

Role of *Cressa cretica* on Serum Lipid Profile and Histopathological Changes of Ovaries in Induced Polycystic Ovary Syndrome in Female Rats

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Abstract

Background Polycystic ovary syndrome (PCOS), also called Stein-Leventhal syndrome, is a heterogeneous endocrine disorder that affects approximately 4-8% of women of reproductive age.

Objective To study the effect of *Cressa cretica* (*C. cretica*) leaves extract on induced PCOS in rat model, to evaluate the effect of *C. cretica* leaves extract on lipid profile and histopathological picture of rat's ovary and to compare the effect of *C. cretica* leaves extract with that of metformin.

Methods Thirty-six apparently healthy female rats weighing (180–200g) at 3-5 weeks of age divided into 6 groups, 6 rats for each. Five groups were administrated with 0.4 ml of oral Letrozole (5.3 mg/kg) daily for 21 days to induce PCOS, followed by a dose of Metformin (500 mg/kg/day) for one group, high dose *C. cretica* (400 mg/kg/day) for 2nd group, low dose *C. cretica* (300 mg/kg/day) for 3rd group and combination of (200 mg/kg/day) from *C. cretica* with (250 mg/kg/day) from Metformin for forth group, the treatment received for 30 days. Other 2 groups, one left without any treatment, and one did not receive any drug (control). Serum samples were taken from each rat to measure the lipid profile, and histopathology of the ovary was done after sacrificing the rats at the end of study.

Results *C. cretica* treated groups showed lower level of lipid profile except that for high-density lipoprotein where it was elevated in comparison to induction group. Ovulation is restored in the *C. cretica* treated group.

Conclusion *C. cretica* treated groups observed better results in comparison with Metformin in improvement of PCOS symptoms in female rats.

Keywords *Cressa cretica*, leaves, PCOS, Female rats, lipid profile.

List of abbreviations: *C. cretica* = *Cressa cretica*, HDL = High-density lipoprotein, LDL = Low density lipoprotein, PCOS = Polycystic ovary syndrome, TC = Total cholesterol, TG = Triglycerides, VLDL = Very low-density lipoprotein

Introduction

Polycystic ovary syndrome (PCOS), also called Stein-Leventhal syndrome, is a heterogeneous endocrine disorder that affects approximately 4-8% of women of

reproductive age ⁽¹⁾. Clinical hyperandrogenism (hirsutism) and biological hyperandrogenemia (elevated androgen levels in the blood), ovulatory arrest (menstrual dysfunction), thicker ovarian stroma, polycystic ovarian morphology on ultrasonography, and infertility are all symptoms of PCOS. PCOS is frequently associated with metabolic problems such as diabetes (increased insulin resistance) and

obesity ⁽²⁾. Non-pharmacological PCOS treatment options include lifestyle changes ⁽³⁾. Using of weight reducing medication (e.g., Orlistat) can help in individuals with high body mass index who wish to reduce their weight ⁽⁴⁾. Clomiphene citrate is utilized as the first-line medication for ovulation induction. A combination contraceptive including estrogens and progestin's has been used to treat hirsutism, acne, and menstrual abnormalities ⁽⁵⁾. Antiandrogens include spironolactone, flu amide, and cyproterone acetate, which reduce androgen output by inhibiting androgen receptors and are regarded as first-line treatments for hirsutism ⁽⁶⁾. Insulin sensitizing drugs, such as metformin and troglitazone, counteract some hyper androgenic symptoms by lowering total and free testosterone levels. It promotes ovulation, alleviates the symptoms of insulin resistance, and regulates overly high levels of androgens. It helps to reestablish the menstrual cycle, ovulation, and fertility ⁽⁷⁾. People have been looking for drugs in nature to treat terrible diseases since ancient times. Natural product-derived antibiotics and antifungal agents include erythromycin, clarithromycin, amoxicillin, and amphotericin B; anticancer agent's paclitaxel, docetaxel, and camptothecin; and cholesterol-lowering medications ⁽⁸⁾.

Cressa cretica (*C. cretica*) is a genus of plants in the Angiosperms family that belongs to the Morning Glory family. *C. cretica* is known to have the following activities such as antiandrogenic activity, nootropic effect, antioxidant, antimicrobial, antidiabetic, antitumor, anti-inflammatory, bronchodilator effect and antiviral properties. Traditionally, the herb was used as an antihelmintic, stomachic, tonic, aphrodisiac, and treatment for constipation, leprosy, asthma, and urinary discharges ⁽⁹⁾.

Current study aimed to study the effect of *C. cretica* leaves extract on induced PCOS in rat model, to evaluate the effect of its effect on lipid profile and histopathological picture of

rat's ovary and to compare the effect of *C. cretica* leaves extract with that of metformin.

Methods

Plant collection and authentication

The leaves of plant harvested on June 2021 from region in the north of Baghdad city in Iraq. It was identified and authenticated by the botanist, Prof. Dr. Khazzal Al-Jubouri at College of Science, University of Diyala.

