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Therapeutic Effects of *Malva sylvestris* Extract on Polycystic Ovary Syndrome in a Rat Model

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Abstract

Objective: Polycystic Ovary Syndrome (PCOS) is known as one of the most usual hormone disorders in women. *Malva sylvestris* is a plant with a potent antioxidant effect. Accordingly, this research aimed to investigate the effect of this herb on ovarian folliculogenesis using a rat model of testosterone enanthate induced PCOS.

Methods: In this experiment, 35 immature female Wistar rats were used. The rats in the control group (Control) only received sesame oil as testosterone enanthate solvent, whereas the rats in the PCOS (PCOS control group) were given daily subcutaneous injections of testosterone enanthate (1 mg/100 g body weight dissolved in sesame oil) for 28 days. Rats in the extract groups were given 50 ($P+E^{50}$), 100 ($P+E^{100}$), and 200 ($P+E^{200}$) mg/Kg BW hydroalcoholic extract of *Malva sylvestris* by gavage for 4 weeks. At the end of the experiment, the animals were anesthetized, then blood samples were collected and the ovary was subjected to histological analyses. The data were analyzed using the one-way ANOVA method considering $P<0.05$/level of significance.

Results: Rats treated with testosterone enanthate (PCOS control group) demonstrated a significant increase in serum testosterone, and LH, and a significant decline in progesterone. Also, disturbing ovarian cyclicity in addition to histopathological alterations, including decreased number of healthy follicles and corpora lutea, increased degenerated, and cystic follicles were detected by light microscopic studies. Rats treated with an extract of *Malva sylvestris* ($P+E^{50}$; $P+E^{100}$; $P+E^{200}$) showed a remarkable reversal in the levels of parameters affected by testosterone enanthate treatment.

Conclusion: In conclusion, *Malva sylvestris* extract may be an effective therapeutic candidate for the treatment of PCOS. It seems that the hydroalcoholic extract of *Melia azedarach* L. seed may lead to a normal ovarian cycle by reducing the androgen concentration.

Keywords: Ovary; PCOS; Wistar rats; Testosterone enanthate; *Malva sylvestris*

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Abbreviations

PCOS: Polycystic Ovary Syndrome; DHEA: Dehydroepiandrosterone; PVC: Persistent Vaginal Cornification; IM: Intramuscular

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common heterogeneous and least understood endocrine disorder [1] which causes an ovulation in animals and women of reproductive age [2]. The prevalence rate of this disease was reported 5.6% to 8% in Europe [3]. According to the latest studies, the prevalence of PCOS in Iran is 19.5% based on the Rotterdam criteria and 6.8% based on the NIH criteria [4]. It is characterized by endocrine, metabolic, and genetic disorders, clinical and biochemical presentations of hyperandrogenism [5], insulin resistance and hyperinsulinemia [6]. This disease represents a condition in which an estimate of 10 small cysts of a diameter ranging between 2 and 9 mm and small subcortical follicles [5] develop on one or both of the ovaries and/or the ovarian volume in at least one of the ovaries exceeds 10 ml in women with enlarged polycystic ovaries. Its characteristic neuroendocrine features include increased serum concentration of Luteinizing Hormone (LH), increased LH/FSH ratio, and increase in amplitude and frequency of pulsatile LH secretion [7]. The exact pathophysiology of PCOS is uncertain, evidence suggests that an excess of ovarian androgen production, either genetically or due to extra-ovarian factors such as hyperinsulinemia or disturbances of the hypothalamic–pituitary–ovarian axis is the main

cause in the pathogenesis of PCOS [8]. Although the etiology of PCOS is still not determined, studies suggest that oxidative stress and inflammation may play an important role in disturbing of metabolic and reproductive found in PCOS [9-11]. The intra-ovarian hyperandrogenism may be the main culprit for follicular excess, anovulation, and atresia of follicles in polycystic ovaries [12]. Understanding the pathogenesis of PCOS could help to make a more effective treatment. Therefore, to study PCOS, animal models are required that have similarities to humans [13]. In this case, exposure of sheep and primates to testosterone has developed models that show similarities to PCOS women such as polycystic ovaries, anovulation, insulin resistance and LH hypersecretion but both models have long developmental periods and are expensive whereas mouse models have a short reproductive lifespan and genetic manipulations are feasible [14-16].

