a statistically significant decrease in the number of corpora lutea (2.6 ±2.7 corpora) compared to control group. (Table 2).

3- Low dose quercetin group:

Microscopic examination of H&E-stained ovarian sections from low dose quercetin treated rats' showed restoration of granulosa (41.2 ± 12.6 μm) and theca layers' thickness (19.9 ±3.4 μm) (Figs. 14,15, 20). There was a significant decrease in the number of cysts (2.5 ±2.3 cyst) and atretic follicles

(2.1±2 atretic follicle) compared to PCOS diseased group. It also showed a statistically significant increase in corpora lutea compared to PCOS diseased group (6.1±3.6 corpora) (Table 2).

4- *High dose quercetin group:*

Microscopic examination of H&E-stained ovarian sections from high dose quercetin treated rats showed restoration of granulosa (42.4±13.8 µm) and theca (19.1±2.6 µm) layers' thickness (Fig. 20) and decreased congestion of blood vessels but with level more that done by low dose quercetin (Figs. 16,17). There was also a significant decrease in the number of cysts (2.3±1.9 cyst) & atretic follicles (2.3±1.5 atretic follicle). It also showed statistically significant increase in corpora lutea compared to PCOS diseased group (7.3±2.9 corpora) (Table 2).

M*etformin group:*

Microscopic examination of H&E-stained sections from metformin treated group showed restoration of granulosa and theca layers' thickness (Figs. 18,19). There was a significant decrease in the number of cysts and atretic follicles compared to PCOS diseased group however, it was still significantly increased compared to control group. It also showed a statistically significant increase in corpora lutea compared to PCOS diseased group but, it was still statistically significantly decreased compared to control group (Table 2). There was increased thickness of granulosa cell layer (about 48.4±7.1 µm) & decreased thickness of theca cell layer (about 19±3.1 µm) compared to diseased group however, it was still significantly increased compared to control group (4.9±3 corpora) (Fig. 20).

Effect on the number of different types of ovarian follicles:

There was no statistically significant change in the number of different types of ovarian follicle including primordial, primary, secondary, graafian follicles & all follicles count except for ovarian cysts and atretic follicles.

Regarding ovarian cysts, there was high statically significant change among all study groups. The diseased group showed a statistically significant increase in number of cysts 6.5±2.6 cyst as compared to control group 0.5±0.5 cyst. High dose quercetin treated group showed statistically significant decrease in the number of cysts 2.3±1.9 cyst as compared to diseased group. The low dose quercetin group showed statistically significant decrease in number of cysts 2.5±2.3 cyst as compared to diseased group. The Metformin treated group showed a statistically significant decrease in the number of cysts 2.87±2 cyst as compared to diseased group but still statistically significant increased as compared to control group. So, high dose quercetin had better effect on reduction of the number of cysts than low dose quercetin and both were better than metformin (Table 2). For atretic follicles, there was high statistical significance among study groups. The diseased group showed statistically significant increase in the number of atretic follicles (7±2.6 follicle) as compared control group (1.4±1.3 follicle). Low dose quercetin showed statistically significant decrease in the atretic follicles (2.1±2 follicle) as compared to diseased group. High dose quercetin showed statistically significant decrease in atretic follicles (2.3±1.5 follicle) as compared to diseased group. Metformin treated group showed statistically significant decrease in atretic follicles (3.3±1.8 follicle) as compared to diseased group. So, low dose quercetin, high dose quercetin & metformin successfully decreased the number of atretic follicles (Table 2). There was no statistically significant change in the number of different types of ovarian follicle including primordial, primary, secondary, graafian follicles & all follicles count except for ovarian cysts and atretic follicles & corpora lutea. Regarding corpora lutea, there was statistically significance among study groups. The diseased group showed a statistically significant decrease in the number of corpora lutea (2.6±2.7 corpora) compared to control group. High dose & low dose quercetin treated groups showed statistically significant increase in corpora lutea compared to PCOS diseased group (7.3±2.9, 6.1±3.6 corpora respectively). The Metformin treated group showed statistically significant increase in corpora lutea compared to PCOS diseased group but, it was still statistically significantly decreased compared to control group (4.9±3 corpora) (Table 2).

Morphometric measurement of thickness of granulosa & theca layers:

Morphometric measurement to granulosa cell layer of diseased group showed statistically significant decreased granulosa cell layer thickness compared to control group. Low dose quercetin, high dose quercetin & metformin treated groups showed statistically significant increase in granulosa cell layer thickness (Fig. 20).

Morphometric measurement to theca cell layer showed that the diseased group had statistically significant increased theca cell layer thickness compared to control group. Low dose quercetin, high dose quercetin & metformin treated groups showed statistically significant decrease in theca cell layer thickness but, they were also statistically

significant increased compared to control group (Fig. 20).

Masson's trichrome stain (MTC):

Masson trichrome staining of ovarian section from control group showed normal collagen distribution in the cortex between and surrounding growing follicles and in the medulla. Ovarian sections from PCOS induced group showed increased green color caused by fibrosis about 72.9±6.4% (Figs. 21-B,22). Masson trichrome staining of ovarian section from low dose quercetin treated group showed decreased greenish area compared to PCOS diseased group (Figs. 21-C,22) about 50.4±0.34%. High dose quercetin treated group showed decreased greenish area compared to diseased group & low dose quercetin treated groups (Figs. 21-D,22) about 43 ±3.8%. The green distribution area of metformin treated group was 61.7±3.7% and that was statistically significant decreased compared to diseased group but, it was statistically significant increased compared to control & high dose quercetin treated group (Figs. 21-E,22).

Periodic Acid Schiff (PAS) stain:

Control group showed strong positive PAS reaction in the intercellular spaces between granulosa cells, within theca cells, in the basement membrane & in zona pellucida. It also showed average theca thickness (Fig. 23-A). PCOS diseased group showed low positive PAS reaction in the intercellular spaces between granulosa cells, within theca cells, in the basement membrane & in zona pellucida. It also showed increased theca thickness (Fig. 23-B). Low dose quercetin treated group showed moderate positive PAS reaction in the intercellular spaces between granulosa cells, within theca cells, in the basement membrane & in zona pellucida. It also shows moderate increased theca thickness (Fig. 23-C). High dose quercetin treated group showed strong positive PAS reaction in the intercellular spaces between granulosa cells, within theca cells, in the basement membrane & in zona pellucida. It also showed average theca thickness (Fig. 23-D). Metformin group showed strong positive PAS reaction in the intercellular spaces between granulosa cells, within theca cells, in the basement membrane & in zona pellucida. It also showed average theca thickness (Fig. 23-E).

Anti TGF-β immunohistochemical staining:

Control group showed negative expression of TGF-β with only few weak positive granulosa cells (Fig. 24-A). PCOS induced group (diseased group) revealed significant TGF-β immune-expression, that was prominent in granulosa and theca cells (Fig. 24-B). Area percentage of anti TGF-β was about 72.9±6.4% (Fig. 25). The low dose quercetin treated group showed moderate TGF-β reaction in granulosa cells and theca cells and it was indicated that the granulosa cells of the ovarian follicles and corpora lutea expressed higher TGF-β (Fig. 24-C). Area percentage of anti TGF-β was about 50.4± 0.34% (Fig. 25). High dose quercetin treated group showed minimal TGF-β reaction in granulosa cells and theca cells and it was also indicated that the granulosa cells of the ovarian follicles and corpora lutea expressed higher TGF-β (Fig. 24-D). Area percentage of anti TGF-β was about 43±3.8% (Fig. 25). Metformin treated group showed moderate TGF-β reaction in granulosa cells and theca cells and it was also indicated as in the other groups that the granulosa cells of the ovarian follicles and corpora lutea expressed higher TGF-β (Fig. 24-E). Area percentage of anti TGF-β was about 61.7± 3.7% (Fig. 25).

Table (1): Insulin sensitivity.

	Normal group (n=4)	Diseased group (n=4)	Low dose quercetin group (n=4)	High dose quercetin group (n=4)	Metformin group (n=4)	*p*-value
FBS (mmol/l)	6.5±0.78	16.44±0.70**N**	6.18±0.80**D**	5.8±1.09**DT**	10.2±0.91**NSTU**	0.002***1**
Insulin (mIU)	0.102±0.015	0.167±0.01**N**	0.140±0.01**ND**	0.123±0.01**NDT**	0.152±0.004**NDU**	<0.001***2**
HOMA IR	0.028±0.0023	0.120.5±0.054**N**	0.040±0.010**ND**	0.030±0.011**DT**	0.065±0.009**NDTU**	0.002***1**

- Data was non-parametric except for insulin was parametric & described as mean ± SD.
1- Kruskal-Wallis test, followed by post hoc, (LSD).
2- ANOVA test followed by Tukey's post hoc teat.
* Statistically significant.
(N, D, T, U) Significant in comparison to control, diseased, low & high dose quercetin group respectively.

Table (2): Follicles count among the study groups.

Mean ± SD	Normal group (n=8)	Diseased group (n=8)	Low dose quercetin group (n=8)	High dose quercetin group (n=8)	Metformin group (n=8)	*p*-value
Primary	5.75±1.58	6±2.98	6.25±4.46	5.38±3.46	5.88±3.48	0.989[1]
Secondary	4.25±3.7	1.63±1.18	3.25±3.7	2.13±2	1.38±1.4	0.272[2]
Graffian	0.5±0.5	0±0	0.38±0.5	1.0±1.0	0.5±0.8	0.104[2]
Primordial	8.25±4.5	2.38±01.8	4±3.4	5.88±5.9	5.5±2.9	0.064[1]
Corpus luteum	7.3±3.3	4.6±2.7	6.1±3.6	7.3±2.9	4.9±3	0.291[1]
Cysts	0.5±0.5	6.5±2.6**N**	2.5±2.3**D**	2.3±1.9**D**	2.87±2**ND**	0.001[*2]
Atretic follicles	1.4±1.3	7±2.6**N**	2.1±2**D**	2.3±1.5**D**	3.3±1.8**D**	<0.001[*1]
All follicles count	27.9±10.9	28.1±9.6	24.6±13.8	26.1±13	24.3±9.3	0.940[1]

- Data were parametric except for secondary and graafian follicles & cysts were non-parametric. Data was described as mean ± SD.
1- Kruskal-Wallis test, followed by post hoc, (LSD).
2- ANOVA test followed by Tukey's post hoc teat.
* Statistically significant.
(N, D, T, U) Significant in comparison to control, diseased, low & high dose quercetin group respectively.