Preparation of plant extract

The leaves of plant material were cleaned, dried in the shade at room temperature, electrically milled, and weighed. Firstly, the crushed plant material (approximately 150 g) was defatted separately for 24 h in a suitable amount of hexane solvent to remove chlorophyll and any hexane-soluble substances like waxy material via filtration and evaporation. A Soxhlet apparatus was used to extract the dried, defatted material. In the round flask of Soxhlet, 450 ml of 80% methanol were added. For around 12 h, the material was removed until it was completely exhausted. To eliminate the marc, the alcoholic extract was filtered through filter paper. The filtrate was concentrated to about 15 ml using a rotary evaporator under decreased pressure, and then combined with 50 ml of distilled water for leaf extraction. Chemical tests are performed to do a preliminary qualitative phytochemical study on leaf extracts, and high-performance thin layer chromatography (HTLC) analysis is used to determine the quantity of phenolic and flavonoid components ⁽¹⁰⁾.

Phytochemical investigation

The preliminary qualitative phytochemical investigation of *C. cretica* extract was performed to detect phytoconstituents such as alkaloids, flavanoids, tannins, saponins, glycosides, steroids, and by using the standard procedure ⁽¹¹⁾.

A. Test for cardiac glycosides

A 0.5 ml of extract was dissolved in 2 ml glacial acetic acid containing one drop of 1% ferric chloride (FeCl_3). This solution was under layered with 1 ml of sulphuric acid (H_2SO_4). A brown ring was formed at the interface that indicated the presence of deoxy-sugar.

B. Test for steroids

A 0.5 ml of sample extract was dissolved in 3 ml of chloroform (CHCl_3) and filtered. A few drops of concentrated (H_2SO_4) were added to the filter, forming a lower layer. Appearance of a reddish-brown color ring showed the positive presence of steroids.

C. Test for flavonoids

10 ml of the aqueous filtrate of leaves extract was treated with 0.5 ml of diluted ammonia (NH_4OH) solution, to this solution, 0.5 ml conc. (H_2SO_4) was added. The presence of flavonoids was revealed by the development of a yellow color. When standing, the yellow color normally vanishes.

D. Test for saponins

Frothing test, the best test for the detection of saponins was applied. 0.5 ml of sample extract was added to 5 ml of distilled water in a test tube. The solution was forcefully shaken, and the presence of saponins was detected by the stable, persistent appearance of foam.

E. Test for tannins

A 0.5 ml of extract was boiled in 10 ml of distilled water before filtering. After a few drops of 1% ferric chloride have been added (FeCl_3), brownish green or blue-black coloration established the existence of tannins.

F. Test for alkaloids

Meyer's test; 0.5 ml of sample extract was dissolved in 5 ml of 1% hydrochloric acid (HCl) and deposited in boiling water-bath, followed by filtration. A few drops of Meyer's reagent were added to 1 ml of filtrate. The presence of

alkaloids was indicated by the presence of white precipitate or turbidity.

Dry extract precipitate test: A 0.5 g of dry sample extract was treated with 4 ml of methanol, 400 ml of glacial acetic acid, and a few drops of ammonia (NH_4OH). The precipitate showed the presence of alkaloids.

G. Test for terpenoids

The Salkowski test was used to detect terpenoids in the extract. A 0.5 g of sample extract was treated with 2 ml of CHCl_3 , and then 3 ml of concentrated (H_2SO_4). A reddish-brown color at the interface established the presence of terpenoids.

High performance thin layer chromatography (HPTLC)

Phenols and flavonoids in *C. cretica* leave extract were investigated. The primary chemical was isolated on a fast liquid chromatographic (FLC) column under optimal conditions ⁽¹²⁾.

Experimental design

A- Animal grouping

Sixty apparently healthy female albino rats weighing (180–200 g) at 4 weeks of age were chosen for the study and obtained from the animal house of College of Pharmacy, University of Baghdad before being housed in the animal house of Al-Nahrain University. Animals were housed (each of ten animals) in standard metal cages, with a maintenance environmental temperature of about (22.8 ± 0.7) °C, a humidity of (41.6 ± 6.6) percent, and a diurnal change of around 12 h light/dark, and the air of the room was changed continuously by using a ventilating vacuum, and they were fed fresh water and standard chow food (ad libitum). It was important to adapt them to the animal house atmosphere for at least one week before we started working on them ⁽¹³⁾.

B- Study design

Apparently healthy sixty female albino rats were randomly assigned into six groups as following:

Apparently healthy group: Ten female rats received normal feeding for 51 days.

Induction group: Ten female rats induced PCOS by oral administration of Letrozole (5.3 mg/kg/day) for 21 days, then receiving normal feeding for 30 days.

Metformin group: Ten female rats induced PCOS for 21 days then treated by oral administration of Metformin (500 mg/kg/day) for 30 days.

High dose *C. cretica* group: Ten female rats induced PCOS for 21 days then treated by oral administration of (400 mg/kg/day) from *C. cretica* for 30 days.

Low dose *C. cretica* group: Ten female rats induced PCOS for 21 days then treated by oral administration of (300 mg/kg/day) from *C. cretica* for 30 days.