Various experimental models for PCOS have been developed in rats like administration of Estradiol Valerate, Dehydroepiandrosterone (DHEA), and prepubertal androgen excess [17]. Even though these models induce PCOS, none of them are fully convincing and identify with the conditions of human PCOS completely. The administration of androgens (testosterone, testosterone propionate, androstenedione) is a useful tool to generate animal models developing a type of physiopathology similar to that observed in women with polycystic ovary syndrome [18]. Testosterone enanthate is an anabolic steroid with androgenic properties which has been considered as a PCOS inducer [19].

Currently, clomiphene citrate, metformin, and tamoxifen are the most widely used drugs to treat PCOS [20]. With regards to the side effects of such drugs and the relative treatment of PCOS by them, it is essential to identify and develop alternative drugs [21], out of which plant-based drugs are considered to be comparatively more effective [22]. Plants, especially medicinal plants are very useful for curing various disease ailments for humankind [23]. Herbal medicines have long been used for the treatment of female reproductive disorders as they contain pharmacologically active components showing promising effects in patients with PCOS, diabetes, and cardiovascular diseases [24]. *Malva* (*Malva sylvestris* L.), a traditional medicinal plant, was used in traditional phytotherapy and cosmetic treatments [25]. In Iran, *Malva sylvestris* is known as "Panirak" in folklore. *Malva sylvestris* has high mucilage content and polysaccharides that are used for many purposes [26-28]. This plant exhibits antioxidant, anti-inflammatory, anticancer, wound healing, hepatoprotective, antinociceptive, and antimicrobial activities [29]. DellaGreca et al. reported strong antioxidant activity of the extract and also isolated eleven compounds responsible for the activity [30]. studied the antioxidants and free radical scavengers as well as the anti-inflammatory effects of different parts extracted from *M. sylvestris* (leaves, flowers, immature fruits, and leafy flowered stems). *M. sylvestris* leaves revealed very strong antioxidant properties including radical scavenging activity, reducing power and lipid peroxidation inhibition in liposomes and brain cells homogenates. The biological activity of this plant may be attributed to its richness in antioxidants [31]. Phytochemical studies on mallow have shown that its various parts contain flavonoids [30], terpenoids [32], polyphenols, phenol derivatives [30,32], polysaccharides [27], Mucilages, coumarins [33], vitamins C and E, beta-carotene and other important phytochemicals [30]. Major flavonoids constituents of Gossypetin 3-sulfate-8-O-β-D-glucoside, hypolaetin 3'-sulfate, three 8-hydroxyflavonoids, terpenoids such as sesquiterpenes, diterpenes, and monoterpenes were identified in *M. sylvestris* [32,33]. Besides, this plant also appears to have therapeutic properties [34,35]. It can have anti-diabetic [36], anti-inflammatory [37], anti-ulcerogenic [38], and anti-oxidant effects [30,39]. Indeed, *Malva sylvestris* leaf extract is known for its ability to scavenge Reactive Oxygen Species (ROS), exert a neuroprotective effect by reducing lipid per-oxidation levels in the kidney and enhance the efficiency of the endogenous antioxidant system.

With regards to the increasing prevalence of PCOS and associated physical and mental problems as well as the effects of changes in sex hormones in the development of this disease, we aim to investigate the protective effects of *Malva sylvestris* on (*Malva sylvestris* L.) on testosterone enanthate induced Polycystic Ovarian Syndrome (PCOS) in the rat.

Materials and Methods

Animals

This study was conducted on 35 immature female Wistar rats weighing 150 ± 10 gram (7 to 8 wk. of age). The animals were obtained from Pasteur Institute in Tehran, Iran. Then housed in clean and sterilized polypropylene cages at room temperature (22 ± 2°C), humidity at 45% to 55% with 12-h light and dark cycles. Animals were provided with standard commercial diet (Pars Food Com., Iran) and water ad libitum. The rats under study were maintained for at least 7 days in the above-mentioned conditions before drug administration, so that they can completely get accustomed to the environment. All procedures were carried out according to the Guide for the Care and Use of Laboratory Animals (Razi University Research Committee). The study was started after obtaining the ethical clearance by the Razi university Ethics Committee (RUEC) (Reg. no. IAEC/11/07/IA dated 19/11/2014).

Preparation of hydroalcoholic extract of *Malva sylvestris* L.

