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RESEARCH ARTICLE

Potential effects of *Mentha piperita (*peppermint*)* on Letrozole- induced polycystic ovarian syndrome in female albino rat

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Abstract

Polycystic Ovary Syndrome (PCOS) is a common condition of infertility in females and its major hallmark features are hyperandrogenemia and hyperinsulinemia. *Mentha piperita* (peppermint) has antiandrogenic properties in both animals and women. This study aims to evaluate the protective role of peppermint on Letrozole induced PCOS in female rats. 40 Wistar female rats were divided into 5 groups of 8 each. G1: served as a control group. G2 (vehicle):rats were daily received oral doses of 2ml/kgb.w. of 1% carboxymethylcellulose for 3 weeks. G3: rats were daily administered oral doses of 1mg/kg b.w. of letrozole for 3 weeks. G4: rats were treated with letrozole for 3 weeks to induce PCOS & supplemented with peppermint (40g/L) for further 3 weeks. G: rats were treated with letrozole plus peppermint for 3 weeks. After the treatment, the rats were killed; uteri and ovaries then excised and weighed. Serum hormone levels and histological changes in ovaries & uteri were examined. The results revealed that PCOS-females exhibited marked alterations in serum testosterone, estrogen, LH and FSH activity. PCOS group showed ovarian cysts with a diminished granulosa layer, atretic follicles and a few number of corpora lutea. Also, PCOS group induced massive alterations in the uterine tissue manifested by necrosis in stromal mesenchymal cells, hyperplasia of luminal epithelial cells. All these alterations in ovarian & uterine tissues were ameliorated by supplementation with peppermint. In conclusion, the peppermint found to have a good potential as alternative therapy in the treatment of PCOS.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a multifactorial, heterogeneous, complex genetic, endocrine and metabolic disorder, diagnostically characterized by chronic anovulation, polycystic ovaries, biochemical and clinical manifestations of hyperandrogenism (**Homburg, 2009**).PCOS is detected in approximately 4–10% of women of reproductive age (**Strowitzki *et al.*, 2010; Teede *et al.*, 2010**). PCOS has a tremendous negative impact on the physiology and metabolism of the body as it may evolve into a metabolic syndrome with insulin resistance, hyperinsulinemia, abdominal obesity, hypertension, dyslipidemia and cardiovascular disease (**Allahbadia and Merchant, 2011**).

The best known treatment for PCOS at present is by using allopathic medicines such as clomiphene citrate, metformin, letrozole, tamoxifene and troglitazone. All these allopathic medicines have mild to severe side effects such as hot flushes, arthritis, joint or muscle pain and psychological side effects such as irritability, mood swings, depression and bloating **(Jadhav and Bhutani, 2005; Sasikala and Shamila, 2009)**. Due to the adverse side effects

caused by the allopathic medicines, alternative medicines are gaining importance such as Laparoscopic ovarian surgery, acupuncture, naturopathy and herbal medicines.

Peppermint (*Mentha piperita* L.) belongs to *Labiatae* family and originated from Mediterranean Regions and widely cultivated throughout all regions of the world. It is well-known plant that is used in numerous forms (i.e., oil, leaf, leaf extract, and leaf water). It is a hybrid mint, a cross between water mint (*M. aquatica L*.) and spearmint *(M spicafa* L.) (**Frampton, 2009**). *Mentha* is commonly used in folk medicine, in Iran, turkey, India, Middle East, Europe and Canada for flatulent colic, appetite, to relieve abdominal pain, fever, nausea, vomiting and digestion (**Westfall, 2004; Tayarani-Najaran *et al*., 2013**). Peppermint leaves (fresh and dried) and the essential oil extracted from the leaves are used in many foods, cosmetic and pharmaceutical products (**Pino *et al.*, 2002; Ruiz del Castillo *et al.*, 2003)**

It was investigated that peppermint has antioxidant, antitumor, antiallergenic, anti-inflammatory, antiviral, antibacterial, and antifungal activity. It has also anti-androgenic properties that reduce the level of free testosterone in the blood **(Guimaraes *et al*., 2011; Grant and Ramasamy, 2012)**.*M. spicata* contains about0.21–2.1% volatile oil, 29–74% carvone, 4–24% limoneneand 3–18% cireole. Carvone is the most important constituents of *M. spicata* (**Akdogan *et al.*, 2004**). Furthermore, effect of *M. piperita* and *M. spicata* herbal teas on testicular function were investigated in an experimental rat model and found that testosterone levels were decreased. Some studies have been shown that peppermint tea has anti-androgenic properties in both animals and women (**Guney *et al.*, 2006; Akdogan *et al.*, 2007**).

As the result of various beneficial medicinal effects of peppermint have been found, the current study aims to investigate the possible therapeutic and ameliorative efficacy of peppermint (leaves) in the PCOS induced by chemical agent, letrozole in the rat model.

MATERIALS AND METHODS

All experiments were conducted in accordance with the national laws for the use of animals in re-search and approved by the local ethical committee at Damietta University.

Chemicals

Letrozole (trade name Femara®) was purchased from El Tarshoby Pharmacy, Damietta, Egypt, manufactured by Henguri Medicine Co., Ltd., Shanghai, China. Carboxymethylcellulose (CMC) was purchased from scientific office for laboratory accessories, Damietta, Egypt, manufactured by Oxford laboratory, Mumbai, India.

Preparations of peppermint herbal tea:

Peppermint was cultivated from gardens in New Damietta, Damietta, Egypt. Fresh mature peppermint leaves were taken and washed with water then left to dry. The herbal teas of peppermint were made by pouring 250 ml (1 cup) of boiling water over approximately10 g (40 g/l) of the dried leaves and steeping for 5–10 min. All teas were prepared daily (**Akdogan *et al.*, 2007**).

Animals

Fertile female albino rats (Wistar strain) weighing100 to 120 g were used in the present study. Rats were obtained from Helwan Company for vaccines and medicines, Cairo, Egypt. Animals were maintained in the animal house of Zoology Department, Faculty of Science, Damietta University, with food and water *ad libitum*. They were housed in well-ventilated cages under standard environmental conditions (25 ± 2°C, 45 - 55% relative humidity, and 12 h dark/light cycle) and allowed 2 weeks for acclimatization.