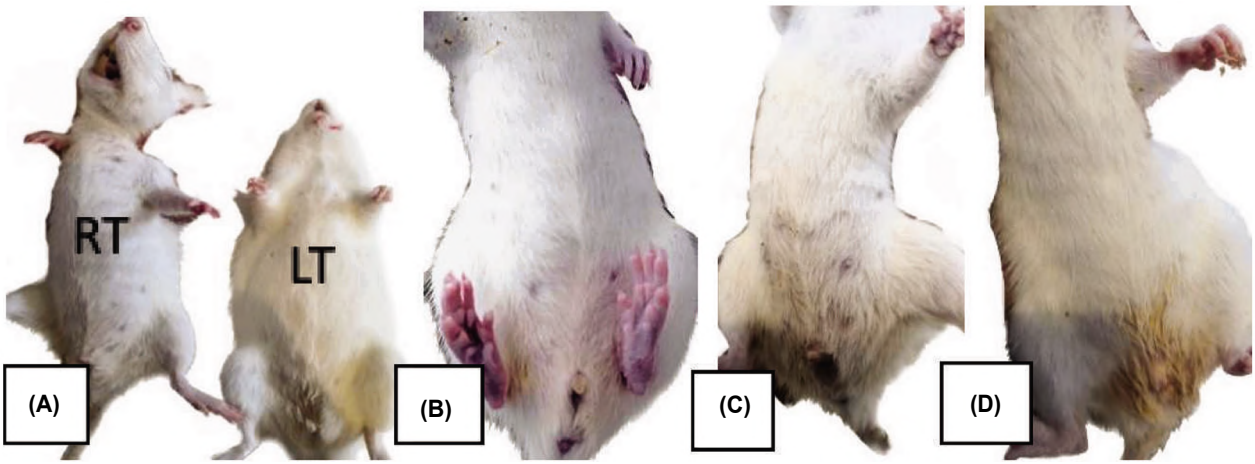


Fig. (1): Hair distribution among study groups (A) shows the difference in hair distribution between normal rat (LT) & PCOS rat (RT). (B,C&D) show hair distribution in low &high dose quercetin and metformin respectively.

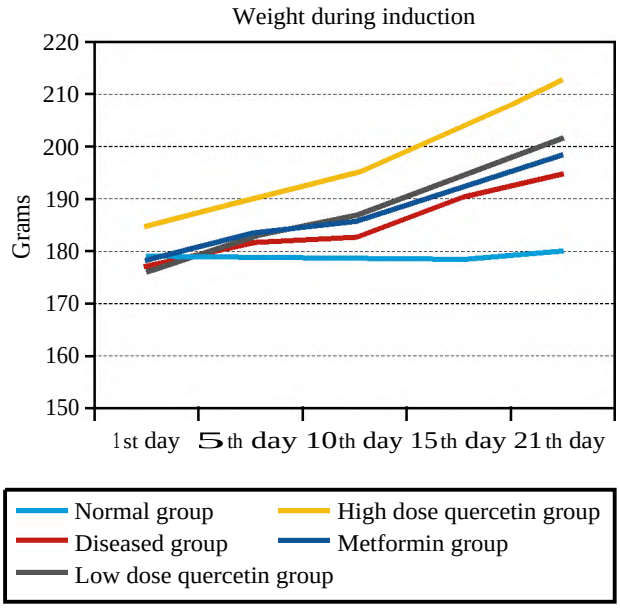


Fig. (2): Serial measurements of weight during induction among the study groups. Data are expressed as mean ± SD using Friedman's & Kruskal Wallis tests followed by post hoc (LSD).

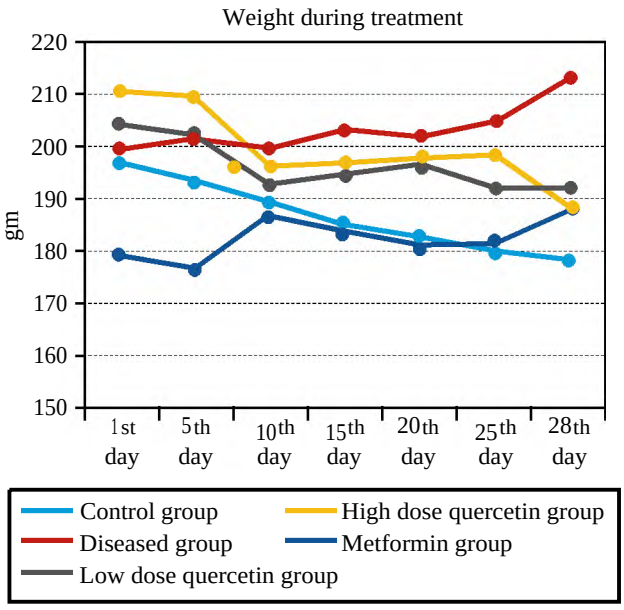


Fig. (3): Serial measurements of weight during treatment among the study groups. Data are expressed as mean ± SD using Repeated measures ANOVA & ANOVA test Followed by Tukey's post hoc.

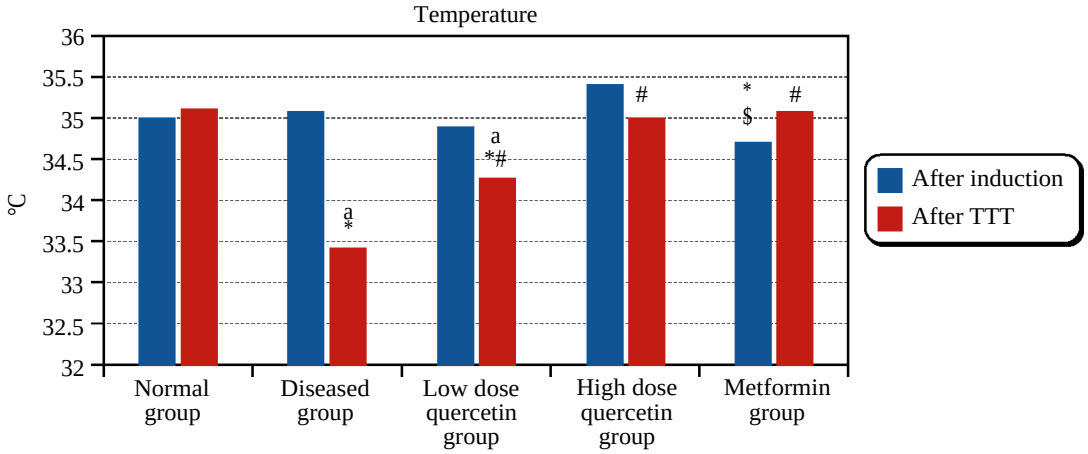


Fig. (4): Temperature measurements after induction and treatment among the study groups. Data are expressed as mean ± SD using Friedman test & Kruskal Wallis test followed by post hoc (LSD) . (*, #, $) Significant *p*-value in comparison to control, diseased & low dose quercetin groups respectively. (a) Significant *p*-value after induction in the same group ($p \leq 0.05$).

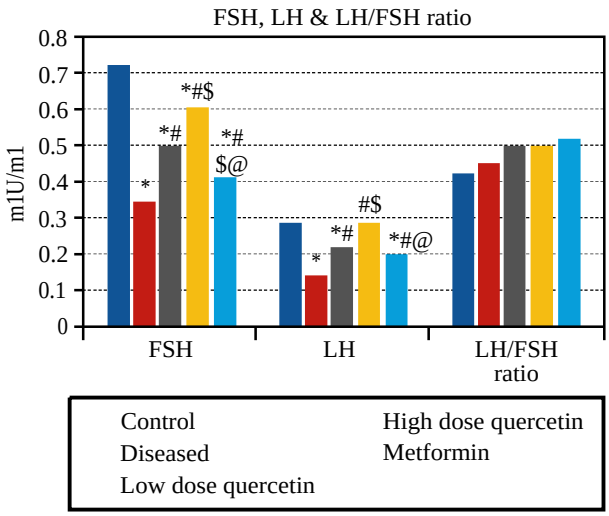


Fig. (5): FSH, LH & LH/FSH ratio.

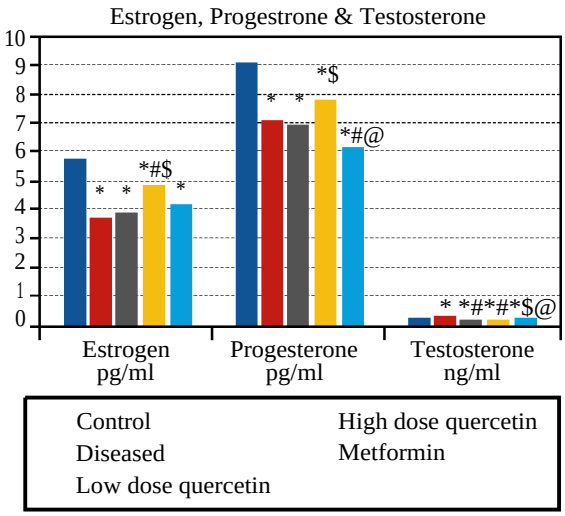


Fig. (6): Estrogen, progesterone & testosterone.

Data in Figs. (5,6) are expressed as mean ± SD using Kruskal-Wallis test followed by post hoc (LSD). (*, #, $, @) Significant *p*-value in comparison to control, diseased, low & high dose quercetin group respectively.

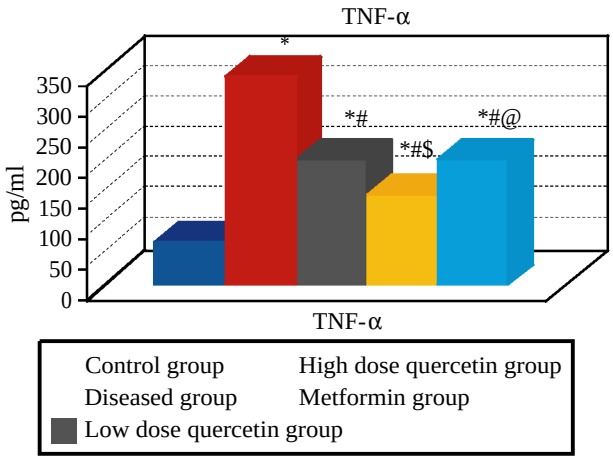


Fig. (7): TNF-α measurements.

