# Therapeutic Effects of Milk thistle Seeds (Silybum marianum) and Red Ginseng roots on Polycystic Ovary Syndrome Induced by Letrozole in Female Rats

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# Therapeutic Effects of Milk thistle Seeds (Silybum marianum) and Red Ginseng roots on Polycystic Ovary Syndrome Induced by Letrozole in Female Rats

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***Abstract:***

The purpose of this study was to assess the effect of milk thistle seeds (MTS), red ginseng roots (RGR) and their mixture (Mix) on the biochemical and histopathologic indicators of polycystic ovarian syndrome (PCOS). 42 adult albino female rats Sprague-Dawley strain weighting (160±10g) were classified randomly into two groups: Group (I) 12 rats received a baseline diet served as negative control group; Group (II) 30 rats were given LTZ (1 mg/Kg b.wt) dissolved in saline by the gavage tube once daily for 21 days to induce PCOS. 6 rats from each two groups were slaughtered to confirm the occurrence of polycystic ovaries. Then, rats reclassified into 4 equal groups (6 rats each) as following: control positive group and 3 treated rat groups which administered MTS at (5%), RGR at (5%) and at (10%) of their Mix at (1:1), respectively with basal diet. The treatment period was designed for 42 days. The results indicated that basal diet supplementation with MTS or RGR and Mix reduced body weight and induced significant decrease in glycemic, glucose tolerance, insulin resistance, lipid profile, antioxidant enzymes and inflammatory indices. Histopathological examination also confirmed the results of the biochemical analyzes in restoring estrous regularities, hormone regulation and alleviation abnormalities in ovarian tissue, due to this herbs anti-androgenic, anti-inflammatory qualities. So, it is recommended to use MTS or RGR and their mix as a dietary supplement to avoid PCOS incidence and its complications.

**Keywords:** *Silybum marianum*, *Panax ginseng Meyer*, Polycystic ovary syndrome, Letrozole, Insulin resistance, Female rats.

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## Introduction

About 15% of women in reproductive age have polycystic ovary syndrome (PCOS), the most prevalent endocrine disorder and the main cause of infertility **(Heshmati *et al.*, 2021)**. Menstrual irregularities, acne, hirsutism, frequent anovulation, and recurrent miscarriages are some of the disease's clinical symptoms **(Maliqueo *et al.*, 2014)**. Increased amounts of androgens, luteinizing hormone (LH), and lower levels of progesterone (P4) are among the endocrine disorders **(Hachey *et al.*, 2020)**. Additionally, insulin resistance (IR), obesity, and type 2 diabetes mellitus are metabolic abnormalities associated with PCOS **(Rababa'h *et al.*, 2020)**.

A non-steroidal aromatase inhibitor called letrozole (LTZ) was recently utilised to induce PCOS in rats **(Ghafurniyan *et al.*, 2015)**. The mechanism of LTZ-induced PCOS is explained by blocking the conversion of testosterone (TS) and androstenedione to estradiol and estrone, respectively. With accumulation of androgen, hormonal changes, and the production of intraovarian androgen, which results in the development of PCOS and metabolic disturbances. The treatment by LTZ in female rat's results in altered estrous cyclicity, increased ovarian weight, ovarian cysts, atretic follicles, absence of corpora lutea in adulthood, increased levels of LH and TS, and decreased levels of P4, and metabolic disturbances **(Rababa'h *et al.*, 2020)**.

Herbs, phytochemicals, and nutritional supplements can lessen the negative effects of PCOS-related **(Alqahtani *et al.*, 2022)**. The milk thistle (*Silybum marianum* L.). It contains a combination of flavonolignans known as silymarin, with silybin (also known as silibinin) serving as the main ingredient and being directly extracted from dried seeds of *S. marianum* **(Marmouzi *et al.*, 2021)**.The silymarin has medicinal properties that include decreasing blood cholesterol levels, hepatoprotective ,antidiabetic, acting as an anti-inflammatory, antioxidant and anti-fibrotic **(Wang *et al.*, 2020a)**.

The ginseng is used in traditional medicine as Korean ginseng or the "Queen of Herbs" and is believed to have healthful effects on oxidative

damage reduction, anti-inflammatory, and glucose-lowering and improved insulin sensitivity because have the bioactive chemicals such as Polysaccharides, polyacetylenes, phenols, alkaloids and ginsenosides that can be isolated from the plant's roots, stems, leaves, flowers, and fruits. These substances show pharmacological properties that can be used to treat many diseases **(Fan *et al*., 2020** and **Kang *et al*., 2021)**. The present study was undertaken to evaluate the utilization of MTS, RGR and their Mix as natural antioxidants to prevent the side effect of PCOS in the female's rats.

## Materials and methods

### Materials

- **Chemicals**: Basal diet, Casein, cellulose, vitamins and minerals were supplied from General Company for Commerce and Chemicals, Cairo, Egypt.
- **Kits**: Letrozole (LTZ), formalin, diethyl either and kits for biochemical analysis of serum were acquired for chemicals from Gama Trade Company, Cairo, Egypt.
- **Herbs**: Milk thistle seeds and Red ginseng roots were obtained from herbs market in Cairo, Egypt.
- **Rats**: Forty two Female Sprague-Dawley rats (weighing 160±10 g) were obtained from animal house of National Research Center, Giza, Egypt.

### Methods

### Preparation of Milk thistle seeds (MTS)

MTS were cleaned with tap water, then distilled water, then dried at 60$^{o}$C in an air drying oven, and grinded into fine powder using a laboratory electronic mill (Broun, Model 2001 DL, Germany) at speed 2 for 3 min. At -20°C, the powder was held in polyethylene bags in the deep freezer for further analysis and study **(Atta and Imaizumi, 2002)**.

### Preparation of Red ginseng roots (RGR)

According to **Lee *et al*., (2020)** RGR were soaked in tap water for 30 minutes and scrubbed with a brush to remove any soil residue. After that,

roots were sliced into small pieces and dried at 40ºC in oven and ground for 2 minutes with 30-s grinding pulses and a 10-s break to make a fine powder, then were packaged in a flexible polyethylene bags until use.

**Experimental design and Induction of PCOS**

The duration of this study was 63 days. LTZ was used to induce PCOS for the first 21 days, and the following 42 days were devoted to the specific interventions. 42 adult female Sprague-Dawley rats weighing 160±10 g were taken. Before the experiment began, water and food were supplied to the animals for one week to allow for adaption.

**Ethical approval:** The study received approval from the research ethics committee of the faculty of nursing at Port Said University, code number: NUR (4-12-2022)(20).

The basal diet was established based to **Reeves *et al*.,(1993)** to meet recommended nutrients levels for rats. Following acclimation, rats were divided randomly into two groups: Group (I) 12 rats received a baseline diet served as negative control group ; Group (II) 30 rats served were given LTZ (1 mg/Kg b.wt) dissolved in saline by the gavage tube once daily for 21 days to induce PCOS **(Ndeingang *et al*., 2019)**.