Inducing PCOS:

Female rats were received oral dose of 1 mg/kg letrozole dissolved in 1% CMC (2 mL/kg) for 21 days to induce PCOS model (**Kafali *et al.*, 2004**).

Experimental design:

40 female rats were divided into 5groups (8each): Group 1(G1): rats were kept as the control group. Group 2 (G2, vehicle): rats were administered oral doses of 2 mL/kg b.w. of 1% aqueous solution of CMC once/day for 3 weeks. Group 3(G3): rats were received oral doses of1 mg/kg b.w. of letrozole once/day for 3 weeks. Group 4(G4): rats were received oral doses of1 mg/kg b.w. of letrozole once/day for 3weeks and then supplemented orally with peppermint (40g/L) for further 3 weeks. Group 5(G5): rats given orally letrozole at a dose of1 mg/kgb.w.in combination with 40 g/l of peppermint once/day for 3 weeks.

At the end of treatment, the animals were anesthetized and sacrificed according to the guidelines of the Egyptian Bioethics Committee, and assessed for various biochemical, histological, histochemical and immunohistochemical investigations.

Measurements of body weight and organs weight:

The body weight of control and experimental animals were recorded daily. Bilateral ovaries & uteri of all experimental animals were weighed, in which the mean values were regarded as the ovary & uterus weight.

Blood sampling:

At 24 hours post the last dose of treatment and after 18 hours fasting period, all animals were weighed then anesthetized by inhalation of diethyl ether. At the time of sacrifice, blood was collected in non-heparinized tubes and centrifuged at 4000 rpm for 10 min at 4°C and sera were collected. Sera were kept in clean well-Stoppard plastic vials at -20± 2 ºC for subsequent hormone determination (follicle-stimulating hormone [FSH], luteinizing hormone [LH], estrogen[E2], total testosterone[T]) and lipid profile determination (total lipids, cholesterol and triglycerides).

Biochemical assessments:

FSH, LH, E2 and T were measured using correspondent commercial radioimmunoassay (RIA) kits (Northern biological technical institute. Bejing, P.R. China)

The serum cholesterol levels was measured according to the method of **Deeg and Ziegenhorn, 1983**, triglyceride by using method of **Fossati and Prencipe, 1982** and total lipids by using method of **Akins *et al.*, 1989,** using kits from Diamond Diagnostic Chemical Company (Egypt).

Light microscopic investigations:

A-Histopathological examinations:

The ovaries and uteri of both control and experimental groups were excised immediately after treatment, cleaned from fats and weighed. The excised ovaries and uteri were fixed in 10% neutral formal saline, embedded in paraffin wax, and then sectioned serially at 5-µm thickness. Sections were mounted and stained by the hematoxylin and eosin procedure (**Durry and Wallington, 1980**).Also some sections was stained by Masson's trichrome (M.T.) to determine the collagenous fibers.

B-Immunohistochemical investigations:

For immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in ovary and vascular endothelial growth factor (VEGF) in uterus, Formalin-fixed wax sections 5µm thick were mounted on slides coated by Histo-Grip. In order to block endogenous peroxidase activity, the sections were incubated for 10 – 15 min in 0.5%H_2O_2. If required, incubate tissue in digestive enzyme. Apply Ultra V Block and incubate for 5 minutes at room temperature to block nonspecific background staining. The sections of ovary were then incubated with mouse monoclonal anti-PCNA as primary antibody (clone PC10, DAKO, Carpinteria, CA) at a dilution of 1:800 (0.5μg/ml) for1 hour at room temperature whereas the sections of uterus were then incubated with primary rabbit monoclonal anti-VEGF at a dilution of 1:200 overnight. To eliminate nonspecific binding, apply biotinylated goat anti-polyvalent and incubate for 10 minutes at room temperature. Then apply Streptavidin Peroxidase and incubate for 10 minutes at room temperature. The binding of primary antibody was visualized using diaminobenzidine for 3-5 min. After washing with distilled water, sections were counterstained with hematoxylin. Finally, sections were dehydrated and cover-slipped with DPX. Between the steps of the staining procedure slides were washed in phosphate-buffered saline for 5 minutes at RT.

Statistical analysis:

One-Way Analysis of Variance (ANOVA) with Tukey as a multiple comparison test and Tuckey's-b procedure was applied to test the significant differences between groups. Statements of significance are based on probability (p) levels of ≤ 0.05was considered statistically significant**(Hayes, 1994)**. All the analysis was performed using SPSS software (version 18.0).

RESULTS

Table 1: Effects on body, ovarian and uterine weights:

Experimental group treated with letrozole exhibited a significant increase in the body and ovarian weights ($p<0.001$) while a highest reduction in uterine weight was observed ($p<0.001$). A significant decrease in body weight ($p<0.001$) and ovarian weight($p<0.05$ and $p<0.001$)were observed in peppermint-supplemented or in combination groups respectively while a significant increase ($p<0.001$) in uterine weight was observed.

Table 2: Effect on hormones:

The data presented in Table 2 showed that the treatment of female rats with letrozole alone induced a significant increase in serum LH, FSH and testosterone levels ($p<0.001$, $p<0.001$, $p<0.001$ respectively) while a significant decrease in estrogen level was observed ($p<0.001$). On the other hand, there was a significant decrease in LH and testosterone levels ($p<0.01$, $p<0.001$) while estrogen level was significant increase in group of animals supplemented with peppermint ($p<0.001$). No significant effect on FSH level was observed.

Table 3: Effect on lipid profile:

A significant ($p<0.001$) increase in serum total lipids while a significant decrease ($p<0.001$) in triglycerides and no significant effect on cholesterol was found in group treated with letrozole. However, a significant decrease of total lipids ($p<0.05$) and a significant increase in cholesterol & triglycerides was found in the group of animals

supplemented with peppermint ($p<0.01$).Administration of animals with letrozole in combination with peppermint caused also a significant increase in cholesterol & triglycerides ($p<0.05$, $p<0.001$ respectively).

Histopathological observations:

A- Histopathological observations of the ovary:(Plate 1, Figs. A-H) and (Plate 2, Figs. A-F)

The ovary of the control and vehicle groups exhibited normal follicles in various stages of development including secondary follicles, Graafian follicles and fresh corpora lutea. Medullar area presented loose connective tissue, rich in blood and lymphatic vessels (Plate 1, Figs. A&B).In experimental group treated with letrozole, showing intense follicular atresia at high degree of degeneration, having formation of cysts. These follicular cysts with virtually no granulosa cell layer or large cystic follicles with scant granulosa cells (Plate1, Figs. C&D).

