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EFFECT OF FENUGREEK AND PALM POLLEN EXTRACT ON INDUCED POLYCYSTIC OVARIES IN FEMALE RATS

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Abstract

Polycystic ovarian syndrome (PCOS) is a common disease widespread among women of reproductive age (12–45 years). The prevalence of the disease is about 5–10%, though its pathogenesis is still unclear. Hormonal disorders of the ovarian and pituitary glands or insulin resistance may be the contributing factors, which lead to defects in the ovarian tissue and deformation and necrosis in ovarian tissue cells.

This study aimed to determine the effect of extracts of herbs (fenugreek and palm pollen) on induced polycystic ovaries (PCO) compared to the untreated control group. Twenty female rats were used in this study; they were divided into four groups, where the 1st group included 5 females as controls and the other 15 females constituting the treatment groups received an intramuscular injection of estradiol valerate (EV) (0.50 ml/kg) daily for two months to induce PCO. The females with induced PCO were divided into three groups of 5 females each. The 1st group received no treatment and was considered the negative control for PCO; the 2nd group was orally administered 0.50 ml/kg of fenugreek water extract. The 3rd group was orally treated with 0.50 ml/kg palm pollen water extract for five weeks. The results of statistical and histological tests and hormonal assays showed that treatment with the fenugreek and palm pollen water extracts had an effective and noticeable impact in reducing the symptoms of PCO in ovarian tissues with resumption of ovarian follicle development and decrease in the number of ovarian cysts compared to that in the untreated PCO group. These results indicate that fenugreek and palm pollen water extracts have a positive effect on ovarian tissue in PCO. The reduced side effects in the treated PCO groups may be attributed to the chemical compounds in these herbs influencing the structure and function of ovarian tissues. This study, thus, suggests the use of fenugreek and palm pollen as safe treatments for PCO without negative side effects or ovarian tissue damage.

Keywords: *Fenugreek, Palm pollen, Polycystic Ovary Polycystic ovaries Syndrome, Estradiol valerate estate.*

Introduction

Polycystic ovarian syndrome (PCOS) is a common disease, widespread among women of reproductive age (12–45 years). The prevalence of the disease is about 5–10%. PCOS affects the ovaries leading to ovulation disorders, possibly due to hormonal imbalances or genetic reasons Al-deresawi, *et al* (2014), Hussein *et al* (2014). Although its pathogenesis is still unclear, hormonal disorders of the ovarian and pituitary glands or insulin resistance may be major contributing factors, leading to defects in the ovarian tissue and deformation and necrosis in ovarian tissue cells.

The important symptoms of the syndrome include absence of ovulation, infertility, weight gain, polycystic ovaries, dysfunction of reproductive hormones from the hypothalamus–pituitary gland axis, increased production of androgens, along with other identifying factors Ehrmann (2005), Atiomo *et al*., (2003). Type 2 diabetes, high blood pressure and cardiovascular diseases are also considered long-term complications of the syndrome Sabuncu *et al*., (2003). The main goal of PCOS treatment is restoration of normal ovulation and ultimately restoring fertility. Treatments for the syndrome include a low-calorie diet, exercise, medication (spironolactone, glitazones, clomiphene, and metformin), and surgery Karimzadeh *et al.,* (2012). Evidence suggests that medicinal plants have been considered for years to treat various diseases. Therefore, considering the adverse complications and side effects of synthetic medicines in the body, many studies worldwide are investigating the therapeutic effects of various plants. The fruits and various other parts of the palm tree are used for different purposes. The plant is native to tropical regions, such as African and Arabic countries, and is planted in some regions of Iran. Palm pollen contains various vitamins (e.g., vitamin A, E, and C) as well as elements (e.g., zinc, copper, selenium, cobalt, iron, nickel, and manganese), along with essential and non-essential amino acids, fatty acids, flavonoids, sterols, estradiol, estrone, beta sitosterol, and cholesterol Teixeira Filho *et al.,* (2002), Hunter *et al.*, (2000). Palm pollen has been recommended in traditional medicine to treat infertility and was used in ancient Egypt to treat infertility in women. In addition, recent studies have also indicated the effectiveness of palm pollen in female reproductive processes. The study by El-Desoky *et al*. (1995) on mice whose ovaries had been removed, showed that palm pollen significantly increases the levels of estradiol and progesterone with a non-significant increase in the levels of LH and FSH. The effects of palm pollen extract on the levels of sex hormones in female rats showed increased levels of estrogen and progesterone hormones Mann and Truswell (2012). Fenugreek is an annual plant of the family Fabaceae, with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semiarid crop. Fenugreek is planted in the Mediterranean region, southern Europe, and south and western Asia. The seeds are used for cooking, to prepare medicine, or to hide the taste of other medicines. Fenugreek is consumed for the treatment of digestive problems, such as loss of appetite, constipation, upset stomach, and gastritis. In addition, fenugreek is used for alleviation of conditions, like diabetes, painful menstruation, PCOS, and obesity. It is also used for conditions that affect heart health, such as atherosclerosis (hardening of the arteries) and for high blood levels of cholesterol and triglycerides. In addition fenugreek is used for kidney ailments, a vitamin deficiency disease called beriberi, mouth ulcers, boils, bronchitis, infection of tissues beneath the skin surface (cellulitis), tuberculosis, chronic cough, chapped lips, baldness, cancer, and Parkinson's disease. Some men use fenugreek for hernia, erectile dysfunction (ED), infertility, and other male problems. Breast-feeding women sometimes use fenugreek to promote milk flow. It is sometimes used as a poultice, (wrapped in cloth, warmed, and applied directly to the skin) to treat local pain and swelling (inflammation), muscle pain, swelling of the lymph nodes (lymphadenitis), pain in the toes (gout), wounds, leg ulcers, and eczema. In food, fenugreek is included as an ingredient in spice blends. It is also used as a flavoring agent in imitation maple syrup, foods, beverages, and tobacco. Fenugreek extracts are also used in soaps and cosmetics. Fenugreek appears to slow the absorption of sugars in the stomach and stimulates insulin release; both these effects lower blood sugar levels in people with diabetes.