Vaginal samples were taken from all rats every morning, stained with Toluidine blue dye, and inspected under a microscope to identify the stage of the estrus cycle and confirm the PCOS induction seven days prior to the end of PCOS induction (i.e., from day 15 to day 21). On day 21, which marked the end of the PCOS induction period, 6 rats from each two groups were slaughtered to confirm the occurrence of polycystic ovaries **(Zheng *et al*., 2022)**.

Then, rats that displayed regular estrus cycle in a cyclical pattern every 4–5 days were used. The treatment period was designed for 42 days. The remaining group (I) (n=6) was the health control and received a basal diet (negative control (NC)) while, the remaining PCOS rats groups(II)(n=24) were randomly divided into 4 equal groups as follows: Group (II) was supplied only the basal diet and given LTZ as PCOS rats (positive control (PC),and the three other groups (IV,V,VI) were fed on

basal diet with supplements of dried MTS at (5%), RGR at (5%) and at (5%) of their Mix at (1:1), respectively.

**A- Gross chemical composition**

According to the method described in the **A.O.A.C (2005)**, moisture, protein, fat, fibre, and ash contents of MTS and RGR were determined. By using the differential, total carbohydrates were computed.

**Determination of total phenolic compounds (TPC) of MTS and RGR**

Folin-Ciocalteu colorimetric method was used to estimate TPC by **Eghdami and Sadeghi, (2010)**. Gallic acid equivalents (mg of GAE/g) on a dry weight basis were used to express the TPC.

**Determination of total flavonoids compounds (TFC) of MTS and RGR**

TFC were determined using a colorimetric method of **Menichini, *et al*., (2009)**. The results were presented as mg quercetin per gram of sample's dry weight.

**Antioxidant activity assay by DPPH**

The level of antioxidant activity of MTS and RGR were measured by spectrophotometric estimation based on the decreasing of methanol extract of DPPH according to **Lim and Quah (2007)**.

**Biological Evaluations**

The amounts of food consumed and/or wasted, were recorded every day while total feed intake (FI) was calculated. In addition, body weight (BW) of rat's was recorded weekly. Body weight gain percentage (BWG%) were calculated according to ***Champman, et al., (1959)*** using the next equation:

$$\mathbf{BWG\%} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\mathbf{FER} = \frac{\text{weight Gain (g)}}{\text{Feed intake (g)}}$$

**Oral glucose tolerance test**

All rats in the groups were given glucose (2 g/kg) after 12 hr fasting. The oral glucose tolerance test (GTT) in the blood samples obtained from the tail vein of mice were analyzed at 0, 15, 30, 60, 90 and 120 min after glucose administration **(Rohling *et al*., 2019)**.

**Tissue and serum sampling**

Rats were fasted for 12-hr, except of water at the end of the study period, and then serially anesthetized with diethyl ether. Rats were euthanized and organs were dissected blood sampling and ovary sampling. The posterior vena cava was used to collect blood samples into dry, clean centrifuge tubes, which were then allowed to clot at room temperature before being spun for 10 min at 3000 rpm to separate. Serum samples were frozen at -20°C for biochemical analysis and hormonal analysis.

**Biochemical analysis**

Testosterone (TS) was analyzed according to **Salameh *et al*., (2010)**. Serum hormone analysis, Progesterone (P4) was analyzed according to **Bychowski and Auger, (2012)**. Luteinizing hormone (LH) was determined according to **Sherman *et al*., (1976)**. Estradiol (E2) was estimated according to **Scott *et al*.,(1993)**. Follicle Stimulating Hormone (FSH) was analyzed according to **Rose *et al*., (2000)**.

Fasting blood glucose (FBG) was determined according to **Burrin and Price, (1985)**. According to **Chevenne *et al*., (1998)** enzyme linked immunosorbent assay ELISA was used to estimate insulin activity. Homeostasis Model Assessment of Insulin Resistance index (HOMA-IR were calculated by **Salgado *et al*., (2010)** using the next equation:

HOMA-IR= {[fasting insulin (μU/ml)]×[FBG (mmol/L)]} /22.5.

Serum lipid profile involving total cholesterol (TC), triglycerides (TG) and cholesterol contents of high density lipoprotein (HDL) were evaluated in accordance to **Allian *et al*., (1974)**, **Fossati and Principe, (1982)**, **Albers *et al*., (1983)**, respectively. Calculations of very low-density

lipoprotein cholesterol (VLDL) and low density lipoprotein cholesterol (LDL) by the equation of **Fruchart, (1982)**.

LDL-c =TC-[HDL-c + (TG/5)] VLDL-c = TG/5.

For assessing lipid peroxidation, plasma level of Malondialdehyde (MDA) was estimated in the supernatant of rat brain following the approach of **Draper and Hadley, (1990)**. The activity of superoxide dismutase (SOD) was assessed according to **Spitz and Oberley, (1989)**. Total antioxidant capacity (TAC) were assayed according to the method of **Woodford and Whitehead, (1998)**. Serum tumor necrotic factor-α (TNF-α) was determined according to **Kandir and Keskin, (2016)**.

**Histopathological examinations**

The ovaries were cleaned of fat, weighed, and fixed in 10% formalin for 48 hours then ovaries underwent routine histological processing, sectioning at a thickness of 5m, and staining with Hematoxylin and eosin (H&E) to be examined **(Ibrahim *et al*., 2018)**. Follicle count was performed in accordance with **Amini *et al*., (2016)**.

**Statistical analysis**

Data were presented as mean ± standard deviation. Utilizing tests for normality (SPSS version 25), the distribution of the data will be confirmed to be normal. One-way analysis of variance will be utilized to assess statistical significance (ANOVA). Statistical significance is determined by the probability of $p \leq 0.05$ **(Snedecor and Cochron, 1989)**.

**Results and Discussion**

Data in Table (1) represent values of moisture, protein, fat, ash, carbohydrate and fiber (on dry weight basis), for milk thistle seed and red ginseng roots. The obtained results from table (1) indicated that, MTS had the higher percentage of protein, fat and fiber which their values were (23.79, 26.01 and 20.69) % respectively compared with (10.11, 5.9 and 4.67) % for RGR. On the other hand, it could be also noticed that, RGR had higher content of moisture, ash and carbohydrates (8.90, 8.8 and 61.62%), respectively than MTS seed which their values were 7.03, 4.17 and 18.31%,

respectively. MTS proximate analysis partially agreed with the results presented by **Aziz *et al*., (2020)** showed moisture , ash , fat , fiber, protein content varied from 6.27-5.01%, 2.37-1.25%, 23.19-19.74%, 7.4-4.39% and 30.09-20.74%,respectively. Similarly, **Abd-Elhady and Arafa, (2019)** demonstrated that the protein content of MTS is 23.43%, the ash content is 4.92%, the fibre content is 25.74%, and the accessible carbs are 19.77%. At the same line, **Elnaggar *et al*., (2022)** found that the most important major compounds of powder ginseng were varied from moisture, ash, lipids, fiber, and protein content 9.6, 5.1, 5.5, 7.8 and 11.6% respectively. The results are consistent with **Jin *et al*., (2019)** found that ginseng contains 8-14% protein, 60% carbohydrates, 4-6% ash lipids 1-3% and 3-8% fiber. It is observed from the data that MTS and RGR are rich in its content of nutrients which can use it as functional ingredient for supplementation foods and medical purposes.