In peppermint supplemented group, the ovarian tissue exhibited marked amelioration of the histological picture but was still not closely resemble to the control group, some amelioration in its architecture exhibited a decrease in atretic cysts compared to letrozole group. The ovary showed a marked recovery of follicles with intact structure of granulosa layer and thecal layer as well as the presence of healthy developed follicles with oocytes (Plate 1,Figs. E&F). While in experimental group treated with letrozole in combination with peppermint, the ovarian tissue showed also some improvement in its architecture but not the same as peppermint treated group(Plate 1, Figs. G&H).

Plate 2 showing distribution of collagenous fibers in ovarian tissue of different treated groups. Control & vehicle groups exhibited normal distribution of fibers in normal histology of the ovary (Plate 2, Figs. A&B). In experimental group treated with letrozole, there was intensive distribution of collagenous fibers in polycystic ovary which revealed fibrosis in ovarian stroma (Plate 2, Figs. C&D). While in peppermint treated groups, showed improvement in ovarian architecture and mild distribution of collagen fibers (Plate 2, Figs. E&F).

B- Histopathological observations in uterus: (Plate 3, Figs. A-E), (Plate 4, Figs. A-J)& (Plate 5, Figs. A-F)

A histological examination of uterus from control and vehicle groups exhibited normal histological structure of rodent uterus. Endometrium consisted of pesudostratified columnar cells and underlying highly cellular connective stroma filled with blood vessels and endometrial glands lined by simple columnar epithelial cells (Plate 4, Figs. A, B, C& D).

A microscopic examination of the uterus in group treated with letrozole, showed histopathological lesions in the uterus include necrosis in stromal mesenchymal cells, few numbers of glands, damage and hyperplasia of luminal epithelial cells(Plate 4, Figs. E & F).The observations on experimental groups revealed that administration of peppermint after letrozole treatment or in combination with letrozole resulted in improvement of the uterine tissue exhibited normal stroma, glands and luminal epithelial cells(Plate 4, Figs. G, H, I& J).

Plate 5 showing distribution of collagenous fibers in uterine tissue of different treated groups. Control (A) and vehicle (B) groups exhibited normal distribution of fibers in normal histology of uterus. In experimental group treated with letrozole, showing excessive distribution of collagenous fibers indicating fibrosis in all uterine layers (Figs. C&D). Letrozole group in combination with peppermint, showing mild fibrosis in uterine layers. On the other hand, in letrozole supplemented with peppermint group the fibrosis is less localized in the endometrium than in pre- and myometrium in previous group (Figs. E&F).

Immunohistochemical observations:

A- Immunohistochemistry for PCNA protein expression in the ovary:(Plate 6, Figs A - H)

Sections of control and vehicle groups showed intense positive staining and discrete staining of the cell nuclei of the majority of granulosa and theca cells in the follicular wall (Figs. A& B). On the other hand, such reaction was weak stained nuclei in sections of letrozole treated rats (Figs. C & D). However, in peppermint supplemented groups, showed moderate staining almost reaching to the control group (Figs. E & F). Moreover, positive stained nuclei in letrozole in combination with peppermint were less than that in the peppermint treated group as shown in (Figs. G & H).

B- Immunohistochemistry for VEGF protein expression in the uterus: (Plate 7, Figs A - E).

Sections of control and vehicle groups displayed a general accumulation of positive cells in the uterine tissues. Intense stained in uterine glands, stromal tissues & luminal epithelium were observed (Figs. A & B). On the other hand, such reaction was moderate stained nuclei in sections of letrozole treated rats (Fig.C). However, in peppermint supplemented group showed intense positive cytoplasmic expression of VEGF almost reaching to the control group (Fig. D). Moreover, in letrozole group in combination with peppermint exhibited moderate staining in uterine tissue in comparison with peppermint supplemented group as shown in (Fig. E).

DISSCUSION

PCOS has many clinical manifestations, including oligomenorrhea and hyperandrogenism, leading to metabolic dysfunction (**Dickerson *et al.*, 2010)**. In the present study, the PCOS induction after treatment with Letrozole was

confirmed through polycystic ovarian morphology and serum hormone profiles. Letrozole is a nonsteroidal reversible, competitive third – generation aromatase inhibitor that is highly potent and selective. Aromatase enzyme is widely expressed in human tissues, such as placenta, ovary, and testis. It is the key enzyme which catalyzes the conversion of testosterone and androstendione into E2 and estrone recepectively. Letrozole inhibits the aromatase enzyme by competitively binding to the heme subunit of the cytochrome P450 (**Buzdar *et al.*, 2002)**.Thereby increasing ovarian androgens, leading to hyperandrogenism or abnormal steroidogenesis which result in abnormal follicular development and polycystic ovary (**Diamanti *et al.*, 2005**). Letrozole showed many histological and biochemical findings consistent with human PCOS (**Kafali *et al.*, 2004**).

The present work found a significant body weight gain in letrozole treated rats compared to control rats which were attributed to the deposition of abdominal fat (**Maharjan *et al.*, 2010;Honnma *et al.*, 2006; Barber *et al.*, 2008)**.The results of the present study revealed that ovarian weight in PCOS group was more than that in control group which is in accordance with the earlier findings (**Sasikala *et al.*, 2010; Desai *et al.*, 2012; Jadhav *et al.*, 2013)** and this may be due to thickened ovarian capsule and hyperplasia of theca interna cells in the ovary.

In the present research, the uterine weight was found to decrease in PCOS group. Reduction in the uterine weight of females is associated to the atrophy of organ and changes observed in the tissue structure. This coincides with the previous study (**Kafali *et al.*, 2004; Jadhav *et al.*, 2013)**.

In 80% of PCOS patients, the testosterone levels increase (**Liepa *et al.*, 2008; Desai *et al.*, 2012; Jadhav *et al.*, 2013)**. The similar results were obtained in the present study. Both testosterone and LH levels in PCOS group were significantly increased compared to normal rats indicating hyperandrogenism. Hyperandrogenism is the key feature of PCOS, resulting primarily from excess androgen production in the ovaries and, to a lesser extent, in the adrenals. The primary mechanisms driving increased ovarian androgen production in PCOS include hypersecretion of LH and increased LH bioactivity, hyperinsulinemia due to insulin resistance and increased volume of theca cells in an expanded ovarian stroma (**Fritz and Speroff, 2011**).