Malva sylvestris L. extraction was prepared using Mirazi et al. method [40]. The leaves of this plant were collected from Kermanshah province, Iran and they were identified with the help of experts in the botany Laboratory of Razi University. Then the leaves dried in shade and subsequently grinded. The powder of dried leaves of *Malva sylvestris* L. was mixed with distilled water and ethanol 80% (1:8), and the mixture was put on the shaker (KS500, Germany) for 48 h at room temperature. The resulting hydro-alcoholic extract was filtered through Whatman filter paper (No. 4) and evaporated to dryness on a rotary evaporator (LABOROTA 4001 Efficient, Germany Heidolph) at 45°C until it became creamy. Then, the extract was transferred to glass containers and in order to evaporate the remaining solvent, they were kept without the lid in the oven for 48 h at 30°C to 40°C. For subsequent usages, the extract was kept at the 4°C. Finally, the extract was dissolved in normal saline and stock solutions with different concentrations (50, 100, 200 mg), were prepared.

Testosterone enanthate-induced polycystic ovarian syndrome

The method used in this project for inducing polycystic ovarian syndrome was hormonal induction using testosterone enanthate (1 mg/100 g body weight dissolved in sesame oil). To conduct this procedure, rats were given daily subcutaneous injections of testosterone enanthate (Samisaz Co. Iran) for 28 days. Then, daily vaginal smear test was made [41]. This process continued until variations in the estrous cycle as well as irregular estrous cycles were observed. It should be noted that this process also continued until the

onset of the advance of the Persistent Vaginal Cornification (PVC) step was seen.

Vaginal smear observation

At the end of injections of testosterone enanthate, vaginal secretions were collected with a plastic pipette by inserting the tip in to the rat vagina, filled with 10 µL of normal saline. One drop of collected vaginal fluid was placed on glass slides. A separate glass slide and pipette tips were used for each animal. Collected vaginal fluid was fixed by placing the slides on a slide warming table and stained with methylene blue (aqueous) staining solution. After staining, slides were washed, dried, and observed through an alight microscope (40x). The smears were classified as 1 of the 4 stages of the estrous cycle as reported earlier [42,43]. Briefly, three types of cells could be recognized: Round and nucleated ones were epithelial cells, irregular ones without nucleus were cornified cells, and the little round ones were leukocytes. The proportion among them was used for the determination of the estrous cycle. When the smear consisted of a predominance of nucleated epithelial cells it is considered as proestrus phase. A smear primarily consisting of a nucleated cornified cells is considered as estrous phase. A smear consisting of the same proportion among leukocyte, cornified and nucleated epithelial cells were considered as meta-estrous phase. A smear primarily consisting of a predominance of leukocytes is considered as diestrus phase.

Experimental groups and study design

In this experimental, 35 immature female Wistar rats were divided into 5 groups (n=7/group). The rats in the control group (Control) only received Intramuscular injection (IM) of 0.2 ml of sesame oil (as solvent of testosterone enanthate) dissolved in normal saline for 4 weeks, whereas the rats in the PCOS group (PCOS control group) were given daily subcutaneous injections of testosterone enanthate (1 mg/100 g body weight dissolved in sesame oil) for 28 days. Rats in the extract groups which were given 50 ($P+E^{50}$ group), 100 ($P+E^{100}$ group), and 200 ($P+E^{200}$ group) mg/Kg BW hydroalcoholic extract of *Malva sylvestris* by gavage for 4 weeks. After the treatment period, all rats were anesthetized with ketamine/xylazine (5/1 mg/kg), then the blood samples were taken from the hearts of all rats and the serum was separated by centrifugation at 3500 g. The obtained sera were kept at -70°C until the further analysis. Then, mice were sacrificed and their ovaries were collected for stereological analysis.

Histological study

The excised ovaries from all experimental and control groups were immediately fixed in Bouin's solution. After 24 h fixation, ovaries were dehydrated in ascending series of ethanol, cleared with xylene and embedded in paraffin wax. Isotropic uniform random sections were obtained using the "isector" method [44]. In this method, the filled spherical modules with paraffin are used to insert each ovary. Then modules were rotated randomly manner and 5 and 20 µm thick sections were prepared using a microtome. Sections were stained with hematoxylin and eosin and 12 sections per animal were examined stereologically.

Estimating the total volume of the ovary, follicles and CL

Using the Cavalieri method the total volume of the cortex and medulla as well as different follicles and corpus luteum was estimated [44]. Twelve sections from 5-µm thick sections were transferred to the working table using the micro-projector with 4x magnification. The resulting points from the randomly superimposed probe on the images were then counted and the total volume of the ovary was estimated using the following formula:

$$Vv = \sum P(structure) / \sum P(total)$$

Where $\sum P(structure)$ = the total number of points superimposed on the image, and $\sum P(total)$ = the total number of points superimposed on tissue section.