Therefore, based on the effectiveness of palm pollen and fenugreek on the female reproductive system, the current study evaluated the effects of

both plant seed extracts in the treatment of induced polycystic ovaries, with respect to ovary weight, structure, histology, some blood parameters, and hormone levels in adult female rats.

Material and methods

Experimental Animals

Twenty adult female Wistar rats with body weight ranging between 200–230 gm were used in the study. The animals were obtained from the animal house at King Abdulaziz University; before beginning the experiment, the animals were acclimatized to the laboratory conditions for a week by housing the rats in special cages with standard area at a temperature of 24 ± 1°C under a 12-hour dark-light cycle with free access to daily food and water. The rats were divided into four groups for the experiment; the 1st control group (CG1) included 5 females who were injected daily with 0.5 ml/kg corn oil (the dissolving agent used for estradiol valerate) for 60 days. The experimental groups were injected intramuscularly (im) with 0.5 mg/kg estradiol valerate (EV) prepared by dissolving one white 2 mg EV tablet (Climen drug used for hormone replacement therapy, from Bayer Schering Pharma) in 1 ml corn oil; this drug was administered daily for 2 months to induce the development of polycystic ovaries (PCO) in 15 female rats Lalithamma and Changamma, (2013), Farideh *et al*. (2010), Mahood (2012) and Farzadi *et al*. (2013). After the induction, the animals were then divided into 3 groups with 5 female rats per group. In the 1st experimental group the PCO-induced rats were not any subjected to any other treatment and served as the negative control group (CG2) to study the structure of the (PCOS) ovary. The 2nd PCO experimental group (Ex1) was treated with the palm pollen extract (0.5 ml/kg) daily for five weeks. The 3rd experimental treated group (Ex2) was treated orally with (0.5 ml/kg) fenugreek extract for 5 weeks; both extracts were obtained from the local market.

Ovary and blood sample collection

At the end of the experiment, the female rats were anesthetized and blood samples were collected. Blood serum was then isolated and the serum levels of estrogen, progesterone, LH, and FSH were measured using special enzyme-linked immunosorbent assay (ELISA) kits. The ovaries were removed after dissection, placed in formalin (10%), and sent to the laboratory for preparation of tissue sections. At least three fields of each slide were then examined by light microscopy. The number of primary, preantral, and Graafian follicles as well as the corpus luteum and the number of cystic follicles was determined and the mean value was calculated. One-way analysis of variance (one-way ANOVA) was used to analyze the data. To evaluate the differences between the mean values, Duncan's test was used in cases where statistical differences in the various groups were significant. Statistical analysis was performed using SPSS version 21 and $p < 0.05$ was considered as the significance level. The data from the results were calculated and compared for the different groups as Mean±SEM.

Results

Examination of the morphology of female rats revealed that estradiol valerate (EV) had an effect on their outer shape and mediated hair loss in various parts of the body, whereas the treatment with herbs restored hair loss, and the hair returned to normal. With respect to the mean body weight of the females, there was a statistically significant difference in the average weights of rats in the control group1 (CG1) and the experimental control group (CG2) ($p < 0.009$), while there were no significant differences between the other groups (Ex1 and Ex2; Table 1). There was a significant difference in the ovary weight between the negative control and treated animals (PCO and CG2, respectively; $p < 0.0001$), while there were no significant differences in the mean ovary weight and the mean reproductive system weight for the different treated PCO groups (Ex1and Ex2).