In the present study, we found a reduction in estrogen level in PCOS group and this coincides with earlier studies ((**Maharjan *et al.*, 2010; Rezvanfar *et al.*, 2012)**. High testosterone levels reflected accumulation of androgens because Letrozole as non steroidal aromatatse inhibitor blocks the conversion of androgen substrates to estrogens. The reduction in estrogen weakens the negative feedback on LH production in the pituitary gland, resulting increasing levels of LH (**Kafali *et al.*, 2004**), which further stimulates theca cells to secrete testosterone. FSH level in PCOS group, were significantly elevated which is in accordance with earlier studies of **Kafali *et al.*, 2004; Akdogan *et al.*, 2007**who observed impairment of ovarian folliculogenesis.

Women with PCOS are hyperandrogenemic which is associated with alteration in circulating lipids and lipoprotein levels resulting in dyslipidemia **(Desai *et al.*, 2012; Jadhav *et al.*, 2013)**. In the current study, the PCOS induced rats showed elevated level of total lipids and decreased levels of triglyceride.

The biochemical results are also supported by histopathological observations of light microscopy. It is reported that the histopathological study of PCOS groups showed the presence of atretic follicles, the formation of more than subcapsular cysts lined with a thin attenuated layer of granulosa cells, thickened ovarian capsule and hyperplasia of theca interna cells in the ovary (**Kafali *et al.*, 2004; Baravalle *et al.*, 2006; Rezvanfar *et al.*, 2012;Jadhav *et al.*, 2013**). Researchers indicated that letrozole caused the reduction of corpus luteum indicating oligo-/anovulation and an increase in the number of atretic follicles (**Shi and Vine 2012; Padmanabhan andVeiga-Lopez, 2013**).

In PCOS high local androgen concentrations are responsible for anovulation by direct effect on the ovary. Androgen-induced follicular atresia is thought to occur by entry of androgens into the granulosa layer of pre-antral follicles, where they bind to the cell receptors and cause the cell death. Androgens cause deterioration of follicles by increasing the number of pycnotic granulosa cells and degenerating oocytes (**De Leo *et al.*, 1998**).The similar observations were seen in the current work when the rats were induced with PCOS by Letrozole.

In the current work, it is reported that, the histopathological study of PCOS group rats showed pathological changes of the uterus including necrosis in stromal mesenchymal cells, damage and hyperplasia of luminal epithelial cells. This coincides with previous finding **(Lakzaei *et al.*, 2013)**.

Proliferating Cell Nuclear Antigen (PCNA) is an auxiliary protein of DNA polymerases δ and ε, enzymes necessary for DNA synthesis and is thus a marker of cell proliferation (**Wood and Shivji, 1997**).The presence of PCNA in oocytes of primordial follicles suggested the role of this protein even in earlier stages of folliculogenesis.

PCNA was significantly down regulated in the granulosa cell layers of cystic follicles of PCOS rat indicating reduced cell proliferation or apoptosis of granulosa cells which may underlie the abnormal follicle development in PCOS (**Isobe and Yoshimura, 2000; Salvetti *et al.*, 2009**).**Chen *et al.*, (2015)** found that cystic fibrosis transmembrane conductance regulator (CFTR) down regulated in granulosa cells of PCOS rats, accompanied with down regulation of pro-proliferative gene PCNA. Immunohistochemical demonstration of PCNA in the current research was in agreement with these earlier finding.

The pathogenesis of PCOS is not well understood but accumulating evidence suggests that dysregulation of angiogenic factors may play an important role. Vascular endothelial growth factor (VEGF) is a potent mitogen for endothelial cells and a stimulator of vascular permeability. It plays an important role in developmental, physiological and pathological angiogenesis. Changes related to angiogenesis are ovulatory – related and serve to prepare a receptive site of the blastocyst (**Nardo, 2005; Demir *et al.*, 2006**).In the present work, we observed a moderate cytoplasmic VEGF immmunostaining within endometrial surface epithelial cells, glandular epithelium as well as endometrial stromal cells which may reveal abnormalities in angiogenensis in uterus of PCOS rat.

Several studies have focused on examining the beneficial effects of *Mentha* species. These effects may be related with the presence of substances such as menthol, eugenol, thymol, flavonoids, terpenoids, eriotricin, luteolin, hesperidin rosmarinic, cinnamic and caffecic acids and other compounds that demonstrate antioxidant activity (**Schmidt *et al.*, 2009; Yang *et al.*, 2010**).

Oxidative stress (OS) may play a role in the pathophysiology of PCOS. OS may affect insulin resistance (IR) that is common in young non-obese PCOS women (**Agarwal *et al.*, 2005**). Reactive oxygen species (ROS) generation from mononuclear cells (MNCs) in response to hyperglycemia increased in PCOS independent of obesity. The resultant oxidative stress may contribute to a pro-inflammatory state that induces insulin resistance and hyperandrogenism in women with this disorder (**González *et al.*, 2006**). Peppermint as antioxidants can modulate the reactive oxygen species effect, and also prevent oxidative stress.

Peppermint is an herbal plant which is usually recommended for treatment of many diseases, particularly digestive system problems; however, some studies show that despite its beneficial effects, spearmint has adverse effects on the reproductive system of male rats (**Akdogan *et al.*, 2004**). Spearmint reduces free testosterone concentration of serum and has been introduced as an anti-androgenic agent and proposed for treatment of hirsuitism in polycystic ovarian syndrome (PCOS) of women (**Akdogan *et al.*, 2007; Grant, 2010**).

M. piperita treatment exhibited a significant recovery of testosterone, estrogen, LH levels, ovarian and uterine tissue.The histopathological observations of the peppermint treated group showed marked recovery of the ovarian tissue with the presence of many follicles in the various stages of development indicating normal oogenesis. The follicles showed normal granulosa layer with defined thecal layers. The presence of corpora lutea was also seen after plant treatment suggesting that treatment with peppermint restored normal estrous cycle.