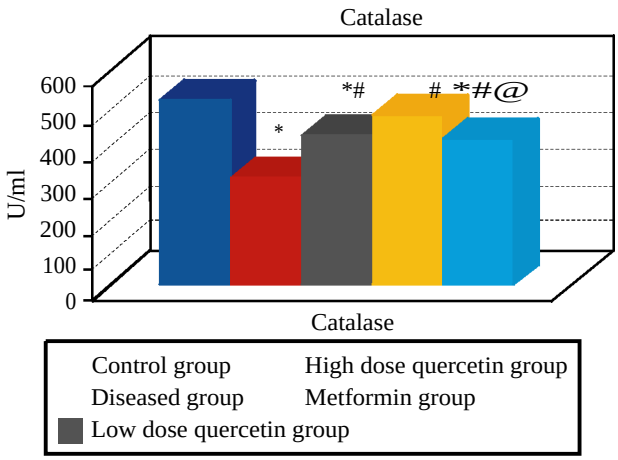
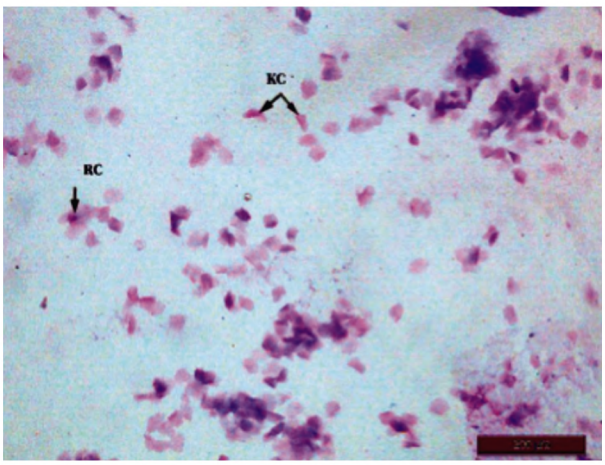
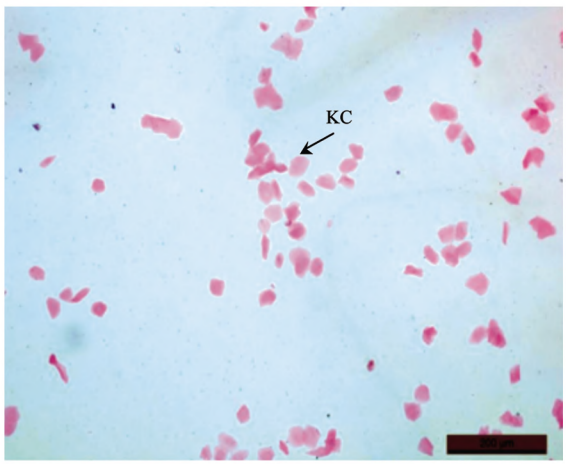


Fig. (8): Catalase measurements.

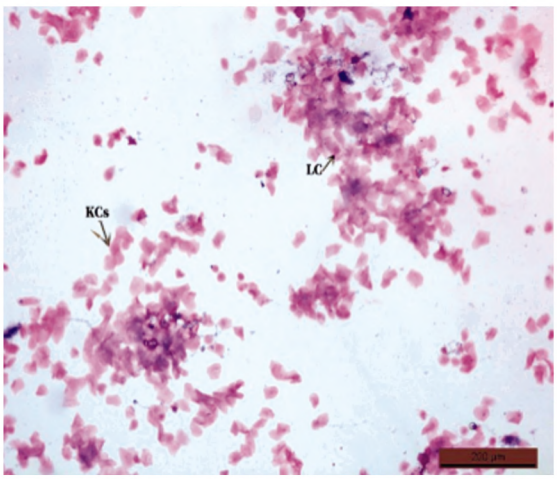
Data in Figs. (7,9) are expressed as mean ± SD using Kruskal-Wallis test are expressed as mean ± SD using Kruskal-Wallis test followed by post hoc (LSD). (*, #, $, @) Significant *p*-value in comparison to control, diseased, low dose & high dose quercetin group respectively.



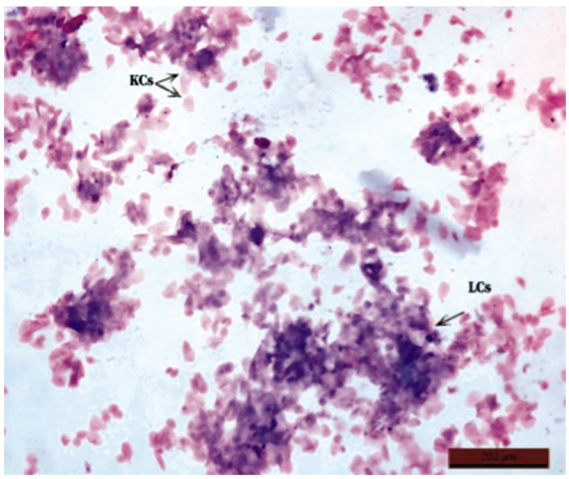
(A)



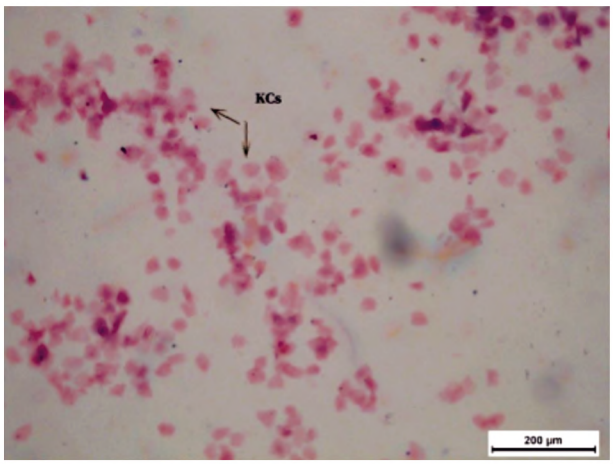
(B)



(C)



(D)



(E)

Fig. (9): A photomicrograph of vaginal smear from control group showing estrous cycle. (A) Proestrus phase of estrous cycle showing round nucleated epithelial cells (RC) a polygonal anucleated keratinocytes (KC). (B) Estrus phase of estrous cycle showing polygonal anucleated keratinocytes (KC). (C) Metestrus phase of estrous cycle showing leukocytes (LC) with the anucleated keratinocytes (KC). (D) Diestrus phase of estrous cycle showing predominance of leukocytes (LC) with some keratinocytes (KC). (E) A photomicrograph of vaginal smear from PCOS diseased group shows persistent estrus phase of estrous cycle. It shows anucleated keratinocytes (KC). (Geimsa x100).

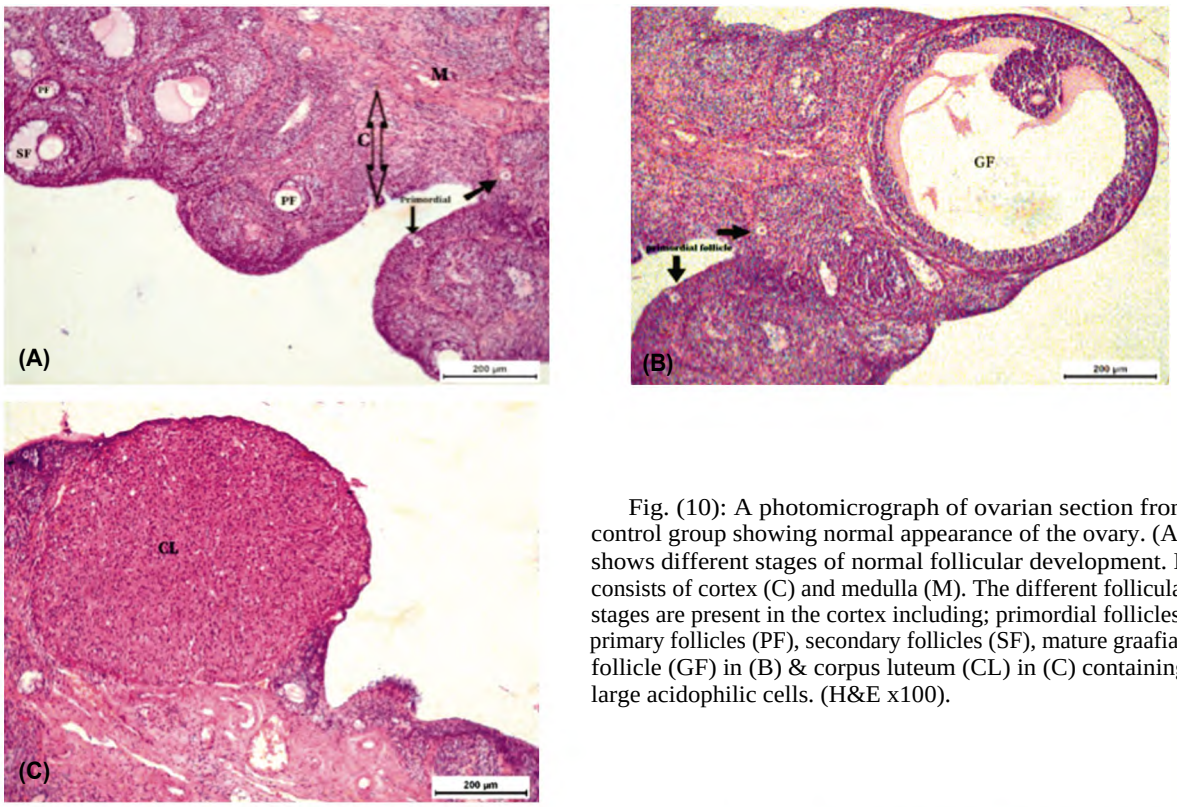


Fig. (10): A photomicrograph of ovarian section from control group showing normal appearance of the ovary. (A) shows different stages of normal follicular development. It consists of cortex (C) and medulla (M). The different follicular stages are present in the cortex including; primordial follicles, primary follicles (PF), secondary follicles (SF), mature graafian follicle (GF) in (B) & corpus luteum (CL) in (C) containing large acidophilic cells. (H&E x100).