**Table (1): Gross chemical composition of raw materials (g/100 g on dry weight basis).**

| Samples | Moisture | Protein | Fat | Ash | Carb. | Fiber |
|---|---|---|---|---|---|---|
| **Milk thistle seeds (MTS)** | 7.03 | 23,79 | 26.01 | 4.17 | 18.31 | 20.69 |
| **Red ginseng roots (RGR)** | 8.90 | 10.11 | 5.9 | 8.8 | 61.62 | 4.67 |

Tabulated data in Table (2) presented the total phenols, total flavonoids, and antioxidant activity of MTS and RGR which a high active component found in raw materials. Table (2) showed that MTS and RGR contained TP (34.79, 33.74) mg GAE/g, TF (23.95, 20.43) mg QE/g and antioxidant activity (88.76, 62.90) respectively. This result is in normal range to those found by **Javeed *et al*., (2022)** and **Aziz *et al*., (2020)** found that M. thistle contained TP (24.17 – 35.07 mg GAE/g), TF (16.01–29.09 mg QE/g) and antioxidant activity in methanol (75.98%). Present results concerning TP, TF and antioxidant activity content of MTS are in harmony with the study of the **Abd-El-hady and Arafa, (2019)** value as (35.65 mg GAE/g, 23.78 mg/g

and 89.73 %). Regarding ginseng, the current study's findings partially corroborate those of **Hussain *et al.*, (2020)** reported that red ginseng roots indicated TP, TF and antioxidant activity content (37.26 g/GAE, 149.4 g/CE and 62.84%). Another study by **Malathy *et al.*, (2020)** observed TPC and TFC in the methanolic root extract (30.21 and 20.25 mg/g) respectively.

**Table (2): Total phenolic, total flavonoid and antioxidant activity.**

| Components | Milk thistle seeds | Red ginseng roots |
|---|---|---|
| **Total Phenolic (mg GAE /g)** | 34.79 | 33.74 |
| **Total Flavonoids (mg QE/g)** | 23.95 | 20.43 |
| **Antioxidant activity (DPPH %)** | 88.76 | 62.90 |

GAE: Gallic acid equivalent, QE: Quercetin

On day 21 were changes of the body weight and hormonal. As presented in Tables (3) and (4) show that, treatment with LTZ for 21 days (PCOS induction) significantly increased BWG, FI FER, ovarian weights, and hormonal abnormalities in comparison to negative control group. These results are consistent with **Younas *et al.*, (2022)** and **Marouf *et al.*, (2022)** revealed that LTZ induced an increase in BWG of PCOS as compared to negative control rats. Similarly, **Ghasemi *et al.*, (2021); Abdelrahman *et al.*, (2021)** noted body weight and ovarian weight of rats dramatically increased after PCOS induction, which may be connected to the presence of cystic follicles. As opposed to the PCOS group, the BWG, FI, FER and ovarian weights significantly decreased ($P \leq 0.05$) in the MTS, RGR, and Mix groups at day 63, the end of the current study. The final body weight difference among three treated groups were mostly statistically non-significant. Results showed the best decreasing of ovarian weight in mixture and nearing with negative control group. This results are consistent with **Zhu *et al.*, (2018)** and **Guo *et al.*, (2016)** reported that *S. marianum* effectively reduced mice body weight. Regarding ginseng, **Huang *et al.*, (2022)** and **Amanat *et al.*, (2021)** showed that red ginseng (RG) treatment improved body weight and ovarian weights. Similarly, **Moradi *et al.*, (2021)** and **Choi *et al.*, (2020)** demonstrated that RGE prevent increased

body weight and ovarian weights by eliminating all ovarian cysts in rats with PCOS.

**Table (3): Effect of MTS, RGR and their mixture on BWG%, FI, FER and ovarian weight in LTZ -induced PCOS**

| Parameters / Sample | IBW | FBW | BWG% | FI (g/d/rat) | FER | Ovarian weight |
|---|---|---|---|---|---|---|
| **Control (-ve)** | 161.87±2.31[a] | 198.28±1.15[c] | 22.49±1.16[e] | 16.00 | 0.036±0.02[e] | 0.026±0.01[d] |
| **PCOS** | 167.64±1.22[a] | 242.29±1.76[a] | 44.53±1.83[a] | 18.00 | 0.066±0.04[a] | 0.047±0.02[a] |
| **MTS-treated PCOS** | 163.43±0.94[a] | 223.84±2.72[b] | 36.96±1.65[b] | 16.50 | 0.058±0.03[b] | 0.036±0.02[b] |
| **RGR-treated PCOS** | 166.02±1.02[a] | 216.72±1.28[b] | 30.54±1.09[c] | 15.40 | 0.052±0.04[c] | 0.032±0.01[c] |
| **Mix-treated PCOS** | 164.81±1.31[a] | 207.60±0.96[b c] | 25.96±0.59[d] | 15.00 | 0.045±0.0[d] | 0.028±0.01[d] |

Initial body weight (IBW), Final body weight (FBW), Body weight gain (BWG %), feed intake (FI) and feed efficiency ratio (FER). Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P \leq 0.05$).

Data in Table (4), on day 21, the levels of TS and LH in LTZ-treated rats increased by (144.76 and 27.61%), respectively while, E2 was decreased by 50.46% compared with negative control. It is clear that the most comparable and consistent hormonal markers to diagnose PCOS are increased serum levels of TS and LH and low E2 **(Marouf *et al*., 2022)**. This findings are in line with earlier studies conclusions that LTZ treatment caused PCOS **(Ghasemi *et al*., 2021** and **Abdelrahman *et al*., 2021)**.

**Table (4): Hormones changes in PCOS rats induced by LTZ on day 21.**

| Parameters / Sample | Control (ve-) - Day 21 | Control (ve+) - Day 21 | Increment % |
|---|---|---|---|
| **TS (Pg/ml)** | 19.99 ±0.50[b] | 48.94±1.10[a] | 144.76 |
| **LH (MIu/ml)** | 5.19±0.32[b] | 6.62±0.25[a] | 27.61 |
| **E2 (Pg/ml)** | 47.10±0.86[a] | 23.33±1.70[b] | - 50.46 |

Testosterone (TS), Luteinizing hormone (LH) and Estradiol (E2). Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P \leq 0.05$).