M. piperita showed anti-androgenic effect by reducing increased androgen levels and prevented ovarian cell dysfunction in PCOS to improve fertility **(Grant, 2010**). The observed recovery of ovarian tissue as well as anti-androgen potential of *M. piperita* may be responsible for its efficacy in the management of PCOS.

CONCLUSION

Infertility is a major concern in PCOS patients and important causes of infertility in PCOS are follicular atresia, anovulation, and consequent hyperandrogenemia. Phytotherapy is frequently considered to be less toxic and free from side effects than synthetic drugs. Thus, *M. piperita* can be used as a therapeutic agent to treat infertile patients whom infertility occurs due to poor oocyte quality and anovualtion. In conclusion, the peppermint as antioxidant found to have a good potential as alternative therapy in the treatment of PCOS.

LIST OF TABLES

Table 1: Effect of various treatments on body, ovarian and uterine weights (gm) in Letrozole induced PCOS rats, based on the results of one way ANOVA with the application of multiple comparison Tucky test.

	Weights parameters				
Organs / Groups	Rate of increase in body weight	Ovarian weight (gm)	Ovariosomatic index	Uterine weight (gm)	Uterinsomatic index
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group 1	1.32 ± 0.22 b***	0.12 ±.009 b***	0.07 ± 0.005 b***	0.21 ± 0.01 b***	0.13 ±0.027 b***
Group 2	1.32 ±0.18 a, b***	0.12 ±0.007 a, b***	0.08 ± 0.005 a*, b***	0.21 ± 0.01 a, b***	0.13 ±0.014 a, b***
Group 3	2.91 ± 0.13 a***	0.15 ±0.007 a***	0.10 ± 0.004 a***	0.07 ± 0.01 a***	0.05 ±0.007 a***
Group 4	0.87 ± 0.09 a***, b***	0.14 ±0.006 a***, b*	0.08 ± 0.004 a*,b***	0.19 ± 0.01 a*, b***	0.10 ±0.013 a**, b***

Group 5	2.06 ± 0 .18 a***, b***	0.14 ±0.006 a***, b***	0.09 ± 0.004 a***,b*	0.07 ± 0.006 a***, b	0.05 ±0.005 a***, b

Comparison with Normal control group NS a, P<0.05a*, P<0.01a**, P<0.001a***
Comparison with Letrozole group NS b, P<0.05b*, P<0.01b**, P<0.001b***

Table 2: Effect of various treatments on serum LH (mIU/ml), FSH (mIU/ml), Estrogen (pg/mL), Testosterone (ng/dL) in Letrozole induced PCOS rats, based on the results of one way ANOVA with the application of multiple comparison Tucky test.

Hormones parameters				
Hormones / **Groups**	**LH (mIU/ml)**	**FSH (mIU/ml)**	**Estrogen (pg/ml)**	**Testosterone (ng/dL)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Group 1	13.35 ±1.6 b***	16.24 ± 2.53 b***	41.11 ± 14.5 b***	0.36 ± 0.11 b***
Group 2	12.35 ±1.8 a, b***	34.34± 1.38 a***,b	30.68 ± 6.1 a***,b***	0.22 ± **0**.03 a ,b***
Group 3	28.08 ±1.9 a***	39.55 ± 4.95 a***	22.08 ± 4.5 a***	2.51 ± 0.09 a***
Group 4	22.61± 1.8 a***, b**	39.04 ± 4.82 a***, b	43.24 ± 13.8 a ,b***	1.27 ± 0.06 a***,b***
Group 5	24.54± 2.8 a***,b	41.13 ± 3.02 a***, b	27.48 ± 8.4 a***,b***	1.84 ± **0**.12 a***,b***

Comparison with Normal control group NS a, P<0.05a*, P<0.01a**, P<0.001a***
Comparison with Letrozole group NS b, P<0.05b*, P<0.01b**, P<0.001b***

Table 3: Effect of various treatments on serum Total lipids (mg/dL), Cholesterol (mg/dL) and Triglyceride (mg/dL) in Letrozole induced PCOS rats, based on the results of one way ANOVA with the application of multiple comparison Tucky test.

lipids parameters			
Lipids / **Groups**	**Total lipids (mg/dL)**	**Cholesterol (mg/dL)**	**Triglyceride (mg/dL)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Group 1	621.3 ± 27.9 b***	359.7 ± 29.4 b	459.0 ±17.3 b***,
Group 2	637.5 ± 13.4 a, b***	373.7 ± 26.9 a, b	334.0 ±38.1 a***, b
Group 3	871.9 ± 20.9 a***	336.6 ± 44.4 a	340.2 ±16.9 a***
Group 4	742.5 ± 27.0 a***, b***	400.4 ± 18.1 a, b**	400.1 ±28.4 a**, b**
Group 5	721.3 ± 27.9 a***, b***	388.4 ± 24.4 a, b*	412.5 ±40.0 a*, b***

Comparison with Normal control group NS a, P<0.05a*, P<0.01a**, P<0.001a***
Comparison with Letrozole group NS b, P<0.05b*, P<0.01b**, P<0.001b***

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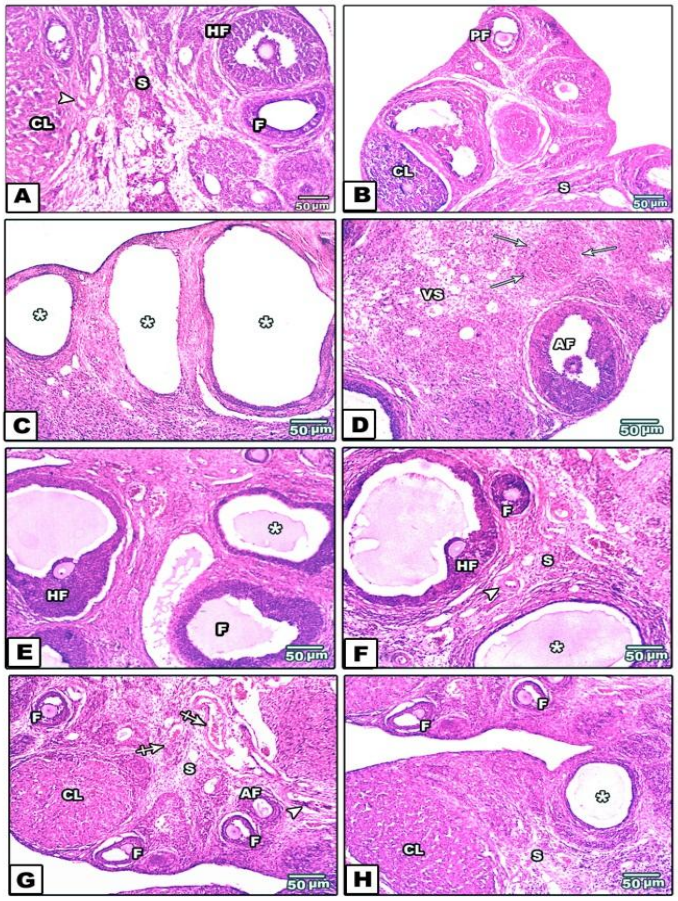