Combination group: Ten female rats induced PCOS for 21 days then treated by oral administration of (200 mg/kg/day) from *C. cretica* with (250 mg/kg/day) from Metformin as in combination for 30 days.

PCOS induction in rats

The induction was done orally with 0.4 ml of Letrozole (5.3 mg/kg) administered daily for 21 days. To approve PCOS induction, a vaginal swab was taken and analyzed every day at 9:00 am until a persistent diestrous phase was observed ⁽¹⁴⁾.

Measurement of serum lipid profile

A. Serum total cholesterol (TC) determination

Richmond's approach was used to calculate serum total cholesterol ⁽¹⁵⁾.

B. Serum triglyceride (TG) determination

The Fassati and Principe's approach was used to determine serum triglyceride levels ⁽¹⁶⁾.

C. Serum high, low and very low-density lipoprotein cholesterol (HDL-C, LDL-C and VLDL-C) determinations

Burstein's approach was used to calculate serum HDL-C levels ⁽¹⁷⁾.

A ready-made kit has been used for these purposes include Cholesterol, Triglyceride rat ELISA kit/ Linear /Spain.

Histopathological study

After animal anesthesia by chloroform in a glass jar, the animal was dissected and the ovaries were cautiously removed from the oviduct using a fine surgical scissor and forceps. The ovaries were subsequently recovered on a petri dish and cleaned of fatty tissue ⁽¹⁸⁾, histopathological changes were microscopically assessed by blind method with assistance of a qualified pathologist Dr. Esraa Mahdi Al-Sudani at the end of induction period and by assistance of a qualified pathologist Prof. Dr. Salim Rasheed Al-Obaidi at the end of treatment period.

Statistical Analysis

Statistical package for social sciences (SPSS) (version 23) was used to examine the data. Data were described as mean $\pm$ standard deviation (SD) and median with range. Unpaired ttest and analysis of variance (ANOVA), was used for normally distributed data according to Shapiro Wilk test, while Mann Whitney U test and Kruskal Walllis test utilized for not normally distributed data. The p value of 0.05 denotes the level of statistical significance.

Results

Phytochemical screening test

The test demonstrated the presence of phenols, flavonoids, alkaloids, terpenoids, saponins and glycosides with absence of steroids as shown in table (1).

Table 1. Phytochemical screening of *C. cretica* leaves extract

No.	Phytoconstituents	<i>C. cretica</i>
1	Phenols	++
2	Flavonoids	++
3	Alkaloids	+
4	Terpenoids	++
5	Saponins	+
6	Steroids	-
7	Glycosides	*

+: present, ++: present in bulk, *: present in less amount, -= absent

HPTLC for *C. cretica* leaves extract

The HPTLC analysis revealed the presence of 7 different phenols and flavonoids compounds with reference to standard, the spots are developed and detected under UV light 335 nm as shows in figures (1 and 2). HPTLC of the

extract shows 7 peaks at different Retention time (R_f) value. The second peak, which represents astragalin, is well-defined with R_f value is 3.10 and the percent area about 21.538% of the extract compositions as showed in table (2).