The volume density of each ovary compartment was calculated as follows:

In turn, the volume density (*Vv*) was multiplied by the total volume of the ovary to estimate each ovary compartment [44].

Estimating the number of corpus luteum and different follicles

To estimate the number of follicles, the optical dissector method was used. Using systematic random sampling 12 sections were selected out of 20-µm thick sections and were studied by a microscope with 100x magnification. Using a microactuator connected to a computer and microscope, the movement of the stage in the z-axis was measured. To avoid cutting artifacts, 5 µm from the bottom and top of the sections were ignored as a guard area. Any nucleus that lay in the frame and had no contact with the lines of the frame was selected. Different types of follicles were identified based on [45]. The number density (N_v) of different types of follicles was estimated as:

$$Nv = \frac{\sum Q}{a(frame) \times h \times \sum P}$$

ΣQ is the total number of the counted follicles in the dissector height (h), a (frame) is the frame area in the true tissue scale and ΣP is the total number of the points superimposed. The total number of the follicles was estimated by multiplying the numerical density (N_v) by the total volume (V_{total}) of the ovary [44]:

$$N_{total} = N_V \times V_{total}$$

Measurement of the Serum Levels of Testosterone, Estrogen, FSH, LH, and Aromatase

At the end of experimental period, serum hormone levels were quantified using ELISA kits for testosterone and progesterone (Demeditec, Germany). The absorbance was recorded at 405 nm for analyzing testosterone and progesterone. Correspondingly, the serum levels of LH were also determined using an ELISA kit (Cusabio, China).

Statistical analysis

Hormonal and histological results obtained from the studies using the rats in all groups were compared by SPSS software and One-Way ANOVA and Tukey post hoc test. The results were expressed as mean ± standard error (Mean ± SEM). The level of significance was considered as $P<0.05$.

Results

Vaginal cytology

We conducted vaginal swab examination (Figure 1) before and after the treatment. A vaginal swab before treatment showed that the female rats were in the estrous cycle, suggesting they are in the normal reproduction phase. In contrast, vaginal swab after treatment with testosterone enanthate was mostly on the diestrus cycle.

The serum levels of testosterone, progesterone, and LH

In PCOS control group, the serum levels of T (ng/ml) and LH (ng/ml) were remarkably increased ($P<0.05$) while those of P4 (ng/ml) significantly decreased ($P<0.05$) compared to the control group.

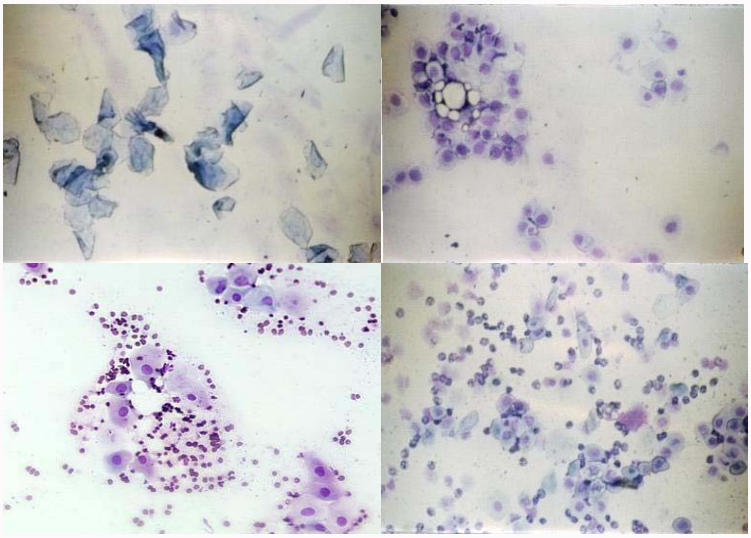

Figure 1: Vaginal smear of rats for detection of reproductive cycle (Giemsa staining).

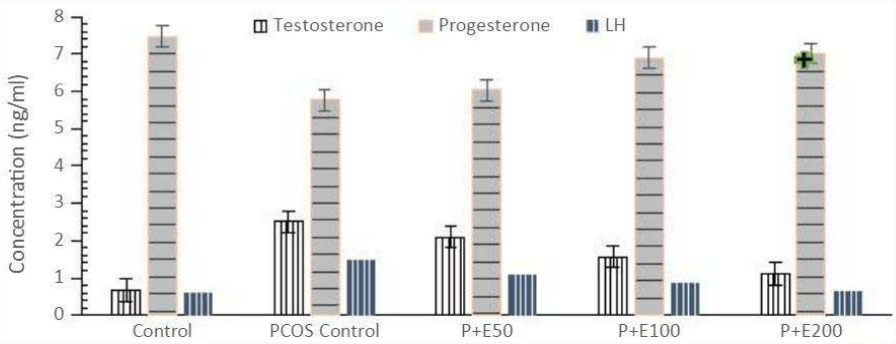

Figure 2: Serum Level of testosterone, LH, and progesterone in different experimental groups. The symbol of shows significant difference with control group and the symbol of means the significant difference with PCO group (One-way ANOVA and Tukey's test, P<0.05).