Plate 1: (**Figs. A-H**): Photomicrograph of transverse histological sections of ovary of both control and experimental groups. **Fig. A:** Control group showing the presence of healthy follicles and corpus luteum. **Fig. B:** Vehicle group showing normal pattern-like control. **Figs. C and D:** Letrozole group showing, many cystic follicles with thin granulosa layer, atretic follicles, stromal hyperplasia, and vacuolated stroma. **Figs. E and F:**Letrozolesupplemented with peppermint group showing healthy follicle and healthy stroma. **Figs. G and H:** Letrozole in combination with peppermint group showing healthy follicles in various stages of development and with congested blood vessel. (Abbreviations: Follicle, **F**; Corpus luteum, **CL**; Blood vessel, (**arrow head**); Congested blood vessel, (**crossed arrow**);Cystic follicles, (**asterisks**); Atretic follicles, **AF**; Stromal hyperplasia, (**arrows**);Vacuolated stroma, **VS;** Healthy follicle, **HF**; Stroma, **S**).(H&E staining).

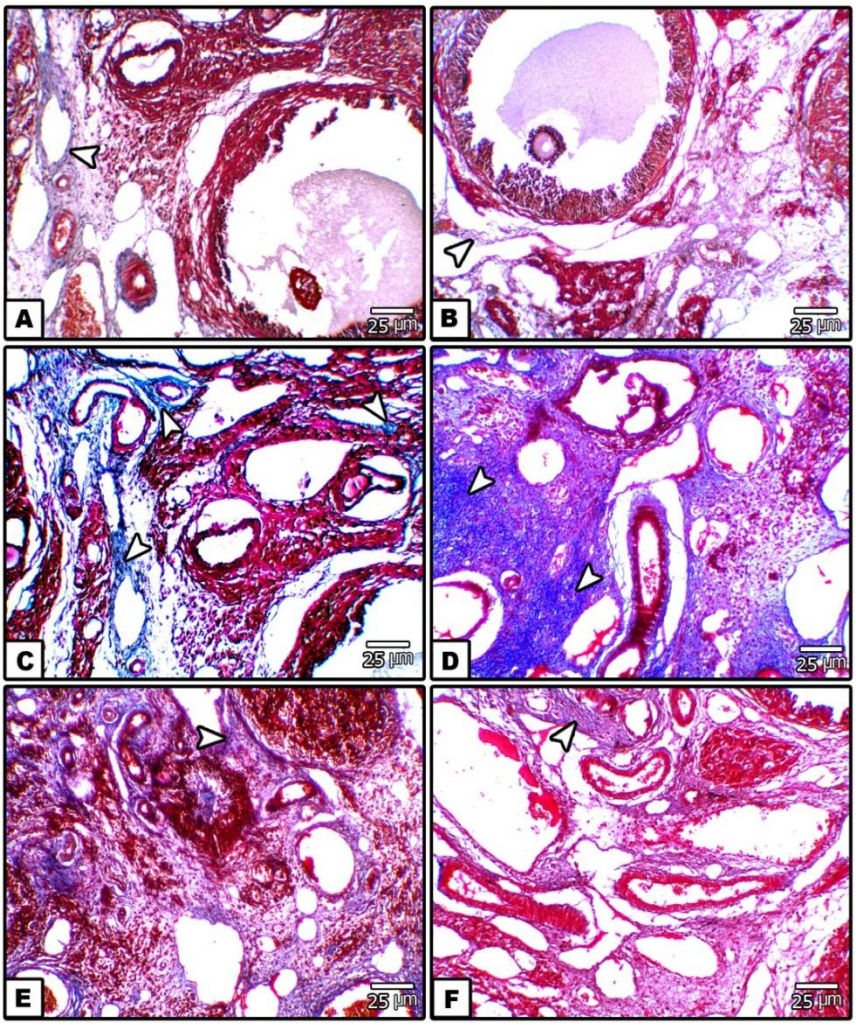

Plate 2: (**Figs A-F**): Photomicrograph of transverse histological sections of ovary of both control and experimental groups. **Fig. A&B**: control & vehicle groups showing normal histological appearance of the ovary with normal distribution of collagenous fibers (arrows heads). **Figs C and D:** PCOS rat ovary showing excessive accumulation of collagenous fibers in the ovarian stroma in the form of network (arrow heads). **Fig E:** Letrozole in combination with peppermint group showing mild appearance of collagenous fibers (arrows heads). **Fig. F:**Letrozole supplemented with peppermint group showing a few scattered of collagenous fibers (arrows heads). (M.T. staining).