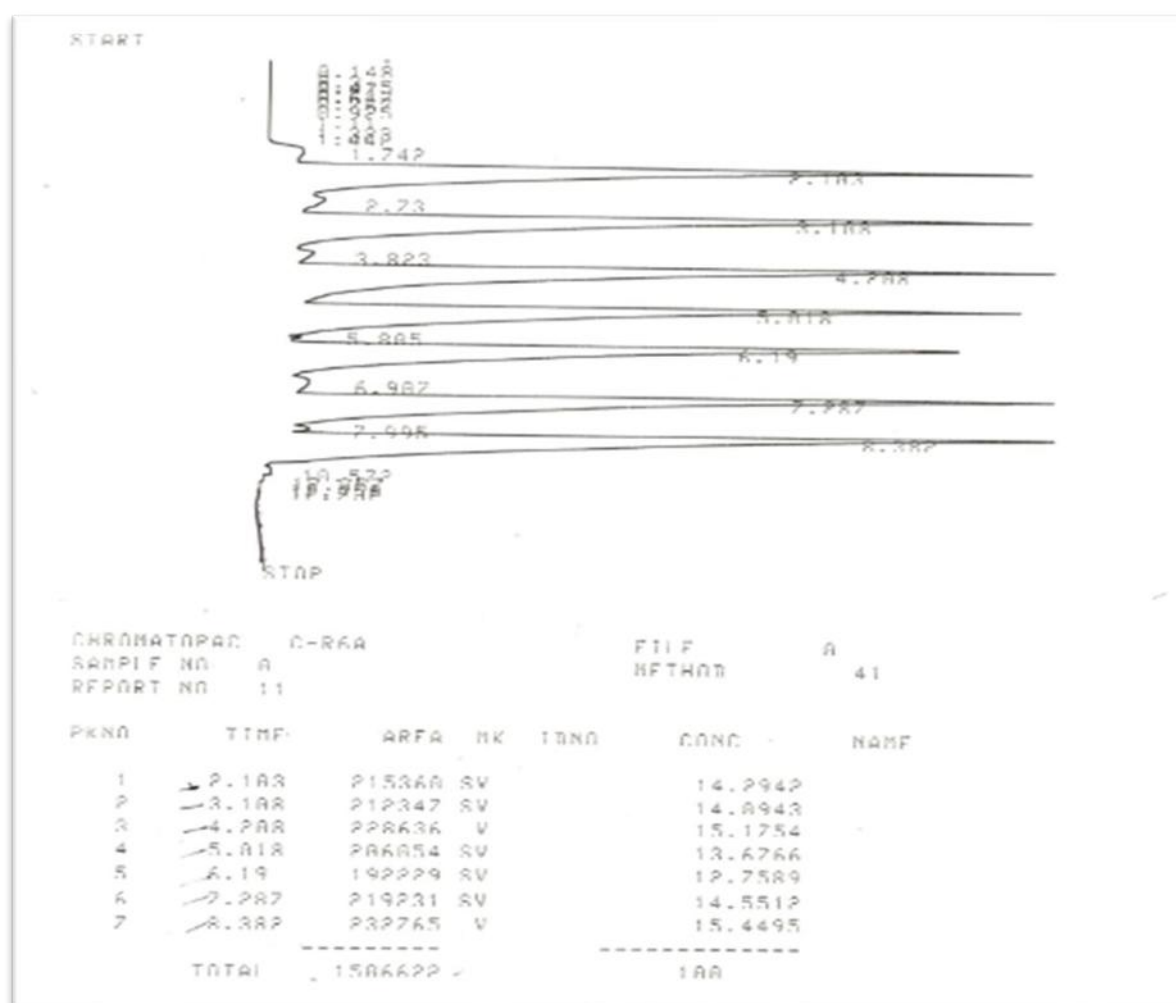


Figure 1. HPTLC of standard phenols and flavonoids

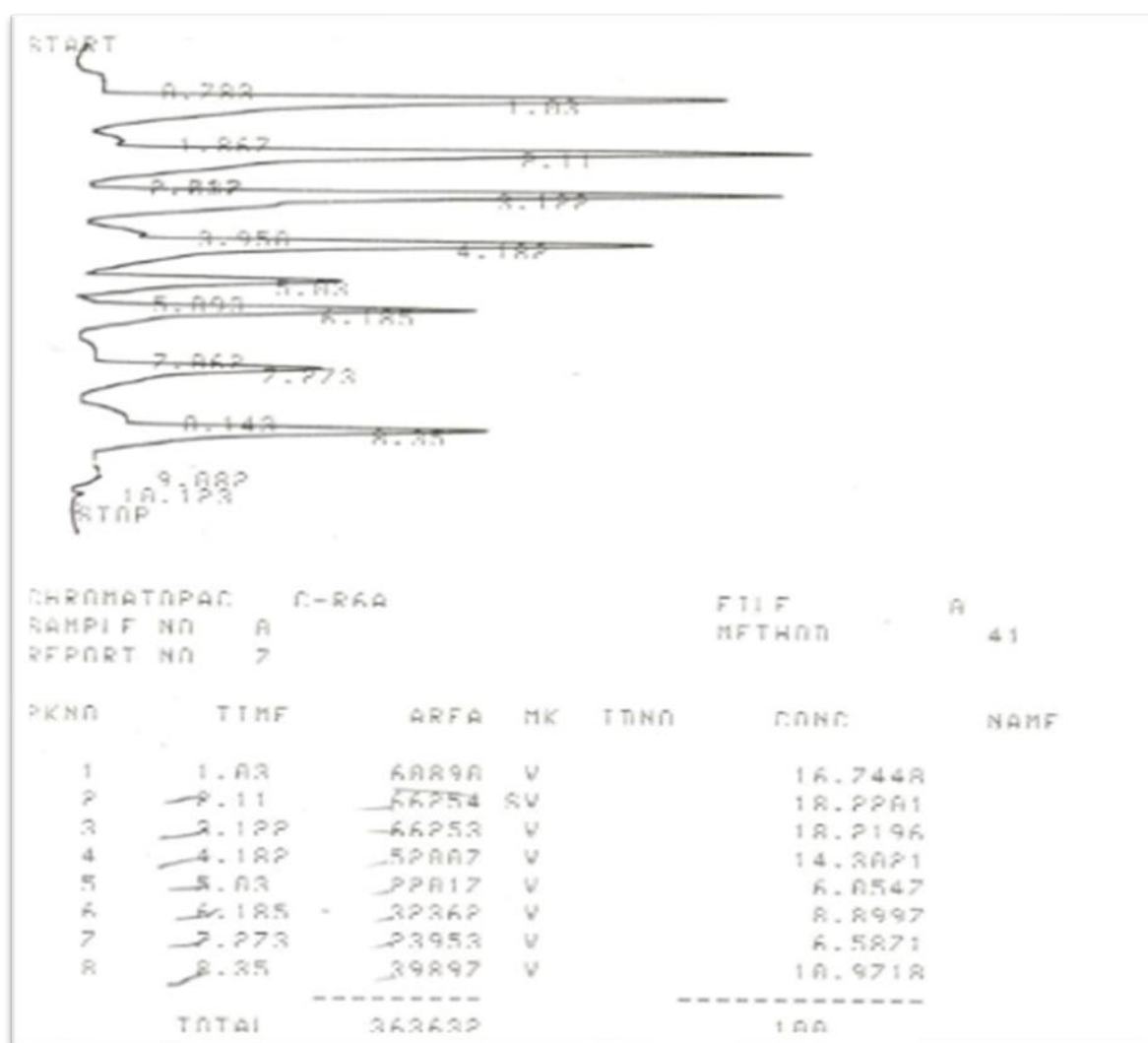


Figure 2. HPTLC of phenols and flavonoids components of *C. cretica* leaves extract

Table 2. HPTLC screening of *C. cretica* leaves extract

No.	Subjects	Retention time R _f	AUC	Conc. 25 µg/ml	Area %
1	Astragalin	3.10	212347	390.00	21.538%
2	Chlorogenic acid	2.10	215360	384.55	21.238%
3	Catechin	4.20	228636	284.32	15.702%
4	Kaempferol-3-o-rhamnogluco-side	5.01	206054	133.56	7.376%
5	Rutin	6.19	192229	210.44	11.622%
6	Isoquercetin	7.28	219231	193.59	10.691%
7	Quercetin	8.38	232765	214.255	11.833%

Effects of *C. cretica* on serum lipid profile

The levels of lipid profile were significantly higher among induction group in comparison with apparently healthy group; except that for

HDL where it was significantly lower than that of apparently healthy group, $P < 0.05$, as shown in table (3).

Table 3. Comparison between apparently healthy group and induction group regarding lipid profile

Parameter		Apparently healthy	Induction	P value
LDL (mg/dl)	Mean±SD	18.88±5.59	69.56±12.89	<0.001*
	Median (Range)	17.74 (13.28-25.63)	65.09 (53.9-85.3)	
VLDL (mg/dl)	Mean±SD	5.56±1.17	13.44±2.32	0.002**
	Median (Range)	6.17 (3.91-6.51)	14.83 (10.32-15.2)	
HDL (mg/dl)	Mean±SD	7.05±2.01	4.02±0.43	0.013*
	Median (Range)	7.85 (3.72-9.12)	4.02 (3.5-4.79)	
Cholesterol (mg/dl)	Mean±SD	31.49±4.17	87.02±14	<0.001*
	Median (Range)	30.15 (27.8-37.72)	82.12 (72.7-104.2)	
Triglyceride (mg/dl)	Mean±SD	27.79±5.87	67.19±11.58	0.002**
	Median (Range)	30.84 (19.55-32.54)	74.14 (51.62-76.02)	