As shown in Table (5), 42 days after PCOS induction, experienced an increase in fasting insulin and fasting blood glucose levels in PCOS group ($p \leq 0.05$) compared to negative group. Consequently, it was accompanied by a rise in IR. The treatment with MTS, RGR and their Mix significantly reduced ($P \leq 0.05$) the elevated FBG, FI and HOMA IR as compared to PCOS group. Mix group showed the best group in all parameters. These results are consistent with **Ghasemi *et al.*, (2021);Abdelrahman *et al.*, (2021)** and **Wang *et al.*, (2020)** observed that induction of PCOS increased FBG, FI and HOMA-IR in rats, which was consistent with the pathological traits of PCOS endocrine and metabolic disorders. These results line up with **Memon *et al.*, (2022)** showed that *silymarin* improves blood glucose levels, reduced FBG, FI, HOMA-IR in type 2 diabetics. Similarly, **Mohammadi *et al.*, (2020)** demonstrated that *silymarin* reduce glucose and insulin levels in diabetic rats. **MacDonald-Ramos *et al.*, (2021)** proved that *Silymarin* an excellent glucose regulator by decreasing the IR and glycemic improvement might be related to anti-inflammatory and anti-gluconeogenesis. Regarding red ginseng, **Huang *et al.*, (2022)** and **Gad *et al.*, (2022)** revealed that were significantly improved serum level of glucose and FI after administration of ginseng to diabetic rats. Similarly, **Xin-Sen *et al.*, (2022)** observed that ginseng extract reduced blood glucose and insulin secretion. Consistent with these reports, **Park *et al.*, (2021)** found ginseng extract supplementation for 8-week led to

significantly decrease of FBG and IR. Ginsenoside, the primary component of P. ginseng, is thought to be responsible for its ability to modulate serum glucose levels by increasing-cell activity and enhancing insulin sensitivity **(Chen *et al.*, 2019)**.

**Table (5): Effect of MTS, RGR and their mixture on glycemic indices in LTZ -induced PCOS**

| Parameters / Groups | Fasting Insulin (FI) (µIU/ml) | Fasting Blood Glucose (FBG) (mmol/L) | Homeostasis Insulin Resistance (HOMA- IR) |
|---|---|---|---|
| **Control (-ve)** | 15.32±1.61$^{e}$ | 4.22±3.07$^{d}$ | 2.87±0.25$^{e}$ |
| **PCOS** | 45.98±2.98$^{a}$ | 7.39±2.64$^{a}$ | 15.10±1.42$^{a}$ |
| **MTS-treated PCOS** | 33.45±1.91$^{b}$ | 6.67±0.71$^{b}$ | 11.40±0.80$^{b}$ |
| **RGR-treated PCOS** | 26.23±1.28$^{c}$ | 6.39±5.32$^{b}$ | 9.92±0.61$^{c}$ |
| **Mix-treated PCOS** | 21.90±1.55$^{d}$ | 5.47±2.33$^{c}$ | 5.32±0.85$^{d}$ |

Results are expressed as mean ± SE.

Values in each column which have different letters are significantly different at ($P \leq 0.05$).

As presented in Figure (1), Glucose tolerance (GT) was measured at the 42 day. In PCOS group, glucose homeostasis is typically compromised, blood glucose levels were significantly higher and that glucose clearance was significantly delayed as compared to control negative group. In contrast to the PCOS group, it showed that GT was significantly better in the MTS, RGR, and Mix treated groups. These results are consistent with **Zhu *et al.*, (2018)** and **Guo *et al.*, (2016)** showed that silymarin (40 mg/100g) lowered GT level and IR in obese mice. Regarding ginseng, **Aminifard *et al.*, (2021)** reported that ginseng can lower blood sugar levels through a number of efficient ways, including improved insulin sensitivity, increased tissue glucose uptake, decreased IR, and improved GT. Similarly,

**Lee *et al.*, (2021)** reported that RGE improved IR and GT while, reduced FBG and postprandial glucose levels.

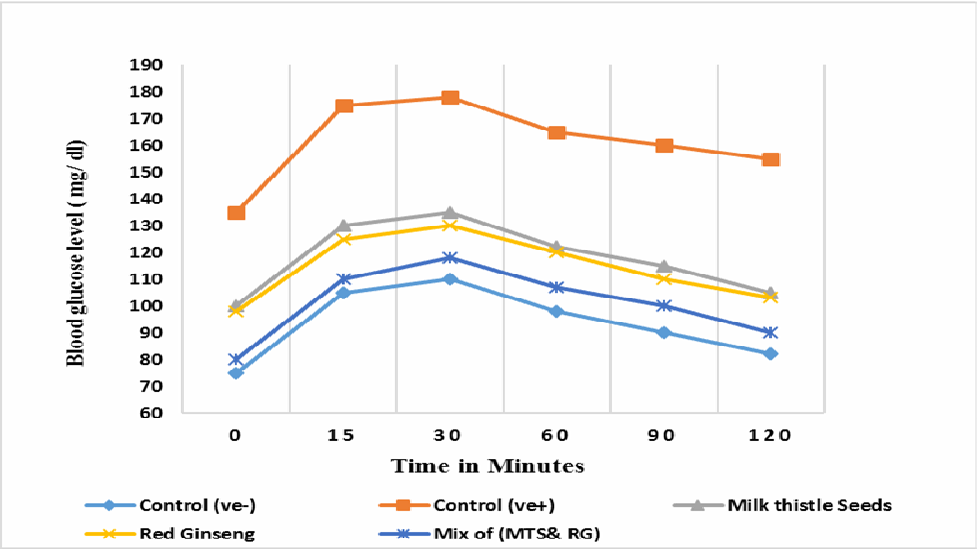



**Figure (1):** Effect of MTS, RGR and their Mix on glucose tolerance of rats.

Based on the table (6), the injection with LTZ caused a PCOS that a significant increase ($P \leq 0.05$) in the levels of TS and LH while, decrease the levels of P4, $E_2$ and FSH comparing with the negative group. This outcomes were consistent with **Ghasemi *et al.*, (2021)**; **Abdelrahman *et al.*, (2021)** and **Wang *et al.*, (2020)** discovered that the levels of TS and LH were dramatically raised while the level of P4, $E_2$ and FSH were lowered in PCOS-IR rats, who used LTZ with the same dose. Besides, MTS, RGR and their Mix-treated group demonstrated a notable improvement in ovulation, an increase in E2, P4, and FSH levels, and a decrease in TS and LH levels compared to PCOS group. These results are consistent with **Marouf *et al.*, (2022)** found that silibinin may be able to alleviate the hormonal and metabolic changes brought on by PCOS by a noticeably lower level of TS, LH, and the restoration of the regularity of the estrous cycle, it's anti-androgenic, anti-inflammatory, and antioxidant qualities might be the cause of this. Also, **Ahmed, (2021)** showed that feeding rats baseline diet supplemented with milk thistle seeds at 10 and15% have improved in the levels of estradiol hormone in female rats with intoxified liver by CCl4.

Regarding ginseng, **Moradi *et al.*, (2021)** showed that RGE lowering TS and LH levels and eradicating ovarian cysts in PCOS that unequivocally supports these estrogenic effects. These results support **Choi *et al.*, (2020)** showed that TS and LH was decreased markedly by pretreatment with RGE. Therefore, it is beneficial in treating ovulation problems in PCOS.