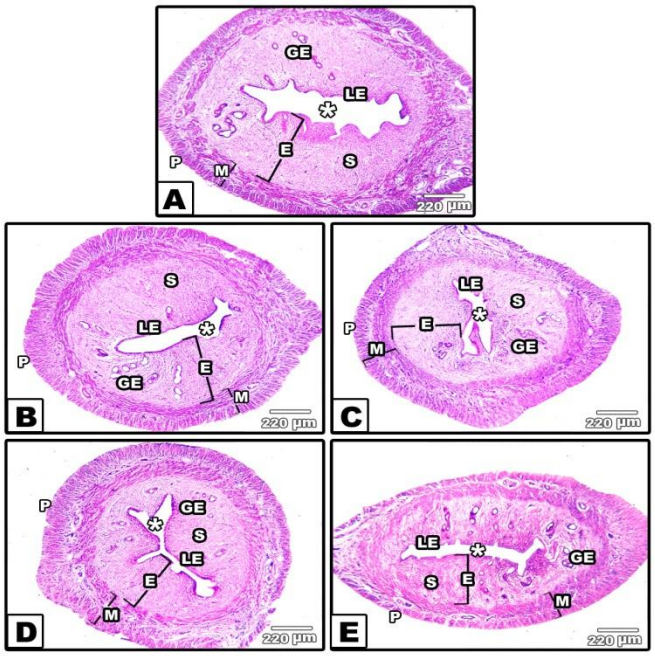

Plate 3: (**Figs. A-E**): Photomicrograph of transverse histological sections of uterus of both control and experimental groups showing layers of uterus. The inner mucosa, or endometrium, consists of surface columnar epithelium overlying a thick lamina propria containing numerous blood vessels and endometrial glands. The middle muscular layer, or myometrium, is composed of an inner circular and outer longitudinal smooth muscle layer. The myometrium is covered by the perimetrium, a thin connective tissue layer overlain by a simple serosa. (Abbreviations: Perimetrium, **P**; Myometrium, **M**; Endometrium, **E**; Glandular epithelium, **GE**; Stroma, **S**; Luminal epithelium, **LE**; Lumen, (**asterisks**)).(H&E staining).

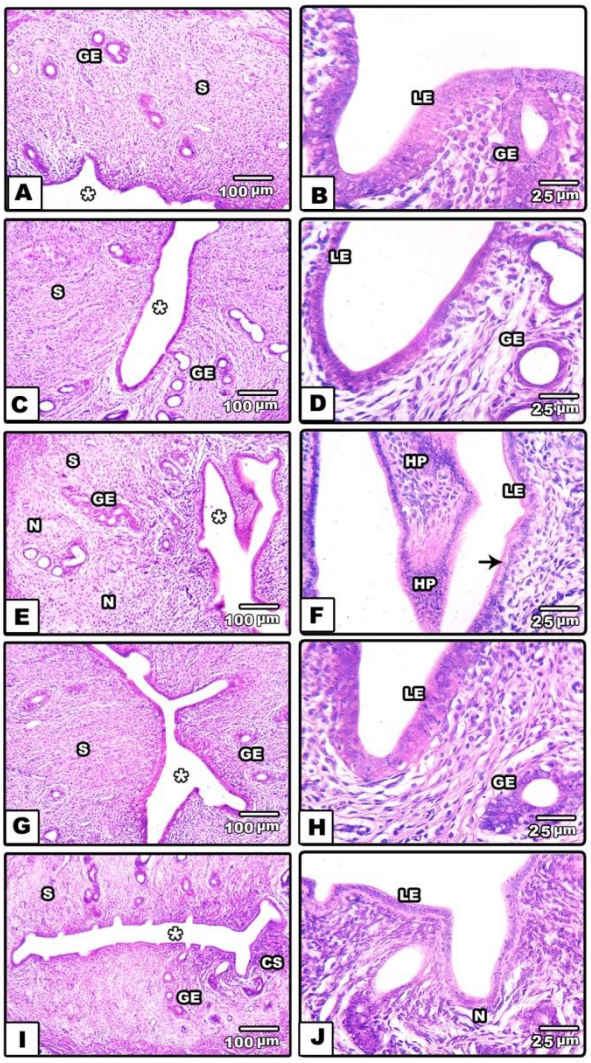

Plate 4:(**Figs. A-J**): Photomicrograph of transverse histological sections of uterus of both control and experimental groups. **Figs. A and B:** Control group showing healthy stroma of endometrium, glandular epithelium and single layer of luminal epithelium exposing to lumen. **Figs. C and D:** Vehicle group showing normal pattern-like control. **Figs. E and F:**Letrozole group showing necrosis in stromal mesenchymal cells, few numbers of glands. **Figs. G and H:** Letrozole supplemented with peppermint group showing healthy luminal epithelial cells, glands and healthy stroma. **Figs. I and J:** letrozole in combination with peppermint group showing normal luminal epithelium but also revealed condensed stroma. (Abbreviations: Stroma, **S**; Glandular epithelium, **GE**; Luminal epithelium, **LE**; Lumen, (**asterisks**);Necrosis, **N**; Damage cells, (**arrows**); Hyperplasia, **HP**; Condensed stroma, **CS**).(H&E staining).