LDL: Low-density lipoprotein, VLDL: very low-density lipoprotein, HDL: High-density lipoprotein. *:P value by unpaired ttest, **: P value by Mann Whitney U test

Comparison between *C. cretica* treated groups and induction group revealed that the levels of lipid profile were significantly reduced among *C. cretica* treated groups in comparison with induction group ($P < 0.05$).

When LDL, VLDL, cholesterol, and triglyceride levels were compared between Metformin treated group and *C. cretica* treated groups, it found that the levels of lipid profile were significantly lower in *C. cretica* treated groups than in Metformin treated group; while HDL was significantly higher after treatment with *C. cretica* treated group in comparison with Metformin treated group ($P < 0.05$). Lipid profile was significantly lower among high dose *C. cretica* treated group in comparison with other groups as shown in table (4).

Effects of *C. cretica* on histopathological study

The number of corpus luteum was significantly lower in the induction group compared to the apparently healthy group; opposite to number of cystic follicles, which were significantly higher in induction group ($P < 0.05$). Whereas the thickness of theca layer, thickness of granulosa layer, and number of graafian follicles were lower in induction group than apparently healthy group; but not reached the significant level. The number of atretic follicles were insignificantly higher in induction group than in apparently healthy group; $P = 0.485$ as depicted in table (5) and figure (1).

Table 4. Comparison between Metformin treated group and *C. cretica* treated groups (high dose, low dose, and in combination with Metformin) regarding lipid profile