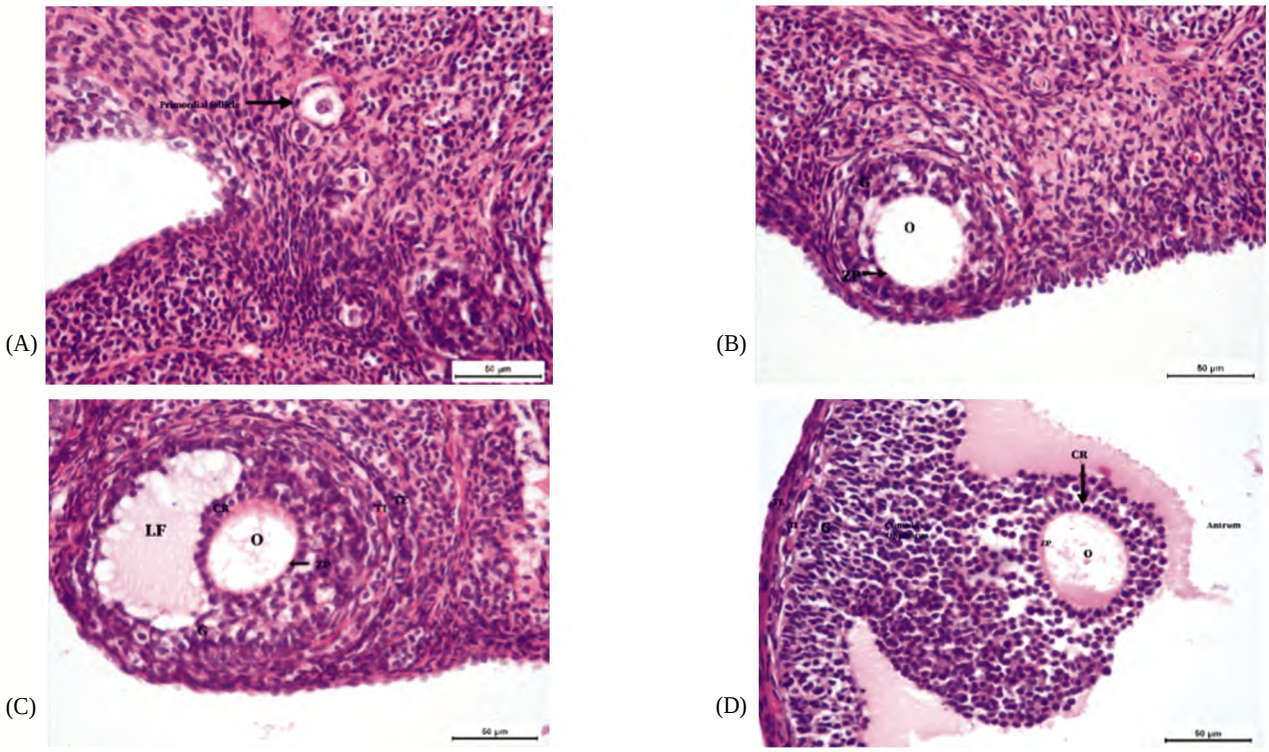


Fig. (11): A high power magnification photomicrograph of ovarian section from control group; (A) Primordial follicle containing small rounded oocyte surrounded by flat stromal cells. (B) Primary follicle containing bigger oocyte (O) surrounded by zona pellucida (ZP) and few layers of granulosa cells (G) and outer flat stromal cells. (C) Secondary follicle (SF) consisting of big oocyte (O) surrounded by zona pellucida (ZP), layer of corona radiata (CR), multiple layers of granulosa cells (G) and theca layer theca interna (TI) and theca externa (TE) and containing fluid cavity liquor folliculi (LF). (D) Part of the wall of mature graafian follicle (GF) consisting of big oocyte (O) surrounded by zona pellucida (ZP), a layer of cells corona radiata (CR) cumulous oophorus connecting it to the outer wall of the follicle which consists of multiple layers of granulosa cells (G), theca interna (TI) and theca externa (TE) surrounding large fluid cavity named antrum (A). (H&E x400).

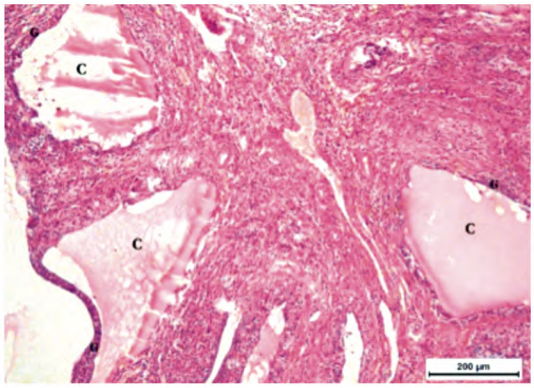
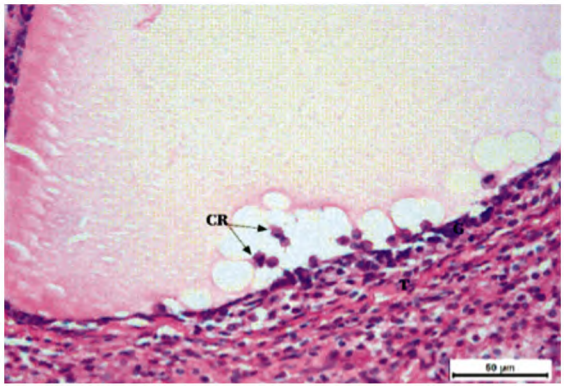


Fig. (12): A photomicrograph of ovarian section from PCOS diseased group. It shows multiple cysts (C) with no oocytes inside them and surrounded by thin granulosa cell layer (G). Absence of different follicular stages is noticed. (H&E x100)

(A)



(B)

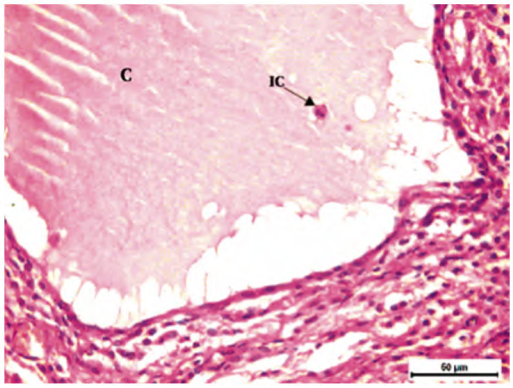


Fig. (13): A high power magnification photomicrograph of ovarian section from PCOS diseased group. (A) Part of wall of a big cyst containing cell remnants (CR) and surrounded by thin granulosa layer (G) and thick theca layer (T). (B) The wall of a big cyst (C) containing multinuclear polymorphs inflammatory cells (IC). (H&E x400).

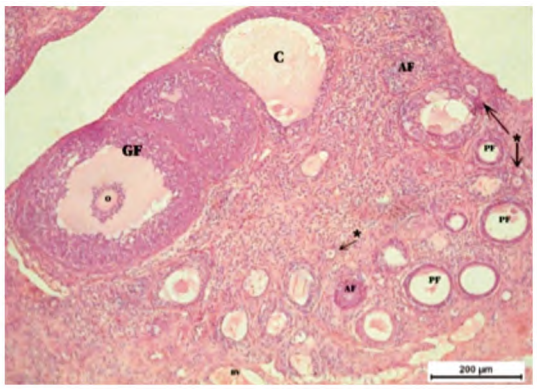
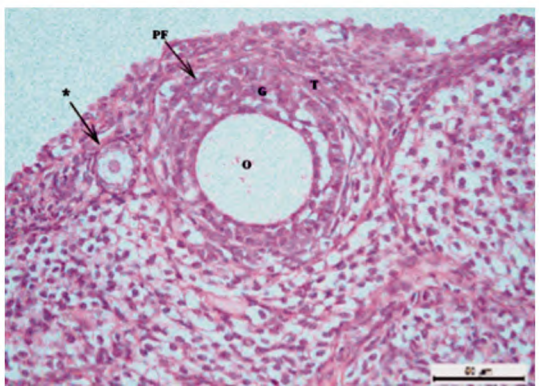


Fig. (14): A photomicrograph of ovarian section from low dose quercetin group. It shows restoration of ovarian structure containing primordial follicles (*), primary follicles (PF), mature graafian follicle but with the presence cysts (C), atretic follicles (AF) and congested blood vessel (BV). (H&E x100).

(A)



(B)

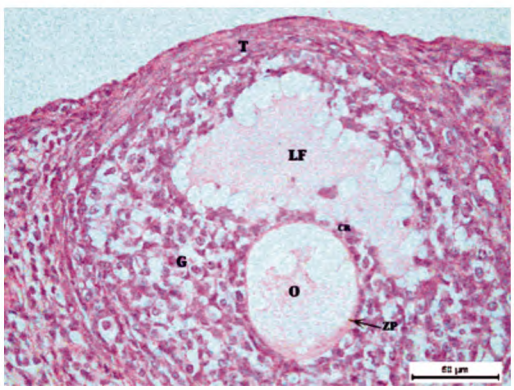
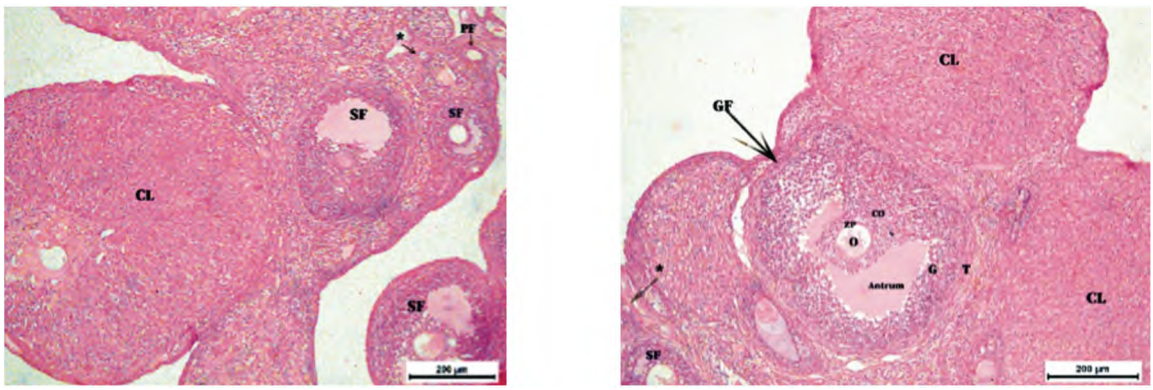
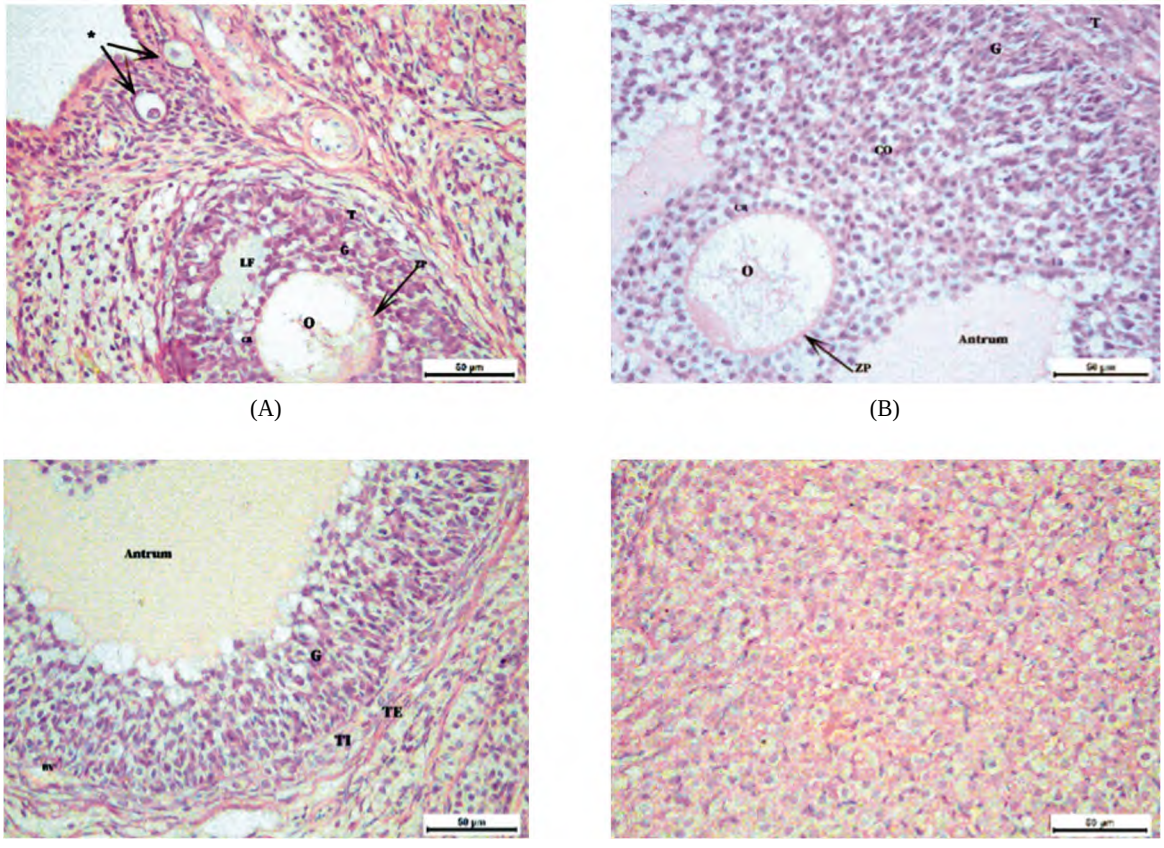