Table 1: Comparing the mean number of primary, secondary, antral, cystic and atretic follicles and corpus luteum number in the study groups.

Variables	Experimental Groups				
	Control	PCOS-control	P+E^{50}	P+E^{100}	P+ E^{200}
Primary Follicle	51.67 ± 9.64^{c}	88.00 ± 5.72^{a}	87.67 ± 13.14^{a}	74.33 ± 18.88^{b}	66.83 ± 9.6^{b}
Secondary Follicle	39.83 ± 10.22^{c}	75.17 ± 10.02^{a}	68.50 ± 14.95^{b}	65.50 ± 14.32^{b}	59.17 ± 6.68^{b}
Antral Follicle	9.83 ± 1.32^{a}	1.83 ± 1.47^{b}	7.83 ± 2.63^{a}	8.83 ± 4.70^{a}	10.33 ± 1.36^{a}
Corpus Luteum	22.67 ± 8.22^{a}	7.83 ± 4.62^{c}	13.50 ± 5.68^{b}	10.33 ± 3.98^{b}	16.67 ± 9.60^{a}
Cystic Follicle	8.83 ± 3.50^{c}	28.67 ± 6.37^{a}	20.00 ± 9.69^{ab}	18.33 ± 7.71^{b}	15.50 ± 8.16^{bc}
Atretic Follicle	23.00 ± 8.92^{b}	34.50 ± 12.88^{a}	31.33 ± 6.62^{a}	24.83 ± 7.05^{b}	19.67 ± 3.38^{b}

Values are means ± S.E. $^{a, b, c}$ The means with different code letters are significantly different (one-way ANOVA and Tukey's test, P<0.05)

A significant fall (P<0.05) in T and LH levels and significant raise in P4 level were observed in rats treated with hydroalcoholic extract of *Malva sylvestris* L. at 100 and 200 mg/kg (Figure 2).

Number of follicles

The number of primaries secondary, antral, cystic, and atretic follicles and yellow bodies is presented in Table 1. Counting the number of follicles in various stages showed that the injection of testosterone enanthate increased the number of primary follicles, secondary follicles, atretic follicles, and cystic follicles; however, it decreased the number of antral follicles and corpus luteum compared to the control group. Treatment with hydroalcoholic extract of *Malva sylvestris* L. at 50 mg/kg did not exhibit any significant change in primary, atretic, and cystic follicles numbers in comparison to the PCOS control group. However, treatment with hydroalcoholic extract of *Malva sylvestris* L. at 100 and 200 mg/kg significantly increased the number of antral follicles and corpus luteum and significantly decreased the number of primary, secondary, atretic, and cystic follicles (Table 1 and Figure 3).

Ovarian histological aspects

The mean weight of the ovary increased in the PCOS control group in comparison to the control group. Treatment by hydroalcoholic extract of *Malva sylvestris* L. at 50, 100 and 200 mg/kg (p<0.05) exhibited a significant decrease in ovary weight in comparison to the PCOS control group ovaries (Table 2).

Table 2: Comparison of the ovary weight and mean total volume of cortex, and medulla of ovary and primary, secondary, antral, cystic and atretic follicles and corpus luteum in different experimental groups.