Parameter		Metformin	High dose <i>C. cretica</i>	Low dose <i>C. cretica</i>	<i>C. cretica</i> & Metformin	P value
LDL (mg/dl)	Mean±SD	36.77±7.48	15.37±1.84	16.05±3.05	17.76±2.28	<0.001*
	Median (Range)	36.31 (26.58- 46.29)	14.9 (13.79- 18.71)	15.71 (12.08- 21.14)	17.02 (15.43- 20.83)	
VLDL (mg/dl)	Mean±SD	10.61±0.67	4.97±0.26	9.09±0.63	5.59±1.36	<0.001**
	Median (Range)	10.59 (9.79- 11.52)	4.9 (4.6- 5.33)	9.31 (7.88- 9.69)	6.12 (3.68- 6.95)	
HDL (mg/dl)	Mean±SD	2.28±1.04	6.1±1.22	5.36±1.67	5.54±1.15	<0.001*
	Median (Range)	2.34 (0.85-3.51)	6.07 (4.21- 7.79)	4.95 (3.55- 7.88)	5.59 (4.14- 6.86)	
Cholesterol (mg/dl)	Mean±SD	49.66±7.75	26.39±1.84	30.5±3.01	28.72±2.64	<0.001*
	Median (Range)	48.94 (39.58- 60.98)	26.66 (24.07- 28.42)	29.49 (27.24- 35.76)	29.51 (25.21- 31.48)	
Triglyceride (mg/dl)	Mean±SD	53.04±3.36	25.19±1.46	45.47±3.15	27.94±6.78	<0.001**
	Median (Range)	52.98 (48.97- 57.62)	25.31 (22.98- 26.66)	46.54 (39.41- 48.43)	30.6 (18.42- 34.73)	

LDL: Low-density lipoprotein, VLDL: very low-density lipoprotein, HDL: High-density lipoprotein. *:P value by ANOVA ttest, **: P value by Kruskal Wallis test

Table 5. Comparison between apparently healthy group and induction group regarding histopathological picture of ovary

Parameter		Apparently healthy	Induction	P value
Theca layer thickness (µm)	Mean±SD	17.24±13.54	0±0	0.065**
	Median (Range)	23.6 (0-28.63)	0 (0-0)	
Granulosa layer thickness (µm)	Mean±SD	24.45±18.99	0±0	0.065**
	Median (Range)	35.13 (0-38.77)	0 (0-0)	
No. of corpus luteum	Mean±SD	4±0.89	1.67±0.82	0.001*
	Median (Range)	4 (3-5)	1.5 (1-3)	
No. of cystic follicles	Mean±SD	1.33±0.82	5.17±2.14	0.005*
	Median (Range)	1.5 (0-2)	5 (3-8)	
No. of atretic follicles	Mean±SD	1.5±1.05	2.17±1.6	0.414*
	Median (Range)	1.5 (0-3)	2 (0-4)	
No. of graafian follicles	Mean±SD	0.67±0.52	0±0	0.065**
	Median (Range)	1 (0-1)	0 (0-0)	

*:P value by unpaired ttest, **: P value by Mann Whitney U test

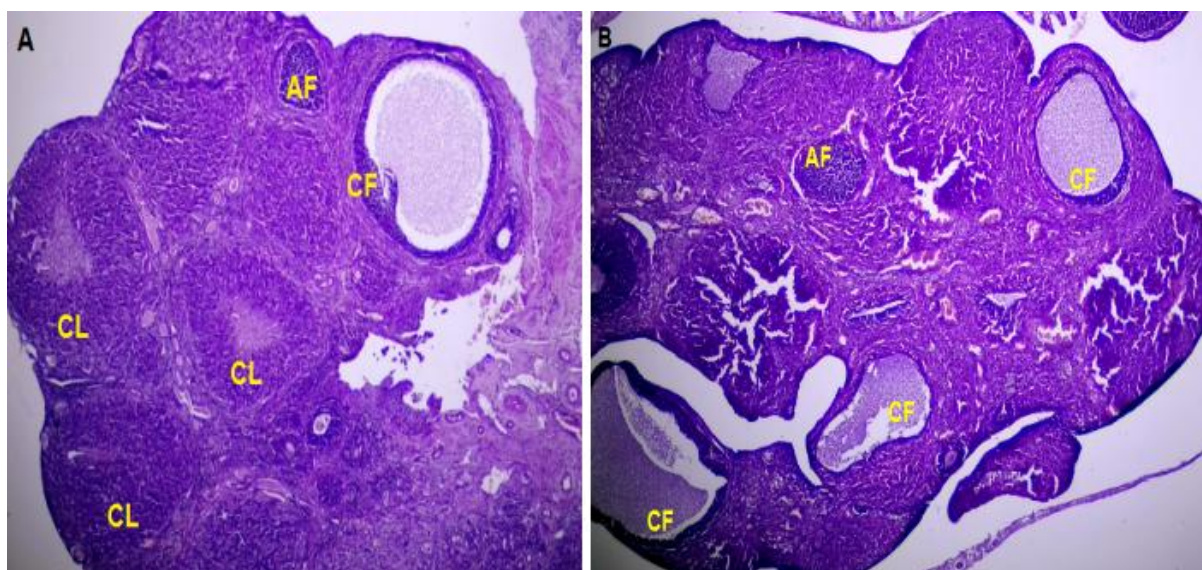


Figure 1. Ovarian indices of rats. (A) Ovary of apparently healthy group, (B) Ovary of induction group, in which CF: cystic follicles, CL: corpus luteum, AF: atretic follicles

In comparison to the induction group, the number of corpus luteum and graafian follicles was significantly higher in the *C. cretica* treated groups, whereas the number of cystic follicles was significantly lower in the *C. cretica* treated groups ($P < 0.05$).