Fig. (15): A high power magnification photomicrograph of ovarian section from low dose quercetin treated group. (A) Primordial follicle (*) containing small rounded oocyte surrounded by flat stromal cells. Primary follicle containing bigger oocyte (O) surrounded by zona pellucida (ZP) and one or two layers of granulosa cells (G) and outer flat stromal cells. (B) Secondary follicle (SF) consisting of big oocyte (O) surrounded by zona pellucida (ZP), layer of corona radiata (CR), multiple layers of granulosa cells (G) and theca layer theca interna (TI) and theca externa (TE) and containing fluid cavity liquor folliculi (LF). (H&E x400).



(A) (B)

Fig. (16): A photomicrograph of ovarian section from high dose quercetin group ovary. It shows restoration of different stages of normal follicular development including (A) primordial follicles (*), primary follicles (PF), secondary follicles (SF), mature graafian follicle (GF) & corpus luteum (CL). (B) Mature graafian follicle (GF) consisting of big oocyte (O), a layer of cells corona radiata (CR) cumulous oophorus (CO) connecting it to the outer wall of the follicle which consists of multiple layers of granulosa cells (G) & theca cells (T) surrounding large fluid cavity named antrum. There are also primordial follicles (*), secondary follicles (SF) and corpus luteum (CL). (H&E x400)



(A) (B)

(C) (D)

Fig. (17): A high power magnification photomicrograph of ovarian section from high dose quercetin group. (A) Primordial follicles (*) containing small rounded oocyte surrounded by flat stromal cells. Secondary follicle consisting of oocyte (O) surrounded by zona pellucida (ZP) and layer of corona radiata (CR), granulosa cell layer (G) & theca cell layer (T). Collection of fluid begins to appear forming liquor folliculi (LF). (B) Part of wall of mature graafian follicle consisting of big oocyte (O) surrounded by zona pellucida (ZP), a layer of cells corona radiata (CR) cumulous oophorus connecting it to the outer wall of the follicle which consists of multiple layers of granulosa cells (G), theca cells (T) surrounding large fluid cavity named antrum. (C) Part of wall of mature graafian follicle consisting of multiple layers of granulosa cells (G), theca interna (TI) and theca externa (TE) surrounding large fluid cavity named antrum (A). (D) Structure of corpus luteum showing normal large acidophilic cells with normal distribution.

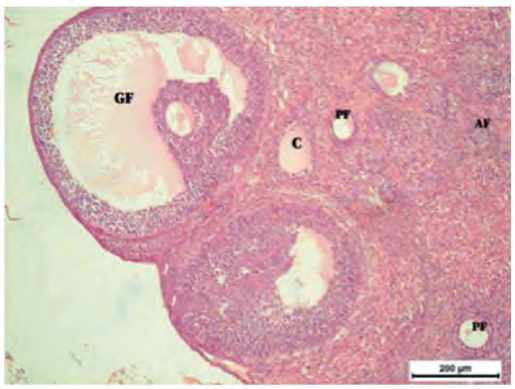
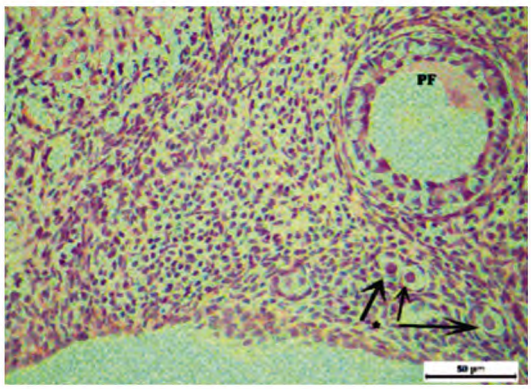
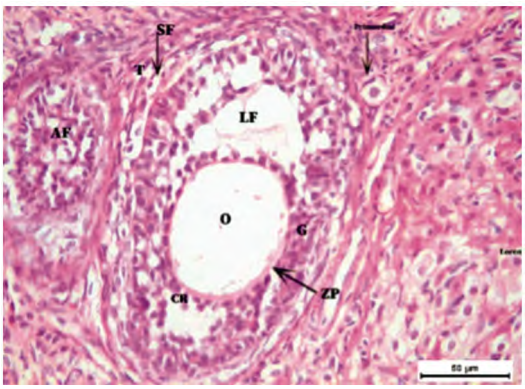


Fig. (18): A photomicrograph of ovarian section from metformin treated group. It shows some follicular stages as primary follicles (PF) and mature graafian follicles with reduced number of primordial follicles, secondary follicles & graafian follicles (GF) and persistence of cysts (C) and atretic follicles (AF). (H&E x100)

(A)



(B)



(C)

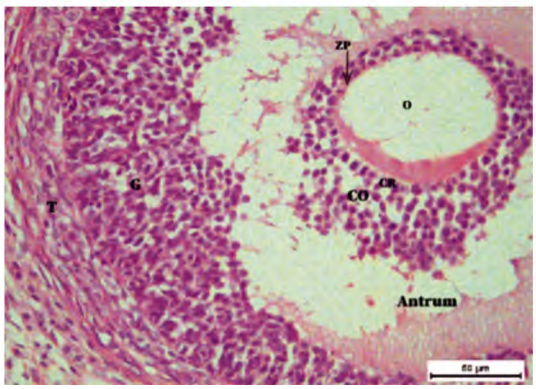


Fig. (19): A high power magnification photomicrograph of ovarian section from metformin treated group. (A) Primordial follicles (*) containing small rounded oocyte surrounded by flat stromal cells. Primary follicle containing bigger oocyte (O) surrounded by zona pellucida (ZP) and multiple layers of granulosa cells (G) and outer flat stromal cells. (B) Secondary follicle (SF) consisting of big oocyte (O) surrounded by zona pellucida (ZP), layer of corona radiata (CR), multiple layers of granulosa cells (G) and theca cell layer (T) and containing fluid cavity liquor folliculi (LF). There is also atretic follicles (AF). (C) part of wall of Mature graafian follicle (GF) consisting of big oocyte (O) surrounded by zona pellucida (ZP), a layer of cells corona radiata (CR) cumulous oophorus connecting it to the outer wall of the follicle which consists of multiple layers of granulosa cells (G), theca cell layer (T) surrounding large fluid cavity named antrum. (H&E x400)

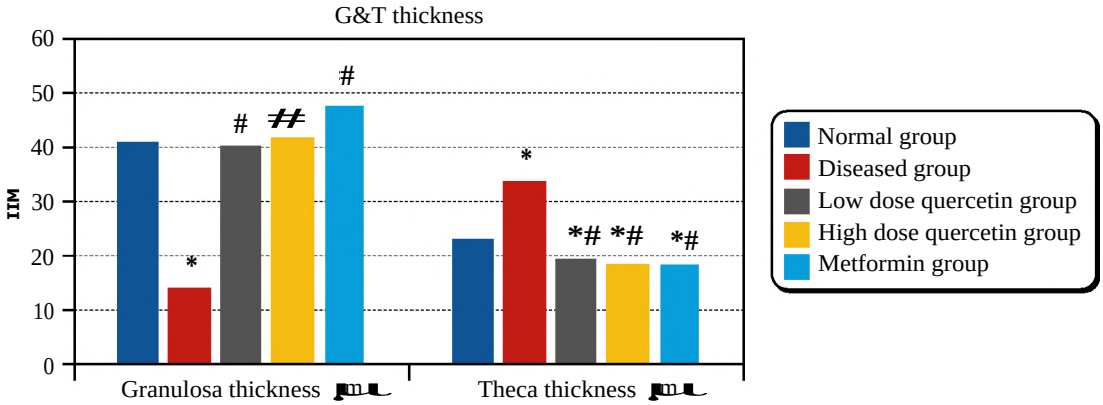


Fig. (20): Morphometric measurement of granulosa & theca cell layers. Data are expressed as mean ± SD using ANOVA test followed by tukey's post-hoc test for pair-wise comparisons (n = 8). (*) Significant p-value ($p \leq 0.05$) in comparison to normal control group. (#) Significant p-value ($p \leq 0.05$) in comparison to diseased group. ($) Significant p-value (p-value <0.05) in comparison to low dose quercetin group. (@) Significant p-value (p-value <0.05) in comparison to high dose quercetin treated group.