Variables	Experimental Groups				
	Control	PCOS-control	P+E^{50}	P+E^{100}	P+E^{200}
Ovary weight (mg)	24.40 ± 0.03^{b}	31.00 ± 0.07^{a}	25.7 ± 0.04^{b}	23.90 ± 0.01^{b}	22.7 ± 0.02^{b}
Cortex Volume (µm^3)	7.20 ± 0.01^{b}	8.90 ± 0.02^{a}	8.70 ± 0.01^{a}	5.50 ± 0.02^{b}	7.70 ± 0.02^{b}
Medulla Volume (µm^3)	3.60 ± 0.07^{a}	4.10 ± 0.06^{a}	2.30 ± 0.08^{b}	1.80 ± 0.09^{b}	2.20 ± 0.08^{b}
Primary Follicle Volume (µm^3)	2.70 ± 0.08^{c}	5.30 ± 0.08^{a}	4.00 ± 0.06^{b}	2.90 ± 0.05^{c}	2.90 ± 0.07^{c}
Secondary Follicle Volume (µm^3)	1.20 ± 0.09^{b}	2.20 ± 0.09^{a}	1.30 ± 0.05^{b}	1.00 ± 0.07^{b}	1.08 ± 0.05^{b}
Antral Follicle Volume (µm^3)	9.60 ± 0.07^{a}	0.60 ± 0.01^{b}	5.30 ± 0.05^{a}	6.10 ± 0.02^{a}	6.80 ± 0.01^{a}
Atretic Follicle Volume (µm^3)	1.30 ± 0.01^{c}	3.90 ± 0.01^{a}	2.40 ± 0.05^{b}	2.30 ± 0.03^{b}	1.80 ± 0.01^{b}
Cystic Follicle Volume (µm^3)	0.10 ± 0.01^{c}	2.80 ± 0.01^{a}	1.30 ± 0.01^{b}	1.50 ± 0.05^{b}	1.20 ± 0.04^{b}
Corpus Luteum Volume (µm^3)	5.20 ± 0.04^{a}	0.70 ± 0.01^{c}	2.40 ± 0.02^{b}	2.80 ± 0.01^{b}	2.80 ± 0.06^{b}

Values are means ± S.E. $^{a, b, c}$ The means with different code letters are significantly different (one-way ANOVA and Tukey's test, P<0.05)

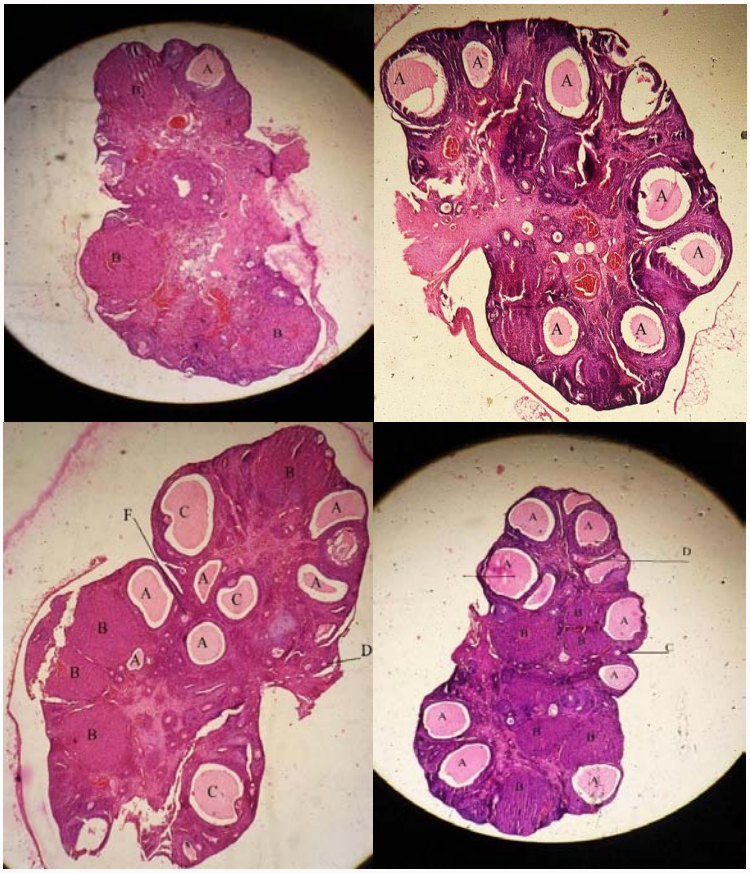

Figure 3: Hematoxylin and eosin-stained sections of ovaries from rats of different experimental groups.
A: Cystic Follicle; B: Corpus luteum; C: Preovulatory Follicle; D: Primary Follicle; F: Secondary Follicle.