Anatomical examination of the ovaries from the rats in the control group (CG1)

Anatomical examination showed that the ovary is located behind the lower pole of the kidney, connected to the ovarian mesovarium peak called the hilum and to the abdominal muscles in the background (figure 1). The ovary is oval shaped and surrounded by the peritoneal capsule consisting of connective tissue covered with an epithelial layer. The ovary can be distinctly distinguished into two areas, the cortex (C) and the internal core or medulla core area (M), which appeared to be composed of fibrous connective tissue with many vascular and lymphatic vessels of various sizes and nerves (figure 2). The cortex area shows that the surface of the ovary was covered with one layer of

intensely stained cubic or ovoid squamous epithelial cells with rounded nuclei; this layer is the germinal epithelial tissue lying on the tunica albuginea formed of thick connective tissue. The histological section of the control ovary shows all kinds of follicles in the ovary at various stages of maturity, the primordial follicle (Pd), primary follicle (Pr), secondary follicle (Sf), and Graafian follicles (Gf); follicular atresia (A) was also observed throughout the stroma (figure 2). The ovarian stroma is composed of connective tissue that forms the body of the ovary and contains cells resembling fiber cells and collagen fibers mixed with the base material. The Pd, which represents the early stage of follicle growth, appears close to the ovarian surface under the tunica layer (T) marked by the presence of primary oocytes (Po), which are surrounded by a single layer of simple squamous epithelial flattened cells with deeply stained rectangular nuclei. The Pr is surrounded by one or two layers of follicle cells and contains the non-central site of primary oocytes (Po) surrounded by the zona pellucida (Zp) membrane. The Pr is surrounded by the theca folliculi membrane composed of the theca externa and theca interna, with blood vessels spread throughout. The secondary follicle (Sf) is larger than the Pr with centrally located Po surrounded by the Zp and containing circular nuclei. Po surrounded by more than one layer with some space appear between the follicle cells. The mature Gf are also observed with a very large space between the follicle cells called the atrium space (An). Its Po were surrounded by cumulus cells forming the corona radiata (Cr) around the ova. After ovulation from the Gf, the Gf turns into the corpus luteum (Cl), which occupies a large area of the ovary, and is formed from large follicle cells that luteinize into granulosa lutein cells, where they form patches of large polyhedral cells called gland mass cells, with blood vessels spread among them. The atritic follicle (A) contains a degenerating ovum, and some degenerating follicle cells with degenerating nucleus can be seen in the follicle antrum space. The A is surrounded by fibrous collagen with blood vessels within it.

Anatomical examination of the ovary in Ev-treated females (CG2) Anatomical examination revealed that the ovary occupies the same position as that in the control group (CG1), but an enlarged uterus was observed that was filled with fluids and fats due to inflammation, which led to a higher ovarian location compared to that in the control group (CG1). When harvesting the ovaries from the female reproductive system, the outer structure of the ovary showed multiple small sacs with deformities and a pale color (figure 9).

Histological examination of the transverse section (TS) in (CG2) female rats injected with Ev showed contraction of the ovary and changes in the shape of the ovaries compared to that of the normal ovary. Ovarian cysts appeared in large numbers and in different forms, with the development of atritic follicles (A) and bleeding inside the ovary. A deformed corpus luteum was also observed (figure 21).

The ovarian surface of samples from CG2 was covered with germinal epithelial tissue (Get) that appeared in some areas as a single layer of cells with a distorted cubic cell structure and vacuoles in the cytoplasm. In other areas, Get appeared to be composed of more than one layer of deformed cells. The tunicate cover layer (T) located underneath the germinal epithelial tissue showed some blood congestion, fibrosis, and few fibroblasts and collagen fiber (figure 22). The primordial follicles (Pd) decreased in number and were packed together properly, they were few compared to the total decomposition area, with deformed cells and blank vacuoles formed(figure 21).