When comparing the high dose *C. cretica* treated group to Metformin treated group, the thickness of the granulosa layer and theca layer thickness were dramatically enhanced in Metformin treated group, while the number of cystic follicles and No. of atretic follicles were decreased in high dose *C. cretica* treated group.

No significant differences were found regarding histopathological picture of ovary between low dose *C. cretica* treated group and Metformin treated group, except that of no. of cystic follicles was significantly low in low dose *C. cretica* treated group ($P > 0.05$). No. of cystic follicles was significantly decreased among combination of *C. cretica* with Metformin treated group in comparison with Metformin treated group ($P < 0.05$). Low dose *C. cretica* treated group had much thickness of granulosa layer and more no. of corpus luteum while no. of cystic follicles was lower in comparison with other groups, as shown in table (6) and figure (2).

Table 6. Comparison between Metformin treated group and *C. cretica* treated groups (high dose, low dose, and in combination with Metformin) regarding histopathological picture of ovary

Parameter		Metformin	High dose <i>C. cretica</i>	Low dose <i>C. cretica</i>	<i>C. cretica</i> & Metformin	P value
Theca layer thickness (μm)	Mean±SD	15.41±12.67	13.96±15.68	23.04±3.84	16.87±13.45	0.601*
	Median (Range)	19.77 (0-31.23)	12.03 (0-34.12)	21.63 (19.32-29.76)	22.31 (0-30.29)	
Granulosa layer thickness (μm)	Mean±SD	28.39±22.05	17.79±19.55	44.12±2.91	26.58±20.67	0.010**
	Median (Range)	41.09 (0-45)	16.89 (0-38.49)	43.65 (41.38-49.63)	38.07 (0-42.37)	
No. of corpus luteum	Mean±SD	3.17±1.47	3.83±1.17	5±1.26	3.83±0.75	0.140**
	Median (Range)	3.5 (1-5)	3.5 (3-6)	4.5 (4-7)	4 (3-5)	
No. of cystic follicles	Mean±SD	2±0.63	1.67±1.03	0.5±0.55	0.67±0.82	0.012**
	Median (Range)	2 (1-3)	1 (1-3)	0.5 (0-1)	0.5 (0-2)	
No. of atretic follicles	Mean±SD	1.67±0.82	0.83±0.75	1.5±1.38	1±0.89	0.431*
	Median (Range)	1.5 (1-3)	1 (0-2)	1.5 (0-3)	1 (0-2)	
No. of graafian follicles	Mean±SD	0.67±0.52	0.5±0.55	1.33±0.82	0.67±0.52	0.170**
	Median (Range)	1 (0-1)	0.5 (0-1)	1 (1-3)	1 (0-1)	