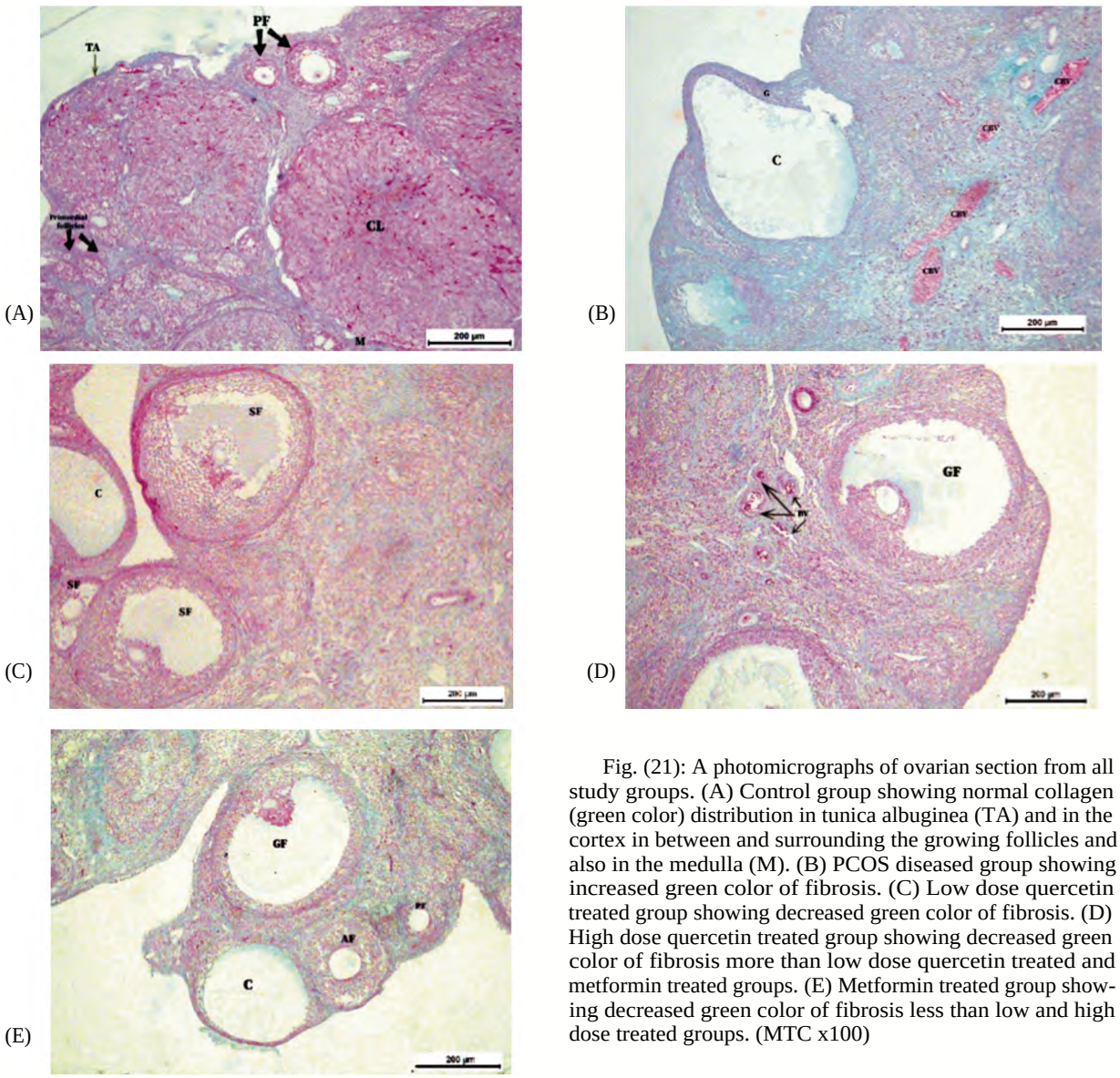


Fig. (21): A photomicrographs of ovarian section from all study groups. (A) Control group showing normal collagen (green color) distribution in tunica albuginea (TA) and in the cortex in between and surrounding the growing follicles and also in the medulla (M). (B) PCOS diseased group showing increased green color of fibrosis. (C) Low dose quercetin treated group showing decreased green color of fibrosis. (D) High dose quercetin treated group showing decreased green color of fibrosis more than low dose quercetin treated and metformin treated groups. (E) Metformin treated group showing decreased green color of fibrosis less than low and high dose treated groups. (MTC x100)

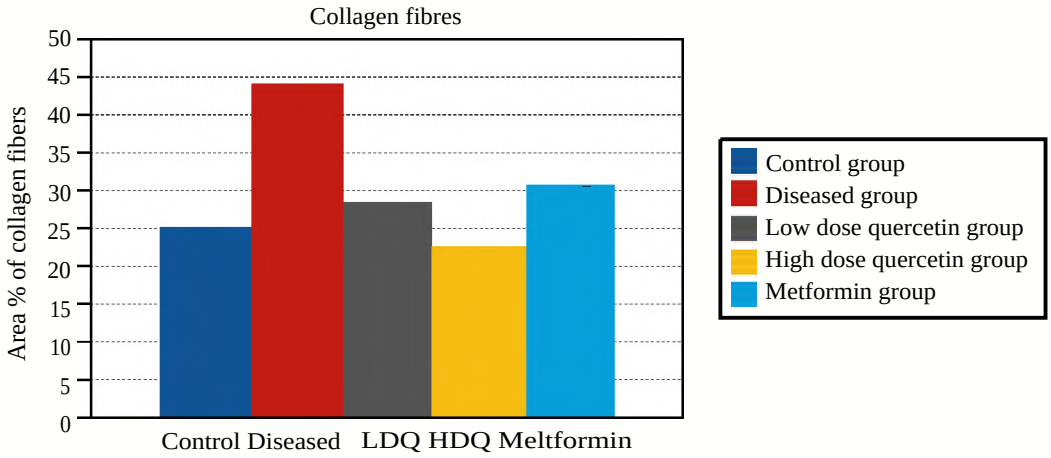
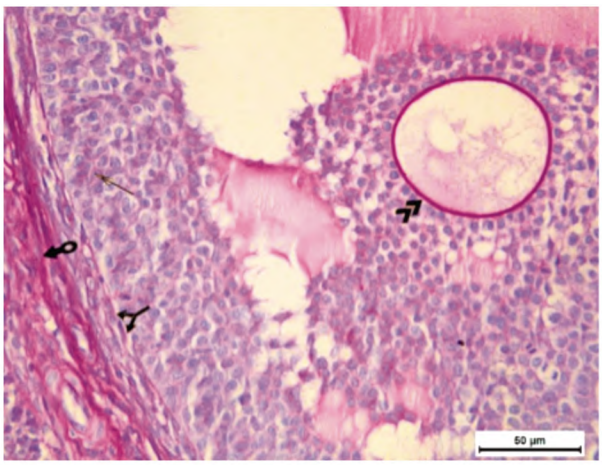
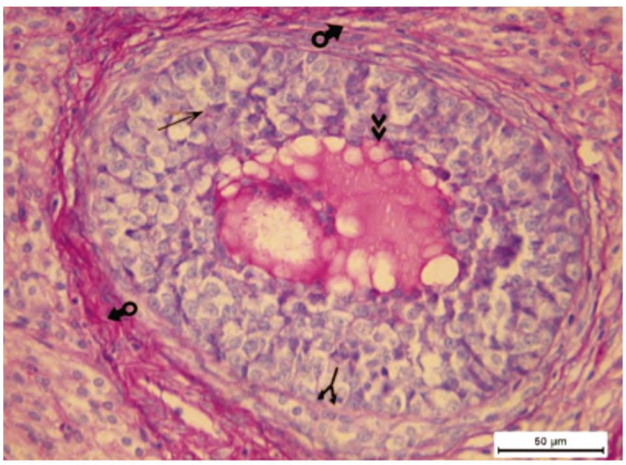


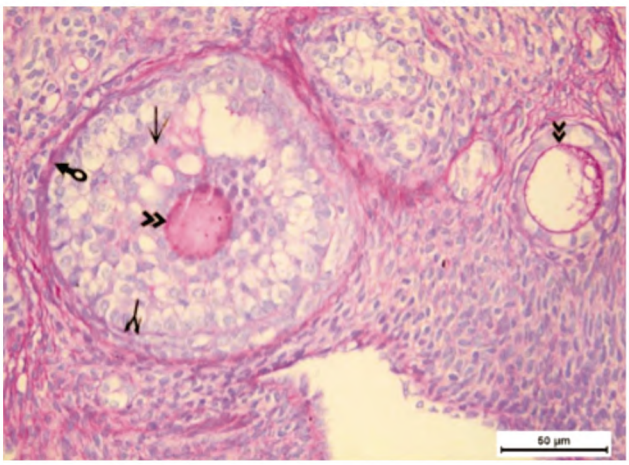
Fig. (22): Morphometric evaluation of the area percent of the greenish colored collagen fibers. Data are expressed as mean ± SD (n = 8). Using one way ANOVA post hoc Tukey test. (*, #,$,@) Significant p-value ($p \leq 0.05$) in comparison to control, disease, low, high dose quercetin groups respectively.



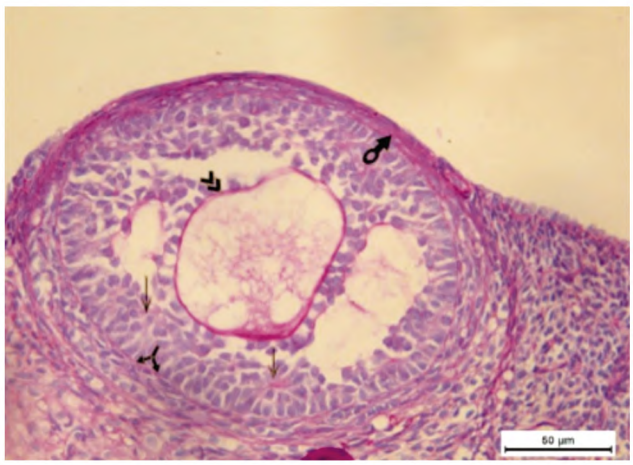
(A)



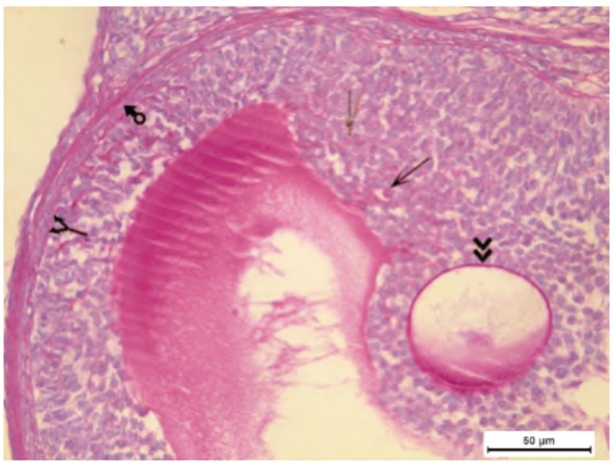
(B)



(C)

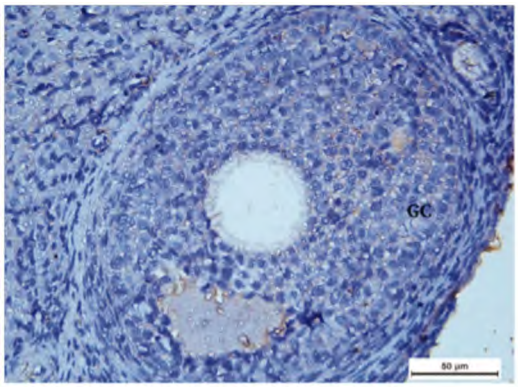


(D)