**Table (6): Effect of MTS, RGR and their mixture on sex hormones in LTZ -induced PCOS**

| Parameters / Groups | Testosterone (TS) (pg/ml) | Progesterone (P4) (ng/ml) | Estradiol (E2) (pg/ml) | Luteinizing (LH) (mIU/ml) | Follicle Stimulating (FSH) (mIU/ml) |
|---|---|---|---|---|---|
| **Control (-ve)** | 22.99±0.97$^{e}$ | 8.34±0.53$^{a}$ | 47.03±1.64$^{a}$ | 4.62±0.21$^{c}$ | 1.18±0.09$^{a}$ |
| **PCOS** | 49.11±2.39$^{a}$ | 3.75±0.18$^{d}$ | 17.77±2.20$^{e}$ | 7.40±0.39$^{a}$ | 0.50±0.08$^{d}$ |
| **MTS-treated PCOS** | 40.03±0.75$^{b}$ | 4.91±0.12$^{c}$ | 28.64±1.40$^{d}$ | 6.51±0.30$^{b}$ | 0.78±0.01$^{c}$ |
| **RGR-treated PCOS** | 35.50±2.68$^{c}$ | 5.04±0.12$^{c}$ | 35.65±1.36$^{c}$ | 5.96±0.09$^{b}$ | 0.93±0.03$^{b}$ |
| **Mix-treated PCOS** | 28.64±1.53$^{d}$ | 6.55±0.24$^{b}$ | 42.83±1.40$^{b}$ | 4.84±0.18$^{bc}$ | 0.99±0.04$^{ab}$ |

Results are expressed as mean ± SE.

Values in each column which have different letters are significantly different at ($P \leq 0.05$).

The current study showed that LTZ has an impact on lipid profile, Table (7) showed that at 42 days after PCOS induction there were significant ($P \leq 0.05$) increase of TC, TG, LDL, and VLDL, while significant ($P \leq 0.05$) decrease in HDL in the PCOS groups as compared with control negative group. MTS, RGR and their mixture significantly suppressed this elevation to normal levels and increases in HDL as compared with the PCOS group. This results were consistent with **Ghanem *et al.*, (2022)** demonstrated that that treatment with milk thistle improved lipid profile. In similar study **Jiang *et al.*, (2022)** showed that silymarin may considerably decrease TC, TG, LDL while, increase the levels of HDL in mice. **Ahmed, (2021)** showed that feeding rats baseline diet supplemented with milk thistle seeds at 10 and 15% have improved in the levels of lipid profile in female rats with intoxified liver.

At the same line, **Huang *et al*., (2022); Xin-Sen *et al*., (2022)** and **Gad *et al*., (2022)** demonstrated Red ginseng a potent antihyperlipidemic effect by decreasing TG, TC, and LDL levels but raising HDL in diabetic rats. In similar study **Park *et al*., (2021)** showed that ginseng raise HDL-c levels while decrease TC, TG, and LDL. Ginsenoside component of ginseng may be responsible for the herb's ability to lower serum cholesterol levels **(Amanat *et al*., 2021)**.

**Table (7): Effect of MTS, RGR and their mixture on lipid profile in LTZ -induced PCOS**

| Parameters / Sample | TC | TG | HDL-c | VLDL-c | LDL-c |
|---|---|---|---|---|---|
| | (mg/dl) | | | | |
| **Control (-ve)** | 159.90±4.87$^{e}$ | 125.75±1.78$^{e}$ | 59.73±0.93$^{a}$ | 25.15±0.35$^{e}$ | 75.01±4.15$^{e}$ |
| **PCOS** | 255.10±2.74$^{a}$ | 183.83±2.04$^{a}$ | 33.27±1.91$^{e}$ | 36.76±0.40$^{a}$ | 185.06±1.15$^{a}$ |
| **MTS-treated PCOS** | 216.06±3.11$^{b}$ | 169.81±2.06$^{b}$ | 40.97±1.73$^{d}$ | 33.96±0.41$^{b}$ | 141.13±4.73$^{b}$ |
| **RGR- treated PCOS** | 196.28±3.32$^{c}$ | 149.57±5.05$^{c}$ | 46.36±1.86$^{c}$ | 29.91±1.01$^{c}$ | 119.99±6.15$^{c}$ |
| **Mix-treated PCOS** | 178.49±2.62$^{d}$ | 136.09±2.09$^{d}$ | 52.53±1.63$^{b}$ | 27.21±0.41$^{d}$ | 98.73±4.23$^{d}$ |

Total cholesterol (TC), Triglycerides (TG), High density lipoprotein - cholesterol (HDL), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C).

Results are expressed as mean ± SE.

Values in each column which have different letters are significantly different at ($P \leq 0.05$).

As shown in Table (8), based on antioxidant enzymes, total antioxidant capacity and inflammatory markers, PCOS induction by LTZ resulted in a higher oxidative status, which increased MDA and TNF- α while decreasing SOD and TAC levels, When compared to the negative group. It is clear that oxidative stress is regarded as a possible stimulator of PCOS and that it is somewhat linked to the many symptoms of this condition **(Marouf *et al*., 2022)**.

In contrast, treatment with MTS, RGR and their Mix significantly decreased serum levels of MDA and TNF-α while increment in the levels of SOD and TAC in treated groups ($p \leq 0.05$) compared to PCOS, could be due to anti-inflammatory and antioxidant. Mixture group showed the best group in all parameters. Silymarin acts as a strong antioxidant agent, and has high ability to scavenge ROS **(Khazaei *et al.*, 2022)**.These results are consistent with **Ghanem *et al.*, (2022)** showed that doses of milk thistle extract (20 and 30 g/day) significantly boosted (TAC) and antioxidant enzymes (SOD and CAT) but decreased the level of MDA. In similar study **Abd Elalal *et al.*, (2022**) and **Ahmed, (2021)** suggested that treatment with milk thistle (rich in silymarin) may contribute to increase in the antioxidant enzymes while, inhibits MDA. **Marouf *et al.*, (2022)** found that silibinin also maintained the TAC of the PCOS-rats. Regarding ginseng, **Gad *et al.*, (2022)** confirmed that Ginseng treatment for two months in diabetic male rats alleviated the oxidative stress by significantly increasing CAT , SOD and TAC while, lowering MDA levels. This results is consistent with that of **Amanat *et al.*, (2021)** reported that RG usage decreases oxidative stress in PCOS rats.

On the other hand, **Alissa *et al.*, (2021)** and **Amanat *et al.*, (2021)** observed that PCOS is associated with higher levels of TNF-α, this is consistent with our study. The current study are in conformity with **Ghanem *et al.*, (2022)** showed that milk thistle extract (20 or 30 g/day) significantly reduced the level of TNF-α due to the high content of silymarin in milk thistle. Similarly, **Marouf *et al.*,(2022)** showed that Silibinin inhibits and reduces the production of the pro-inflammatory cytokine TNF-α and has anti-inflammatory effects on PCOS. Furthermore, polyphenols including silymarin exert a beneficial effect on health of animals through reducing oxidative stress and inflammation.