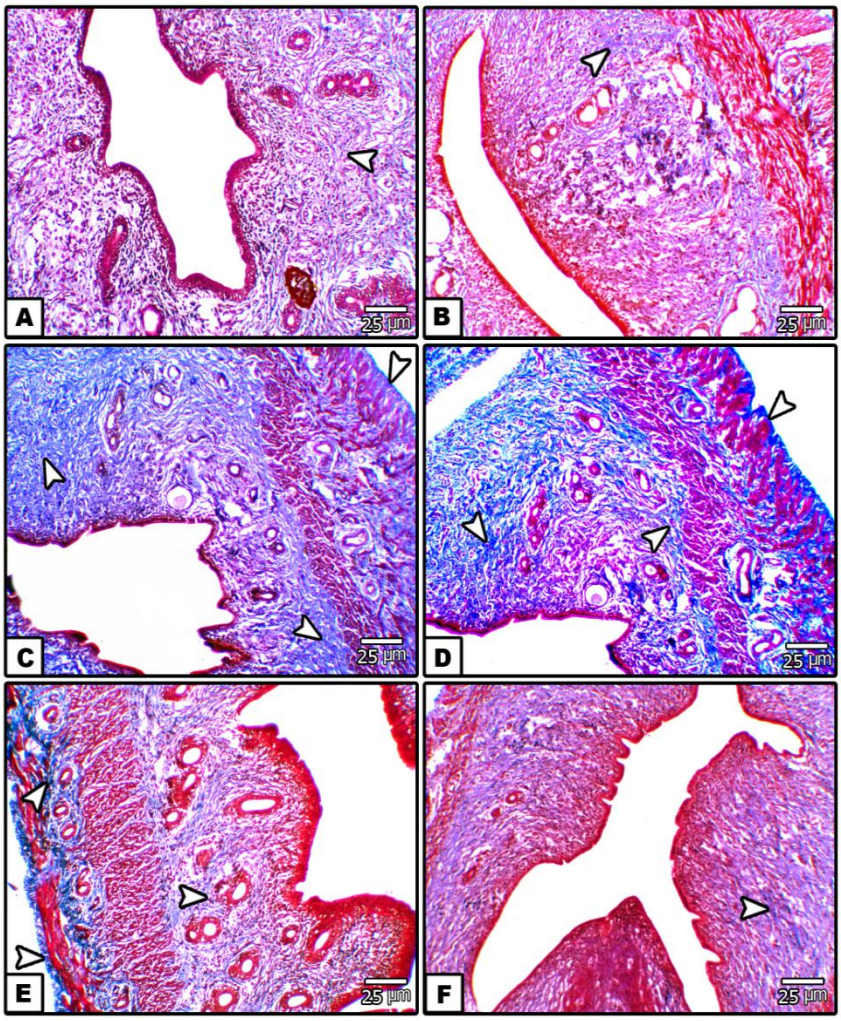

Plate 5: (**Figs. A-F**): Photomicrograph of transverse histological sections of uterus of both control and experimental groups. **Fig. A&B:** Control & vehicle groups showing normal histological appearance of the uterine endometrium and moymetrium with normal distribution of collagenous fibers (arrow heads)**. Figs. C and D:** PCOS rats revealed excessive distribution of collagenous fibers indicating fibrosis (arrow heads)**. Fig E:**Letrozole in combination with peppermint group showing mild appearance of collagenous fibers (arrow heads).**Fig. F:**Letrozole supplemented with peppermint group showing almost normal distribution of collagenous fibers (arrow heads).(M.T. staining).

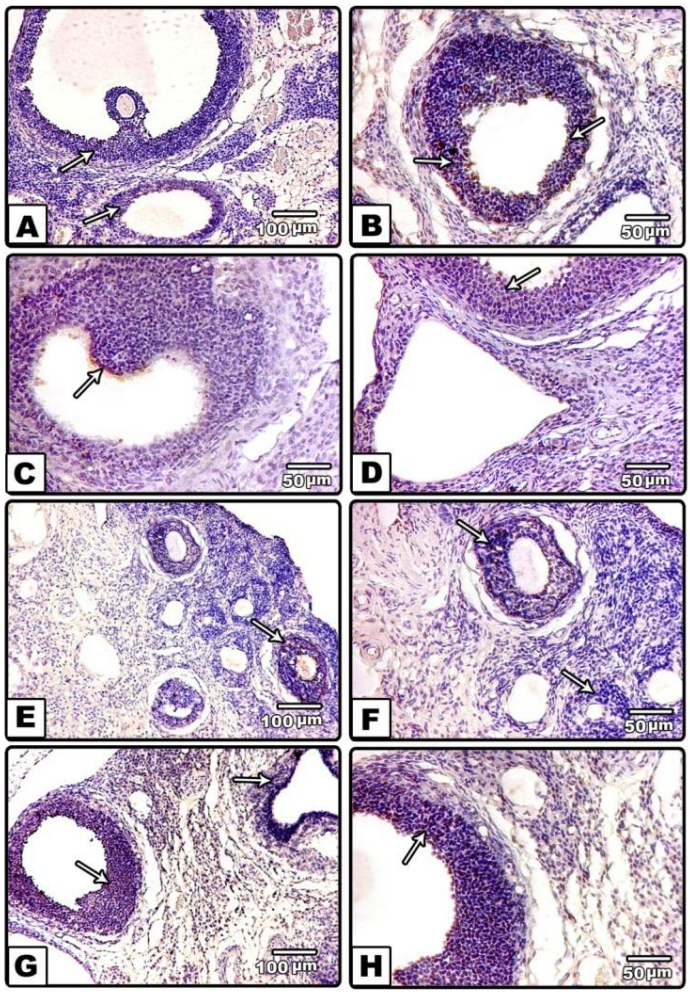

Plate 6: Fig. A: Section of ovary from control rat showing positive brown nuclear staining of follicles. **Fig. B:** Section of ovary from vehicle rat showing positive brown nuclear staining of follicles. **Figs. C&D:** Section of ovary from PCOS showing mild brown nuclear staining of follicles. **Figs. E&F**: Section of ovary from letrozole supplemented with peppermint group showing positive brown nuclear staining of follicles. **Figs. G&H:** Section of ovary from letrozole in combination with peppermint group showing positive brown nuclear staining of follicles. (PCNA immunostaining).

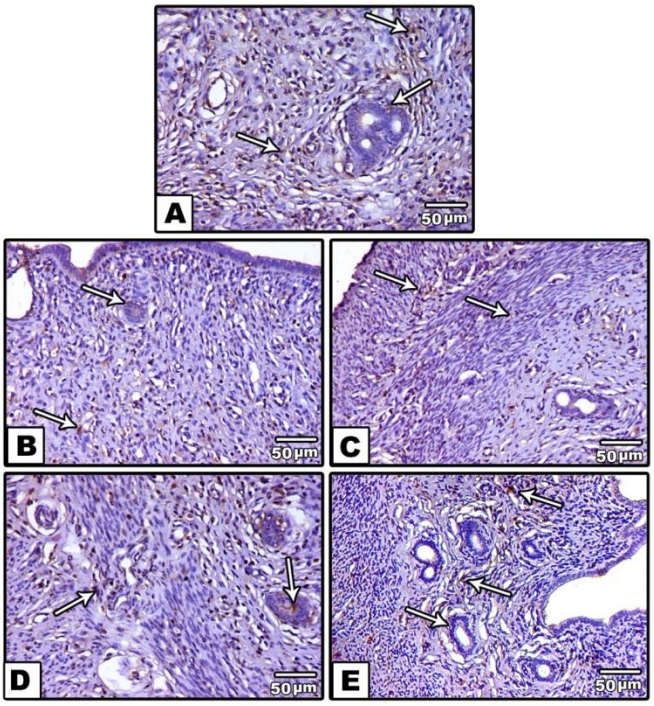

Plate 7:Fig. A: Section of uterus from control rat showing glandular epithelial cells as well as stromal cells with marked immunostaining. **Fig. B:** Section of uterus from vehicle rat showing positive staining of endometrial cells. **Fig. C:** Section of uterus from PCOS rat showing mild brown stain in muscular layer cells and stromal cells. **Fig. D:** Section of uterus from peppermint supplemented group showing intense positive stain in epithelial glandular cells and stromal cells. **Fig. E:** Section of uterus from letrozole plus peppermint group showing mild stain in endometrial stromal cells (VEGF- immunostaining).

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