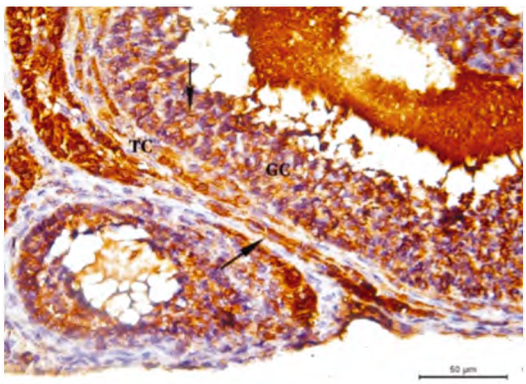


(E)

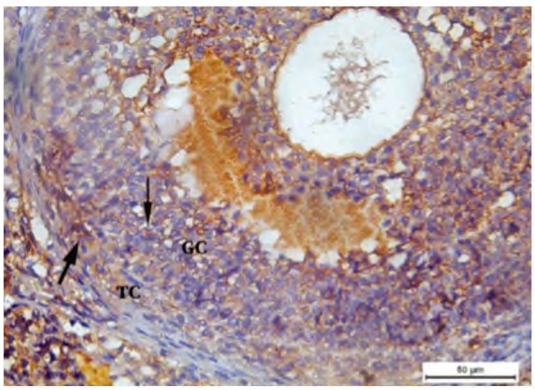
Fig. (23): A high power magnification photomicrograph of ovarian section from all study groups showing positive PAS reaction in the intercellular spaces between granulosa cells (arrow), within theca cells (round tailed arrow), in the basement membrane (bifurcated arrow) & in zona pellucida (double arrow heads) . (A) Control group shows strong positive PAS reaction and average theca thickness (round tailed arrow). (B) PCOS group shows low positive PAS reaction and increased theca thickness (round tailed arrow). (C) Low dose quercetin treated group shows moderate positive PAS reaction and moderate increased theca thickness (round tailed arrow). (D) High dose quercetin treated group shows strong positive PAS reaction and average theca thickness (round tailed arrow). (D) Metformin treated group shows strong positive PAS reaction and average theca thickness (round tailed arrow). (PAS x400)



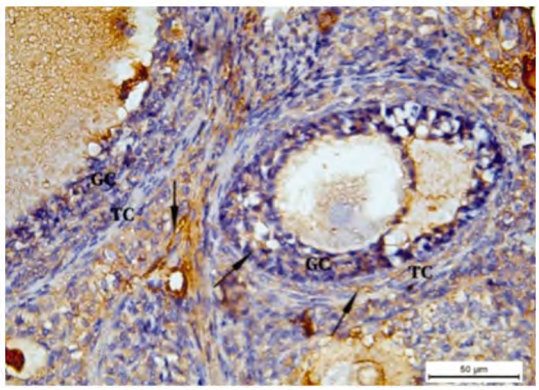
(A)



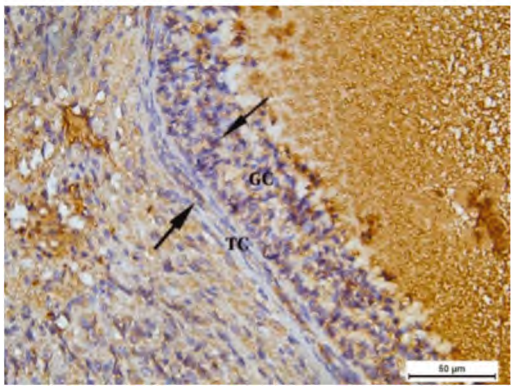
(B)



(C)



(D)



(E)

Fig. (24): A high power magnification photomicrograph of ovarian sections from all study groups; (A) Control group showing negative TGF-β expression with few positive granulosa cells (GC). (B) PCOS diseased group showing marked diffuse positive TGF-β expression as brown cytoplasmic color (arrows) in granulosa cells (GC) and theca cells (T). (C) Low dose quercetin treated group showing moderate positive TGF-β expression as brown cytoplasmic color (arrows) in granulosa cells (GC) and Theca (TC) layer. (D) High dose quercetin treated group showing minimal positive TGF-β expression as brown cytoplasmic color (arrows) in granulosa cells (GC) and Theca cell (TC) layer. (E) Metformin treated group showing moderate TGF-β expression as brown cytoplasmic discoloration (arrows) in granulosa cells (GC) and Theca cell (TC) layer. (Anti TGF-β immunohistochemical stain x400)

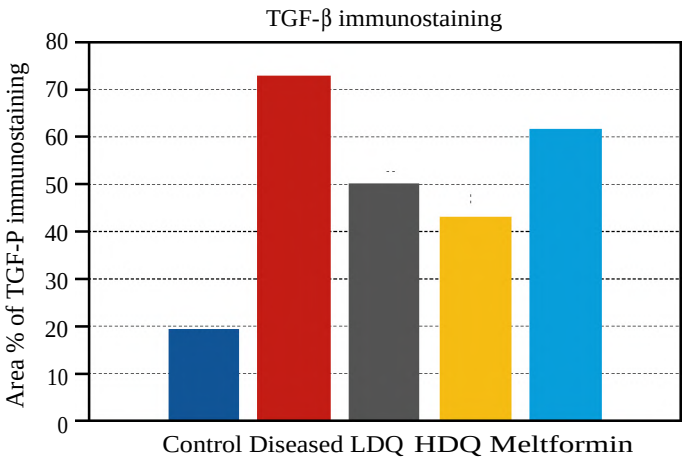


Fig. (25): Morphometric evaluation of the area percent of the TGF-β immunoreaction. Data are expressed as mean ± SD using ANOVA test followed by tukey's post-hoc test for pair-wise comparisons (n = 8). (*, #, $, @) Significant *p*- value ($p \leq 0.05$) in comparison to normal control group, diseased group, low dose quercetin treated group& high dose quercetin treated group respectively.

Discussion

The present study has proven the effectiveness of letrozole induced polycystic ovarian syndrome in blocking aromatase enzyme functions so, ovarian cells can't produce estrogen, and a polycystic ovary with an abnormal follicular cycle develops due to an increased circulating androgen level [5]. 21 days of letrozole treatment in adult female rats led to the development of traits that are strikingly comparable to women with PCOS. The vaginal smear histology was the indicator for ovarian physiology. Normal rats showed cyclic phases of estrous cycle. After induction, we noticed that there is persistent estrus phase in all rats, and this was a positive sign of successful induction. This also was proved by [20] who induced PCOS in rats using 60 days of continuous illumination. In this study after 28 days of quercetin treatment with doses of 50mg/kg and 100mg/kg, it was obvious that estrous cycle returned normal in quercetin treated rats. These results were in line with [21].

According to our study, there was weight gain in PCOS rats explained by androgen excess boosted food intake in rats and promoted obesity by reducing insulin and leptin signaling in the brain mostly by decreasing leptin levels in the CSF [22]. The main features of PCOS patients are unbalanced reproductive hormones and insulin resistance, which caused an imbalance in energy homeostasis, the development of adiposity, and weight gain [23]. After treatment with quercetin there was decrease in body weight as supported by [13,15]. Weight loss was more obvious in high dose quercetin and metformin treated groups than low dose quercetin treated group.

The alterations in body temperature in PCOS rats could be related to the metabolic abnormalities observed in PCOS induced animals [24].

The model showed increase in testosterone, unchanged LH/FSH ratio and decrease in FSH [24], estrogen and progesterone level [25]. Our study showed that there is decreased level of FSH & LH in PCOS diseased group this may be due to negative feedback of increased testosterone level [26] so, quercetin treatment increased their level due to improved testosterone level. Aromatase activity suppression causes a rise in ovarian androgens, which in turn causes hyperandrogenism, a defining feature of PCOS [27]. Estradiol is created in the ovaries by the granulosa cell-made aromatase, which converts C 19 androgens. Letrozole may be anticipated to reduce the activity of this enzyme, which could lead to an increase in ovarian androgen, a reduction in estrogen, and the development of PCOS in animal models.Anovulation and a decrease in the number of corpora lutea cause the blood levels of progesterone to drop [28]. After quercetin treatment, decrease testosterone and increase in FSH, LH, estrogen and progesterone. That also was concluded by [21]. Quercetin showed anti-androgenic potentials by totally blocking the phosphatidylinositol-3-kinase (PI3K) pathway and downregulating the CYP17A1 gene [29]. Metformin proved a crucial role in normalization of disturbed hormones in PCOS [17].

PCOS positive rats showed decreased levels of catalase. Similarly [30]. This finding might be explained by the formation of free radicals, which can disrupt the levels of antioxidants. Free radical excess might result from an immune system affected by an out-of-whack antioxidant level [30]. It was increased in quercetin treated rats as compared to PCOS rats as in [12]. The cause may be explained by that quercetin administration has counterbalanced the reactive oxygen species (ROS) levels and improved the antioxidant activities by inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [12]. Both doses of quercetin in this current study were better than metformin in that.

PCOS rats revealed increased levels of fasting glucose, fasting insulin & HOMA-IR [31] reported the same results. The HOMA-IR was increased but it didn't reach the level of insulin resistance. This also was reported by [32] who concluded that high fat diet + letrozole induced insulin resistance in PCOS rats. This may be due to elevated androgen concentrations. Letrozole has no impact on the routes used by insulin and has no ability to increase insulin resistance or decrease insulin sensitivity [33].

There was improvement in fasting glucose levels, fasting insulin & HOMA-IR after quercetin and metformin treatment as compared to PCOS rats as in [34] using different doses of quercetin [35] declared that quercetin enhances glucose absorption by activating adenosine monophosphate activated protein kinase (AMPK)-dependent and insulin-independent pathways to raise glucose transporter 4 (GLUT-4) levels. Additionally, it inhibits the crucial gluconeogenesis enzymes while safeguarding the islet -cell activity [34].

In this study, PCOS positive rats revealed increased levels of TNF-a as shown in [36]. PCOS is intimately linked to chronic low-grade inflammation, the main cause of which is macrophage

infiltration. At various locations in the ovary, ovarian macrophages emit anti-inflammatory and pro-inflammatory cytokines that are crucial for the control of tissue remodeling and apoptosis related to luteinization, follicular development, and ovulation. Ovarian dysfunction is brought on by the production and release of proinflammatory cytokines, such as TNF-α, which are essential for host defense and are generated and released by activated M1 macrophages [36]. It was observed that TNF-α was decreased after high dose quercetin than low dose quercetin and metformin treatment. This may be mediated by inhibiting Toll-like receptor 4 [13]. When compared to the PCOS group, metformin lowered TNF-α levels in rats treated with it, and additional evidence shows that this impact may have been mediated by activation of AMPK [17].