At the same line, **Amanat *et al.*, (2021)** showed genistein supplementation decreased the levels of serum proinflammatory cytokines including TNF-α in comparison to the PCOS group. Similarly, **Fan *et al.*, (2020)** and **Choi *et al.*, (2020)** suggested that RGE can inhibit inflammatory response in the ovarian tissue of PCOS, indicating both its a

preventive and therapeutic potential for the condition, due to anti-inflammatory and antioxidant properties, or its active ingredients such as ginsenosides, polysaccharides, and gintonin **(Chen *et al.*, 2019)**.

**Table (8): Effect of MTS, RGR and Mix on antioxidative enzyme, total antioxidant capacity and inflammatory indices in LTZ-induced PCOS.**

| Parameters / Groups | MDA (µmol/dl) | SOD (µ/ml) | TAC (mmol/L) | TNF-α (Pg/ml) |
|---|---|---|---|---|
| **Control (-ve)** | $10.30\pm0.30^{e}$ | $86.91\pm1.00^{a}$ | $3.85\pm0.14^{a}$ | $80.38\pm1.84^{d}$ |
| **PCOS** | $36.50\pm0.83^{a}$ | $48.75\pm1.64^{e}$ | $1.61\pm0.09^{d}$ | $300.25\pm3.27^{a}$ |
| **MTS-treated PCOS** | $30.31\pm0.52^{b}$ | $56.71\pm0.75^{d}$ | $2.79\pm0.08^{c}$ | $192.53\pm1.97^{b}$ |
| **RGR- treated PCOS** | $20.90\pm0.61^{c}$ | $65.55\pm1.88^{c}$ | $3.52\pm0.06^{b}$ | $195.11\pm1.84^{b}$ |
| **Mix-treated PCOS** | $14.70\pm0.41^{d}$ | $77.28\pm1.11^{b}$ | $3.68\pm0.13^{ab}$ | $104.24\pm1.86^{c}$ |

Malondialdehyde (MDA), Superoxide dismutase (SOD), Total antioxidant capacity (TAC) and Tumor necrotic factor-α (TNF-α). Results are expressed as mean ± SE.

Values in each column which have different letters are significantly different at ($P\leq0.05$).

**Histopathological examination of a vaginal smear**

The stage of estrous cycles (OC) was identified daily by light microscopic analysis of the prominent cell type in the vaginal epithelial smears. As a result, all stages were visible on their vaginal smear; as shown in Photo 1 (A, B, C and D). Daily inspection of the vaginal smears demonstrated the arrest of the cyclicity in the diestrus phase in all LTZ-treated rats, confirming the induction of PCOS.

Furthermore, as shown in Table (9), results examination of vaginal smear. Rats in all treated groups recovered to their cycles, however the PCOS group was still caught in the diestrus phase after three weeks of treatment. This aligns with the results of **Marouf *et al.*, (2022)** that silibinin was effective in restoring estrous regularities and alleviating abnormalities

of the ovarian and uterine tissues. Similarly, **Choi *et al.*, (2020)** noted that pretreatment with RGE has a positive impact on maintaining a normal estrous cycle.

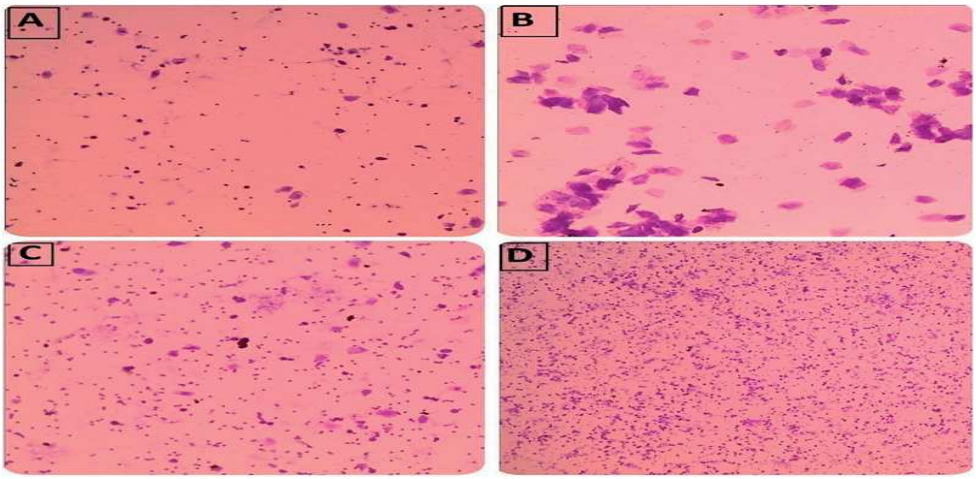



**Photo (1).** The vaginal smear's stages (A) characterised by the predominance of nucleated epithelial cells with distinct borders, the pro-estrus phase. (B) Cornified cells (large a nucleated cells) with irregular margins predominate during the estrus phase. (C) Numerous cornified cells and leucocyte infiltration are visible during the met estrus phase. (D) Leucocytes predominate during the diestrus phase, and cornified cells are absent.

**Table (9):** Results examination of vaginal smear

| Groups | 3rd week (%) | 4th week (%) | 5th week (%) | 6th week (%) |
|---|---|---|---|---|
| Control (-ve) | 100 | 100 | 100 | 100 |
| PCOS | 0 | 0 | 0 | 0 |
| MTS-treated PCOS | 0 | 10 | 55 | 100 |
| RGR-treated PCOS | 0 | 15 | 65 | 100 |
| Mix-treated PCOS | 0 | 30 | 75 | 100 |

The morphometric examination of ovarian follicles, which is shown in table (10) demonstrates that, PCOS group the number of primordial (PF) and cystic follicles (CF) significantly increased while the Grafian follicle (GF) and corpora lutea (CL) decreased compared to the negative control

group and this consistent with **Marouf *et al.*, (2022)**, which confirm the PCOS induction, due to the increase in androgen production. Also, **Ghasemi *et al.*, (2021)** demonstrated that the number of cystic follicles had increased in the ovaries of LTZ induced PCOS rats. Contrarily, the MTS, RGR, and Mix-treated groups showed a decreased number of PF and CF while significantly (P≤0.05) increased GF and CL compared to the PCOS rats. These results are consistent with **Marouf *et al.*, (2022)** showed silibinin resuming the appearance of multiple CL, absence of CF. Due to silymarin antioxidant and anti-inflammatory properties, which minimise the amount of cysts and the natural development of follicles, it may be utilised as an agent to protect ovarian follicles **(MacDonald-Ramos *et al.*, 2021)**. On the other hand **Amanat *et al.*, (2021)** demonstrated that treatment with genistein led to increase the number of atretic follicles and CL, decrease in the number of cysts and the emergence of healthy follicles due to its antioxidant compounds and adaptogenic properties improves ovarian tissue. **Choi *et al.*, (2020)** showed that pretreatment with RGE can successfully prevent the growth of follicular cysts which cause ovarian cysts to multiply and enlarge.