The volume of the ovary, cortex and medulla

The mean volume of the cortex increased significantly in the PCOS control group compared to the control group (p<0.05). The mean volume of the medulla in the PCOS control group showed no significant difference (p>0.05) compared to the control group (Table 2). Treatment with an extract of *Malva sylvestris* L. (50 mg/kg) did not exhibit any significant change in the mean volume of the cortex compared to the PCOS control group. However, treatment with an extract of *Malva sylvestris* L. at 100 and 200 mg/kg exhibited a significant decrease in the mean volume of the cortex. Also, the mean volume of the medulla significantly decreased in groups treated with all three doses of *Malva sylvestris* L. extract (50, 100 and 200 mg/kg) in comparison to the PCOS control group. The mean volume of primary follicles, secondary follicles, atretic follicles, and cystic follicles increased significantly in the PCOS control group compared to the control group (p<0.05). However, the mean volume of antral follicles and corpus luteum decreased in the PCOS control group compared to the control group (p<0.05). Treatment with hydroalcoholic extract of *Malva sylvestris* L. at all three doses of 50 mg/Kg, 100 and 200 mg/Kg decreased the mean volume of primary follicles, secondary follicles, atretic follicles, and cystic follicles and increased the mean volume of

antral follicles and corpus luteum compared with the PCOS control group.

Discussion

In the present study, injections of testosterone enanthate in immature female rats induced reproductive and endocrine characteristics of PCOS such as hyperandrogenemia, LH hypersecretion and multiple cysts. Our results revealed that ovaries in rats treated with testosterone enanthate did not display a regular estrous cycle and exhibited a persistent diestrus phase and manifested follicular cysts which are consistent with the previous studies [46,47]. The rats with PCOS did not exhibit a regular estrus cycle and had a prolonged diestrus phase [48]. The changes in the rat estrous cycle may be linked to alterations in the circulating concentrations of the sex hormones and gonadotrophins. These hormones control ovarian function, including follicular maturation and hormonal imbalance which would have led to an irregular estrous cycle thus affecting ovarian function [42]. In the present study, there was a significant elevation in serum Testosterone (T) levels in the PCOS control group compared to the control group. This increased concentration of testosterone in peripheral blood can be the reason for prolonged diestrus of PCOS control rats in the study [49]. The testosterone administration in female rats interfere with the reproductive function of the female rat [50]. This interference is a change in the normal morphology of the reproductive tract or a disturbance in the duration of particular phases of the estrous cycle. The current study supports the same statement. The changes in the estrous cycle are predominantly seen in the testosterone enanthate-induced group. In this study, the female rats of the control group displayed a regular estrous cycle. Treatment with an extract of *Malva sylvestris* L. gradually reversed the diestrus phase to a normal estrous cycle and resulted in a decreased percentage of vaginal diestrus days which might be due to their ability to decrease testosterone concentration in the peripheral blood. The serum concentrations of T in the PCOS groups treated with hydroalcoholic extract of *Malva sylvestris* L. at 100 and 200 mg/kg significantly decreased.

Data showed that after induction of PCOS, serum Testosterone (T) and LH levels increased, while Progesterone (P4) levels were decreased in the PCOS control group compared to the control group. These findings were in line with those obtained by some researchers [51]. Increased plasma levels of androgen and LH were the most consistent hormonal feature of rats with PCOS [52], and low levels of progesterone were also observed in rats with PCOS [52]. High levels of LH can serve as biomarkers to diagnose PCOS in women [53]. As evidenced, there was a marked increase in T levels when compared to control animals indicating the hyperandrogenism status in the PCOS condition. In a previous study, excessive testosterone was shown to contribute to the pathogenesis of PCOS, and repression of these high levels of testosterone may have beneficial effects on disorders in PCOS [52,54]. High T levels presumably reflected the accumulation of androgens possibly due to the blockade of the conversion of androgen substrates into estrogens. PCOS is also associated with oxidative stress which leads to increased androgen production [55]. Serum level of P4 was decreased in PCOS control group. These results are following the earlier studies [56-59]. Decreased progesterone levels are also indicative of anovulation [60]. In PCOS control group, the elevated LH levels are explained by an increased pituitary sensitivity to hypothalamic Gonadotropin-Releasing Hormone (GnRH), and increased pulse frequency of GnRH which may cause the production of LH [61]. In addition, increased levels of LH lead to an increase in the production of androgens from the theca cells [62,63]. LH stimulation led to androgen hypersecretion by increasing the expression of the key enzymes in androgen synthesis. Compared to the PCOS control group, the serum concentrations of LH, and T in the PCOS groups treated with the hydroalcoholic extract of *Malva sylvestris* L. at 100 and 200 mg/kg significantly decreased; however, the level of progesterone significantly increased. Therefore, hydroalcoholic extract of *Malva sylvestris* L. at 100 and 200 mg/kg was able to reduce significantly serum T and LH levels and increase P4 level compared to the PCOS control group. It can stem from the effects of the hydroalcoholic extract of *Malva sylvestris* L. on serum testosterone concentrations which possibly pertained to the presence of flavonoid, and saponins in *Malva sylvestris* L. It has been indicated that these compounds can prevent the hyperandrogenism [64]. Earlier reports indicate that extract of *Malva sylvestris* L. has decreased testosterone levels by 5a-reductase inhibitory and androgen receptor binding inhibitory activities [65].