*:P value by ANOVA ttest, **: P value by Kruskal Wallis test

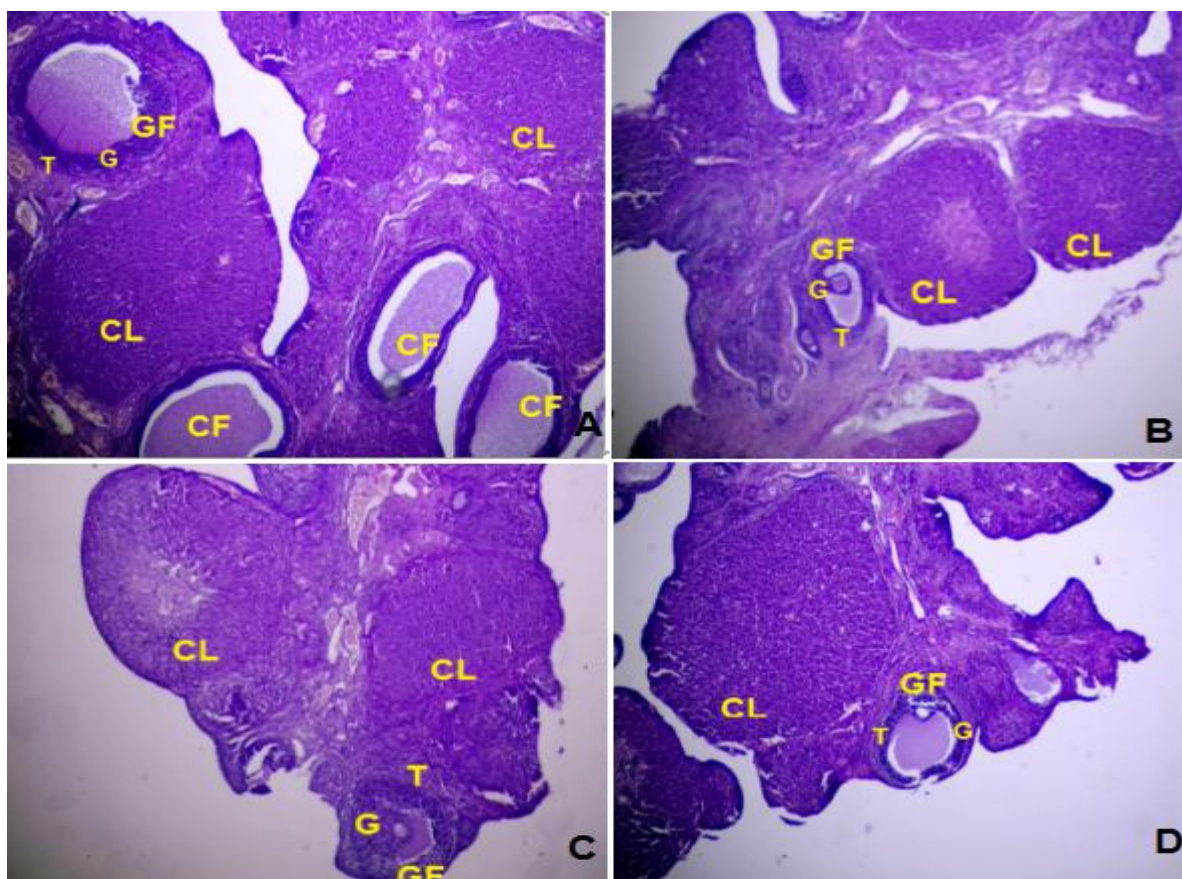


Figure 2. Ovarian indices of rats; (A) Ovary of Metformin treated group (B) Ovary of high dose *C. cretica* treated group, (C) Ovary of low dose *C. cretica* treated group (D) Ovary of combination of *C. cretica* with Metformin treated group, in which CF: cystic follicles, CL: corpus luteum, AF: atretic follicles, GF: graafian follicles, G: thickness of granulosa layer and T: thickness of theca layer

Discussion

PCOS is an ovarian dysfunction that causes a variety of metabolic and hormonal symptoms, as well as reproductive axis disruption. PCOS is categorized based on the source of the dysfunction (hyperandrogenemia, anovulation and polycystic ovarian morphology) ⁽¹⁹⁾.

Regarding cholesterol level in the current study, the induction group had significantly higher cholesterol levels than the apparently healthy group and this could be because insulin resistance can cause dyslipidemia ⁽²⁰⁾. Other lipid profile markers, such as LDL and VLDL, reveal a substantial rise when comparing the induction PCOS group to the apparently healthy group most likely as a result of excessive hypersecretion of apo-lipoprotein B and VLDL from the liver in response to insulin

stimulation, resulting in hypertriglyceridemia ⁽²¹⁾. Letrozole lowered HDL levels throughout puberty due to increased HDL catabolism ⁽²²⁾.

Treatment with Metformin in the current study resulted in significantly lower levels of biochemical markers as compared to the induction group, Metformin increases the bile acid pool within the intestine, predominantly through reduced ileal absorption, and results in the liver using more cholesterol for bile acid synthesis ⁽²³⁾. In comparison to other treated groups high dose *C. cretica* treated group showed significantly lower level of LDL, VLDL, cholesterol, and triglyceride, and show significantly higher HDL level, this could be because high dose *C. cretica* treated group contains a higher concentration of active ingredients (chlorogenic acid, rutin, catechin

and quercetin) than other treated groups. The plant extract's potential to lower LDL levels may be attributable to increased LDL receptors due to attribution of quercetin content ^(24,25). Rani et al. found the same result that *C. cretica* treated groups have significantly lower triglyceride, cholesterol, and LDL levels when compared to diabetic control groups. It may be due to the presence of phenols and flavonoids ⁽²⁶⁾.

These lipid profile could be regarded as a reflection of histopathological changes in ovarian parameters. Jahan et al. revealed that quercetin administration results in a significant healing of ovarian tissue with the formation of graafian follicles, a significant reduction in cysts, and regular luteinization. The presence of greater corpus luteum in the *C. cretica* treated groups suggested that the estrous cycle had been restored to normal functioning ⁽²⁷⁾.

In conclusion, *C. cretica* leaf extract revealed improvement in PCOS symptoms in female rats. *C. cretica* leaf extract significantly decreased serum lipid profile (LDL, VLDL, cholesterol, and triglyceride); while HDL was significantly elevated. Also, *C. cretica* leaves extract decreased atretic and cystic follicles number while increased corpus luteum and graafian follicles number and so restore ovulation. *C. cretica* treated groups observed better results in comparison with Metformin in improvement of PCOS symptoms in female rats. Also, high dose *C. cretica* treated group observed better results regarding lipid profile in comparison with other treated groups, and Low dose *C. cretica* treated group had much thickness of granulosa layer and more no. of corpus luteum while no. of cystic follicles was lower in comparison with other treated groups, this could hold a great promise for future clinical applications. *C. cretica* contain various bioactive compounds, including alkaloids, saponins, terpenes, and phenolics.

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Author contribution

Al-Kabby: performed the experimental work, data collection, statistical analysis, and manuscript preparation. Dr. Gatea supervised the study, interpreted the results, and critically revised the manuscript. Both authors approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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