**Table (10):** The morphometric examination of ovarian follicles

| Parameters / Groups | Primordial Follicle (PF) | Grafian Follicle (GF) | Cystic Follicle (CF) | Corpus Luteum (CL) |
|---|---|---|---|---|
| **Control (-ve)** | $4.8\pm1.24^{c}$ | $5.5\pm0.64^{a}$ | $2.9\pm2.23^{d}$ | $5.7\pm0.75^{a}$ |
| **PCOS** | $6.7\pm1.03^{a}$ | $2.8\pm 0.76^{c}$ | $9.4\pm1.35^{a}$ | $0.7\pm 0.09^{c}$ |
| **MTS-treated PCOS** | $5.6\pm1.03^{b}$ | $4.3 \pm 0.81^{b}$ | $6.9\pm1.98^{b}$ | $4.5\pm0.64^{b}$ |
| **RGR-treated PCOS** | $5.3\pm1.10^{b}$ | $4.5 \pm 0.97^{b}$ | $6.5\pm2.23^{bc}$ | $4.9\pm 0.41^{ab}$ |
| **Mix-treated PCOS** | $5.0\pm0.92^{bc}$ | $4.9 \pm 1.27^{ab}$ | $5.8\pm0.95^{c}$ | $5.4\pm 0.89^{a}$ |

Results are expressed as mean ± SE.

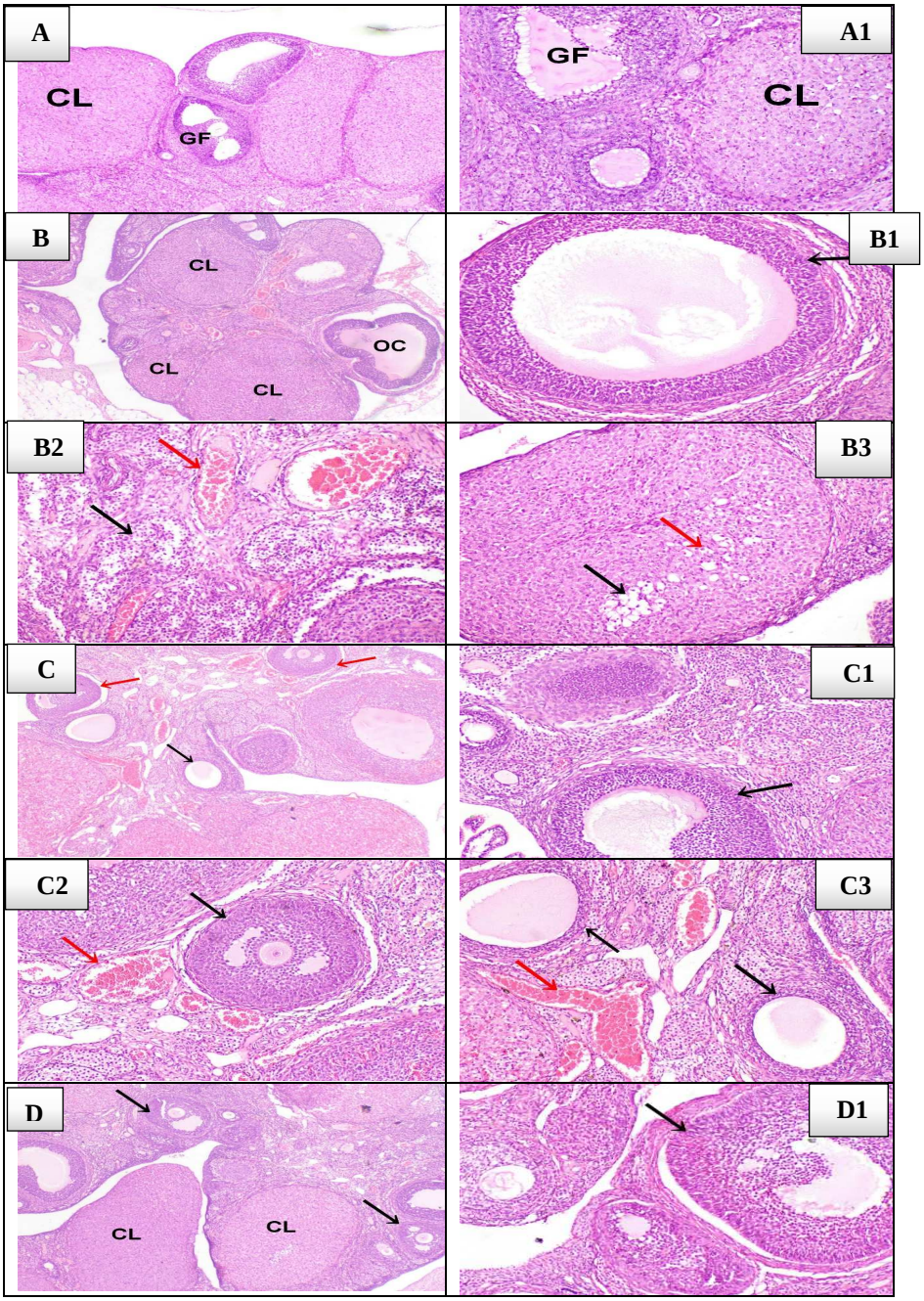
Values in each column which have different letters are significantly different at (P≤0.05).

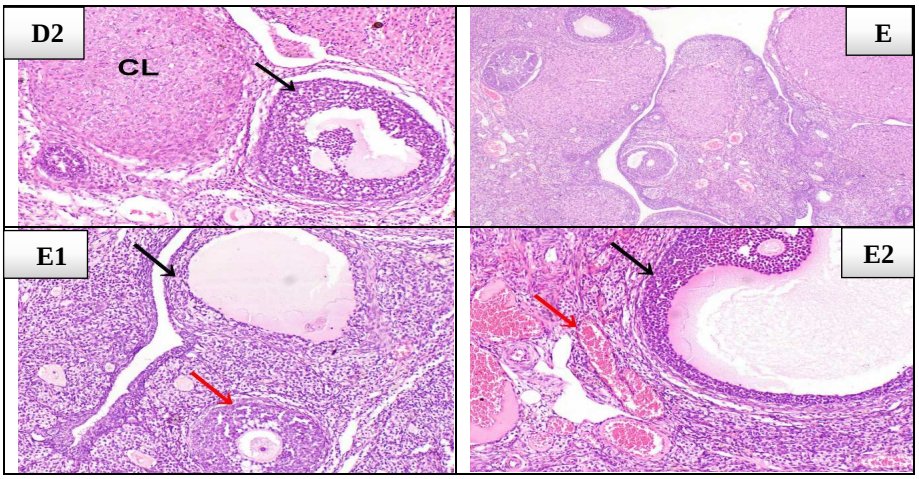
**Histopathological examination of ovarian tissue**

In the present study, microscopically, ovaries of negative control rats showed normal histological structure. Note normal corpora lutea (CL) and normal grafian follicles (GF) (Photo 2 (A & A1). Multiple CL and ovarian cysts (OC) with a thin layer of granulosa cells were seen when the ovaries of the PCOS group were examined histologically (Photo2. B & B1), hyperplasia of interstitial cells, congested blood vessels (Photo5. B2), vacuolization and apoptosis of cells of corpus leutium (Photo2. B3). These results are consistent with **Younas *et al.*, (2022)** demonstrated that histological analysis revealed a clear difference between PCOS-affected and normal control rat ovaries due to LTZ. This is in agreement with **Amanat *et al.*, (2021)** and **Ghasemi *et al.*, (2021)** observed that the PCOS rats had many cystic follicles, a diminished granulosa cell layer, and a markedly reduced volume of CL.