Regarding ovarian histomorphology, induction of PCOS resulted in a significant reduction in the number of antral follicles and yellow bodies and increase in the number of primary, secondary, atretic and cystic follicles compared to the control group. These phenomena might originate from hyperandrogenism that leads to the generation of cystic follicles and the reduction of the number of normal follicles [66]. Manneras et al. [67] reported that PCOS increased the number of atretic antral follicles and decreased the number of healthy antral follicles. Moreover, Badawy et al. [68] reported that PCOS caused a reduction in the number of healthy follicles in rats.

Calabro et al. [69] Showed that after induction of PCOS, follicle growth and development in the experimental group decreased compared to the control group. Also, Desjardins et al. [70] reported that multiple cysts were generated in the ovaries after the induction of PCOS in rats. The origin of these cysts was atretic follicles. These results are consistent with the findings of the present study [69]. The HPG axis dysfunction in PCOS may lead to the increase of GnRH, and improper secretion of LH and FSH causes disruption of ovarian function and the growing of more preantral follicles, which may not convert to antral follicles [71]. Hyperandrogenism also accelerates early follicular growth leading to the excess of primary and preantral follicles [72], which is in agreement with the results obtained by this study. Low serum progesterone levels in PCOS were associated with reduction of corpora lutea because the follicles don't release the egg and become cysts in these ovaries [73]. In polycystic ovaries, the number of antral follicles is lower than in normal ovaries. There is a positive correlation between high levels of androgen and preventing small follicles to become mature. These high androgen levels and follicular cell stimulation by LH might produce high levels of Cyclic Adenosine Monophosphate (CAMP) in the granulosa cells, which causes premature terminal differentiation and arrest follicular growth [74]. Treatment with hydroalcoholic extract of *Malva sylvestris* L. improved the ovarian condition. In other words, the hydroalcoholic extract of *Malva sylvestris* L. is somewhat effective in the growth and development of follicles, and corpus luteum and in reduction of the atretic and cystic follicles after induction of PCOS. The results showed that treatment with hydroalcoholic extract of *Malva sylvestris* L. could increase the number of follicles, and corpus luteum and reduce the number of the atretic and cystic follicles compared to the PCOS group. These findings are in agreement with the results of some other useful herbal extracts for PCOS treatment [75-78]. The

effects of *Malva sylvestris* L. at 100 and 200 mg/kg on the growth and development of follicles, and corpus luteum and reduction of the atretic and cystic follicles may be due to anti-hyperandrogenic properties of this herb [64].

The mean ovarian weight in the PCOS control group significantly increased compared to the control group ($p<0.01$). Increasing the level of follicular fluid and ovarian stroma could lead to ovarian weight gain in the PCOS control group compared to the control group. However, the mean of ovarian weight in the PCOS groups treated with hydroalcoholic extract of *Malva sylvestris* L. at all the three doses of 50, 100 and 200 mg/kg significantly decreased in contrast to the PCOS control group ($p<0.005$) (Table 1). Our results demonstrated that the mean total volume of the cortex, increased in the PCOS control group in comparison to the control group, while the mean total volume of the medulla showed no significant difference compared to the control group. This could be due to an increase in the number and volume of primary, secondary, atretic, and cystic follicles which occurs in the PCOS control group. The antioxidant activity of the high flavonoid content of the *Malva sylvestris* extract may be involved in the protective effect of this plant against the hormonal and ovarian morphology disturbances associated with PCOS.

Conclusion

These results suggest that *Malva sylvestris* may be a potent therapeutic for the treatment of hormonal disturbances associated with PCOS and that this substance has potential applications as a functional food for women. The results presented in our report suggest that the *Malva sylvestris* extract has numerous beneficial effects on hormonal changes in rats with PCOS, and these changes significantly aid the recovery of the estrus cycle, the number of developed follicles and ovarian morphology in rats with PCOS. However, *Malva sylvestris* extract contains various compounds, and further studies on fractions from partial purification of the extract are required to identify the active pharmaceuticals among the phlorotannin components. These findings have uncovered a potential treatment for PCOS; therefore, we believe that the *E. cava* extract could be useful for managing PCOS in women.

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