We observed the occurrence of hirsutism in our PCOS model and it was subsided by high dose quercetin & metformin more than low dose quercetin treatment. Hirsutism, a disorder in which excessive androgens cause uncontrollable hair growth in PCOS [13,29].

In the follicular structures of androgenized rats, several investigations have demonstrated that metformin enhances glucose metabolism, lowers proliferation, and decreases Cytochrome P450-17 (CYP-17) expression [37]. Treatment with metformin decreased the area occupied by degenerating ovarian follicles [38].

H&E stained ovarian sections from the PCOS positive rats showed hyperplasia of theca cell layer; that is the cause of increased testosterone level we measured before, decreased number of growing follicles & corpora lutea together with significant increase in the number of subcapsular cysts bordered with a thin layer of granulosa cells [15]. There was significant increase in atretic follicles. The main cause of these results is disturbance in folliculogenesis & oogenesis. These disturbances were attributed to the absence of the interaction between granulosa and theca cells, which would normally result in ovulation [9].

PCOS diseased group showed significant increase in the number of ovarian cysts and atretic follicles [39] who returned that to the intraovarian hyperandrogenism to the follicular arrest [39]. Both quercetin and metformin produced a remarkable improvement of the ovarian histological structure in PCOS and was confirmed by the significant increase in the mean number of corpora lutea indicating that the estrous cycle had been restored to normal operation [39]. There were also significant decreased cystic follicles. The improved ovarian histoarchitecture could be related to the anti-androgenic property of the treatment options [40].

The follicular structure in the control group was normal as demonstrated by H&E stain and confirmed by PAS stain. PCOS induction caused degenerated follicles as was also confirmed by [18]. Low & high doses of quercetin and metformin led to restoration of normal structure of follicles. Control group showed strong positive PAS reaction due to increased polysaccharides and this was also proved by [41] who concluded that the strong PAS reaction seen in the theca layer, could be attributed to the increased amount of carbohydrates and GAGs associated with the increased amount of collagen present. There is an important role of carbohydrates and GAGs secreted by granulosa cells in the differentiation and development of ovarian follicles. This might explain the significant decrease of carbohydrates and GAGs found in the granulosa of cystic ovaries in comparison to the control and to the treated subgroups.

In the PCOS group, Masson's staining revealed a considerable increase in the area % of collagen fibers, indicating a greater deposition of fibrous tissue. Theca-interstitial cells, which are the major sources of androgen in a normal ovary, are stimulated to proliferate as a result of the abnormally high TGF-β expression in the same environment, leading to an excess of androgen [42]. These Masson's results were confirmed by the TGF-β immune-expression was significantly higher in the PCOS animal group. It was supported by a rise in the mean area percentage of TGF-β immunoreactivity. TGF-β has been linked to a variety of biological processes, including tissue fibrosis which was consistent with our findings [43].

The histomorphometric results showed an increase in the mean theca cell thickness, supporting [42] earlier hypothesis. There was a significant reduction in the granulosa cell layer thickness which might be hormonal dependent. This notion was supported by [44] who demonstrated that the survival of granulosa cells depends on estrogen, so letrozole-induced inhibition of aromatase leads to granulosa cells degeneration. According to [45], interstitial fibrosis may develop from an imbalance of fibrotic factors such as matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) on the extracellular matrix (ECM) caused by an excess of testosterone and excessive TGF-β expression. So, the TGF-superfamily members, which are known to control collagen synthesis and

have been linked to fibrosis, are responsible for the thickening of the ovarian capsule and stroma in the PCOS ovary as a result of increased collagen deposition and fibrous tissue **[46]**.

When compared to the PCOS group, low dose and high dose quercetin treatments significantly reduced the mean area percent of fibrous tissue deposition and TGF-0 immunoreactivity. So, our study revealed that quercetin reduced TGF- 0 . The mechanism may involve inhibition of the TGF-0 signaling pathway as was demonstrated by **[47]** who concluded that quercetin protects renal function and alleviates the progression of glomerulosclerosis in rats. However, there was no significant difference between these treatments and metformin treatment for the amount of Masson-stained collagen fibers and TGF-0 immune-expression. Literature has suggested that metformin has an antifibrotic impact in a number of organs, including the ovarian fibrosis caused by dehydroepiandrosterone **[40]**.

Conclusion:

Our study showed that quercetin is a powerful flavonoid that showed a good ability to normalize metabolic and ovarian histological structure in polycystic ovary syndrome. Quercetin has a good effect on hormonal profile, serum glucose level, insulin & HOMA-IR it also showed that it has a powerful anti-inflammatory & antioxidant effect. It was obvious also that different doses have different effects, and that high dose of quercetin is better than low dose but with the fact that the low dose has a good effect that is comparable to metformin. So, we can conclude that quercetin may will be a promising medication for PCOS.

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التأثير العلاجى للكيرسيتين فى متلازمة المبيض المتعدد الكيسات المستحثة بالليتروزول فى نموذج الجرذان

تعتبر متلازمة المبيض المتعدد الكيسات هى واحدة من اضطرابات الغدد الصماء الأ كثر انتشاراً التى تؤثر على النساء فى فترة الإنجاب. إنها مشكلة صحية رئيسية تؤدى إلى ضعف الخصوبة، واضطراب التمثيل الغذائى، وضعف امتصاص الخلايا للجلو كوز، والمظاهر النفسية مثل الاكتئاب والقلق، والمضاعفات القلبية الوعائية والدماغية الوعائية. تؤثر متلازمة المبيض المتعدد الكيسات (PCOS) على ٢.٢–٢٦٪ من النساء فى سن الإنجاب فى جميع أنحاء العالم.

خيارات علاج متلازمة المبيض المتعدد الكيسات بما فى ذلك، إنقاص الوزن عن طريق اتباع نظام غذائى أو ممارسة الرياضة أو جراحة لعلاج البدانة. الخيارات الأخرى هى تنشيط الإباضة بواسطة سترات كلوميفين ، مثبطات الأروماتاز، تقنيات الإخصاب فى المختبر. كيرسيتين هو فلافونويد موجود فى العديد من الخضروات والفواكة له تأثير مضاد للالتهابات ودور مثبط للمناعة. يعمل الكيرستين كمضاد للأ كسدة من خلال الحفاظ على التوازن التأكسدى.

كان الهدف من هذا العمل هو دراسة تأثير جرعتين مختلفين من كيرسيتين على علاج متلازمة المبيض المتعدد الكيسات من وجهه النظر الفسيو لوجية وتوفير علاج طبيعى و آمن وفعال لمتلازمة المبيض المتعدد الكيسات. أجريت الدراسة فى مختبر قسم الفسيولوجيا وبيت الحيوان بكلية الطب جامعة قناة السويس. استخدمنا ٤٠ أنثى من الجرذان الويستار البالغة فى هذه الدراسة تزن من ١٦٠–١٨٠ جم، ٣٢ جرذا مستحثة بمتلازمة المبيض المتعدد الكيسات باستخدام ١ ملجم/كجم من الليتروزول لمدة ٢١ يوماً. تم تقسيم الفئران المستحثة بمتلازمة المبيض المتعدد الكيسات إلى ٣ مجموعات، مجموعة عولجت ب ٥٠ ملجم/كجم من كيرسيتين ومجموعة عولجت ب ١٠٠ ملجم/كجم من الكيرسيتين، ومجموعة عولجت ب ١٠٠ ملغم/كغم من الميتفورمين. تم تقسيم جميع الجرعات إلى ٤ مرات فى اليوم عن طريق الفم لمدة ٢٨ يوماً.

تم إجراء مسحات مهبلية للتأكد من أن الجرذان لديها دورة استروس طبيعية و كذلك بعد الحث للتأ كد من أنها تم تحريضها على متلازمة المبيض المتعدد الكيسات، تم قياس وزن الجرذان كل ٥ أيام خلال فترة الحث والعلاج، وتم قياس درجة الحرارة عن طريق المستقيم بعد الحث وبعد العلاج، بعد التضحية، تم جمع الدم وطرده مركزياً لقياس FSH و LH والإستروجين والبروجسترون والتستوستيرون ومستوى الجلوكوز فى الدم والأنسولين و TNF-α والكاتالاز. تم استئصال المبيضين للتقييم النسيجى.

أظهرت النتائج أن الجرذان المستحثة بتكيس لديها دورة استروس غير منتظمة، وانخفاض فى LH، FSH وكذلك هرمون الاستروجين والبروجسترون والكاتلاز، وزيادة فى هرمون التستوستيرون، TNF-α، الجلوكوز فى الدم والأنسولين. العلاج مع كيرسيتين والميتفورمين حسن هذه القيم وكانت جرعة عالية من الكيرسيتين أفضل من جرعة منخفضة من الكيرسيتين وكلاهما كان أفضل من الميتفورمين. وقد ثبت أيضاً أن نموذج متلازمة المبيض المتعدد الكيسات الناجم عن اليتروزول أظهر زيادة فى توزيع شعر الجسم عن طريق الملاحظة. نجحت الجرعة المنخفضة من الكيرسيتين، الجرعة العالية من الكيرسيتين وعلاج الميتفورمين فى رجوع توزيع شعر الجسم للوضع الطبيعى.

أظهر التقييم النسيجى زيادة عدد الكيسات والبصيلات الأنثرية فى الشرائح التى صبغت بالهيماتو كسيلين والإيوسين. أظهرت الشرائح التى صبغت بالماسون ثلاثى الكروم زيادة فى نسبة المساحة الخضراء مما يشير إلى زيادة ألياف الكولاجين والتليف، وقد ثبت ذلك أيضاً من خلال الصبغة المناعية المضادة لـ TGF-β. أظهرت صبغة شيف الحمضى الدورى بصيلات حويصلات متحطمة. وكان فى ذلك الجرة المنخفضة من كيرسيتين، الجرعة العالية من كيرسيتين والميتفور مين جعل النتائج قريبة من الطبيعى. الجرعة العالية من الكيرسيتين كان أفضل من الجرعة المنخفضة من الكيرسيتين وكلاهما كان أفضل من الميتفورمين فى ذلك.

لذلك أثبتت الدراسة كفاءة جرعة ١٠٠ مجم من الكيرسيتين فى علاج متلازم المبيض المتعدد الكيسات أفضل من جرعة ٥٠ مجم من الكيرسيتين وكلاهما كان أفضل من جرعة ١٠٠ مجم من الميتفورمين لذلك الكيرسيتين ربما يكون له دور مهم فى علاج متلازمة المبيض المتعدد الكيسات.