On contrast, ovaries of rats treated with MTS revealed multiple normal graafian follicles (Photo2. C), decreased ovarian cyst count, and increased granulosa cell thickness (Photo2. C1), normal graafian follicles and congested blood vessels (Photo2. C2). Some examined sections from this group showed ovarian cyst and congested blood vessels (Photo2. C3). These results are consistent with **Marouf *et al.*, (2022)** showed silibinin restored the existence of antral follicles at various growth stages and eliminated endometrial hyperplasia due to its anti-androgenic properties. At the same line, ovaries of rats treated with RGR exhibited multiple corpora lutea and multiple grafian follicles (Photo2. D), no histopathological alterations and restoration of granulosa cell thickness (Photo2. D1 & D2). Likewise, examined sections from rats treated with Mix revealed normal ovarian histology (Photo2. E), no histopathological alterations, restoration of granulosa cell thickness and normal grafian follicle (Photo2. E1) as well as congested blood vessels (Photo2. E2). These results are consistent with **Amanat *et al.*, (2021)** found that genistein-supplemented rats showed fewer cysts, and the presence of CL in the ovaries indicated follicular development and ovulation. Similarly, **Moradi *et al.*, (2021)** supported that the

effectiveness of RGE in restoring ovarian weight normalcy and eliminating ovarian cysts.






**Photo(2).** Ovaries of (-ve) control rat showing normal histological structure. Note normal CL and GF (A&A1). Ovaries of PCOS rats showed multiple CL and OC (B & B1), hyperplasia of interstitial cells (black arrow), congested blood vessels (red arrow) (B2), vacuolization (black arrow) and apoptosis of cells of CL (red arrow) (B3). Ovaries of rat treated with MTS showing multiple normal GF (red arrow) and OC (black arrow) (C)، decreasing in the number of OC and restoration of granulosa cell thickness (black arrow) (C1), normal GF (black arrow) and congested blood vessel (red arrow) (C2), Some examined sections from this group showed OC (black arrow) and congested blood vessels (red arrow)(C3). Ovaries of rat treated with RGR showing multiple CL and multiple GF (black arrow) (D), no histopathological alterations and restoration of granulosa cell thickness (black arrow) and CL (D1 & D2). Ovaries of rat treated with Mix showing normal ovarian histology, no histopathological alterations. Note restoration of granulosa cell thickness (black arrow) (E), normal GF (red arrow) (E1), and congested blood vessels (red arrow) (E2) were noted too (H&E, x40) or (H&E, x100).

## Conclusion

The promising findings in this study suggest that milk thistle seeds or red ginseng roots and their combinations may be a potential strategy to deal with the various complications of PCOS, such as IR, dyslipidemia,

oxidative stress, and inflammatory. According to histologic analysis of the ovaries and vaginal epithelial cells, these herbs also had positive effects on restoring normal hormonal levels, resuming folliculogenesis, and restoring ovulation. These protective effects are primarily attributable to their potent antioxidant and anti-inflammatory properties. So, it is recommended to use MTS or RGR and their mixture as promising herbs in the future for many important nutritional and therapeutic applications, which can play good role in avoiding PCOS incidence and its complications.

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# التأثيرات العلاجية لبذور شوك الحليب وجذور الجينسنج الأحمر على متلازمة تكيس المبايض المستحثة بالليتروزول فى إناث الفئران

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## الملخص العربي:

كان الهدف من هذه الدراسة هو تقييم تأثير بذور شوك الحليب وجذور الجينسينج الأحمر وخليطهم على المؤشرات البيوكيميائية والنسيجية لمتلازمة تكيس المبايض. تم تقسيم عدد ٤٢ من اناث الفئران البالغة من سلالة الألبينو، وزنها (١٦٠ ± ١٠ جم) بشكل عشوائى إلى مجموعتين: المجموعة (I) ١٢فأر تلقت النظام الغذائى الأساسى كمجموعة ضابطة سالبة ،المجموعة (II) ٣٠ فأر التي أعطيت الليتروزول بتركيز (١ مجم / كجم من وزن الجسم) مذاب في محلول ملحي بواسطة أنبوب التزقيم / التجويف مرة واحدة يوميًا لمدة ٢١ يومًا للحث على متلازمة تكيس المبايض ، تم تشريح ٦ فئران من كلا المجموعتين للتأكد من حدوث تكيس المبايض ، بعد ذلك تم اعادة تقسيم فئران مجموعة تكيس المبايض الى ٤ مجموعات متساوية (٦ فئران لكل منها) على النحو التالى: مجموعة ضابطة موجبة و٣ مجموعات تم تغذيتهم على النظام الغذائى الأساسى المدعم بمسحوق بذور شوك الحليب بتركيز ٥٪ ، جذور الجينسينج الأحمر بتركيز ٥٪ وخليطيهما عند مستوى (١:١) على التوالى. وتم تحديد فترة المعالجة لمدة ٤٢ يوم . أشارت النتائج الي أن النظام الغذائى المدعم ببذور شوك الحليب ، جذور الجينسينج الأحمر وخليطيهما أدى الي حدوث انخفاض فى وزن الجسم وتحسن كبير فى مستوى السكر فى الدم ، تحمل الجلوكوز، مقاومة الانسولين ، صورة دهون الدم ، مستوى الانزيمات المضادة للاكسدة ومؤشرات الالتهابات. كما اكد الفحص الهستوباثولوجي على نتائج التحاليل البيوكميائية فى استعادة انتظام دورة الشبق وتنظيم الهرمونات وتخفيف التشوهات فى أنسجة المبيض. بسبب خصائص تلك الأعشاب المضادة للاندروجين والمضادة للالتهابات .لذا يوصى باستخدام كلا من بذور شوك الحليب ، جذور الجينسينج الأحمر وخليطيهما كمكمل غذائى لتجنب حدوث متلازمة تكيس المبايض ومضاعفاتها .

الكلمات المفتاحية : شوك الحليب ، الجينسنج الأحمر، متلازمة تكيس المبايض، الليتروزول ، مقاومة الأنسولين ، اناث الفئران .

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