The number of ovarian follicles also decreased. Of the primordial follicles (Pd) with a monolayer (Pr1) of follicle cells, some appeared normal with primary oocytes (Oo). The granulosa cells were intact, but few in number and some were distorted because they had lost their oocytes. The multiple layers of Pr2 all exhibited a distorted composition and loss of oocytes and the zona pellucida (Zp). Emergence of cellular debris and a decrease in the number of surrounding granule cells due to the formation of a cyst inside it or multiplication of follicle cells to fill up the cavity to form atritic follicles (A) was observed (fig. 22). The secondary follicles (Sf) of the CG2 group all showed a distorted structure, as their oocytes were winding the core wall with chromatin under the membrane along with the disappearance of the zona pellucida (Zp). The corona radiata cells thickness was below normal with loss in many areas around the oocytes. Of the cumulus oopharous

(CO), some were intact and some were decomposed and large. Follicle cavity (An) expansion, occurred with the absence of a theca interna, whereas the theca externa remained, preparing to form the ovarian cyst. In others the follicle cells were proliferated inside the lumen ready to form the atritic follicles (Af). In some areas, the follicle cells replicated abnormally whereas in other areas, the follicle cells were few in number because of decomposition (figure 25). The Graafian follicles (Gf) appear close to the surface of the ovary in the cortex (C), with large size, but destruction was observed at the structure where they lost the oocytes and the follicle cell forming the ovarian follicles. However, other Gf appeared to contain their oocytes (Po), but lacked the proper and natural composition, where the cavity was filled with cumulus cells and vacuoles, and the surrounding cells showed reduced thickness due to decomposition (figure 24). In other areas the Gf had completely disappeared. The corpus luteum (Cl) in some areas appeared to be composed of granular lutein granulosa cells, but at a rate lower than that observed in the normal group, with bleeding between these cells and the gaps in it (figure 25b). In other areas, the (Cl) had completely disappeared.

Anatomical examination and histology of the ovaries in herbal treated groups

Fenugreek (FR) (Ex1): The ovarian anatomical examination of adult female rats treated with the fenugreek extract revealed a return of the ovary to its normal position as that in the control group due to return of the uterus to it normal shape, with disappearance of uterine swelling. The ovarian cysts disappeared from the surface of the ovary (figure 15). Examination of the ovarian sections revealed return of the ovary to its normal structure and appearance of all types of ovarian follicles compared to that in the CG2 group. Absence of tissue necrosis was observed with very few ovarian cystic sacs observed in a specific region. A lower rate of atritic follicles (Af) compared to that in the CG2 group was observed. Bleeding was still observed in the cortex (C) and the medulla (M) area of the ovary, but the bleeding less than that observed in the CG2 group (figure 14). The germinal epithelial tissue (Get) in most areas appeared close to the normal structure, where it is composes from a single cubed layer of longitudinal regular shape (figure 15), whereas in few other areas, it appeared to be formed from more than one layer of cubed or longitudinal cells that contained vacuoles. Compared to that in the CG2 group, in Ex1, lymphocytes were few in number. The collagen fibers were scattered in the tunica cover (T), and fewer fibers were observed compared to that in the normal control group (CG1) and more than that in the treated control group (CG2). Primordial follicles (Pd) appeared largely similar to those in the control group (CG1); the number of follicles was greater compared to that in the treated control group (CG2). Some Pd had lost their oocytes (Po) but their follicle cells were normal in shape (figure 16). Primary follicles (Pr) appeared in the primary monolayer (Pr1) with normal orderly follicle cells containing more oocytes (Po) compared to those in the treated control group (CG2). Few Pr appeared small in size compared to the normal follicles, and few appeared to have lost their oocytes (Po). The multiple layers of the primary follicles (Pr2) were mostly similar to those in the normal group (CG1) with their oocytes (Oo) and the surrounding zona pellucida (Zp), and both theca layer covers. Quite a few of them appeared to be affected by the drug Ev with increasing gaps between the follicle cells, decomposed oocytes, and cavities filled with cellular debris, along with disappearance of the zona pellucida (Zp) and the theca cover (figure 19). The secondary follicles (Sf) appeared largely similar to the normal structure. Oocytes (Oo) containing the nucleus, surrounded by the zona pellucida (Zp), were clearly surrounded by corona radiata cells (Cr) along with the cumulus oophorus (Co), in addition to showing the antral cavity (An) and the theca interna and externa. The little ones were devoid of oocytes (Oo) and zona pellucida and showed lumen filled with cumulus cells (figure 21). The Graafian follicles (Gf) were still under the influence of the (Ev) drug but less than that in the CG2 group, with only the distorted cell structure and cavities filled with follicle cells. The oocytes (Oo) were popping and the zona pellucida (Zp) was irregular. The corona radiata cells (Cr) were fitted unnaturally and the theca layer cover (Tf) appeared very thin compared to the normal, with disappearance of the cumulus oophorus (Co). The antral cavity was filled with scattered follicle cells (figure 22), whereas the medulla (M) still showed blood congestion, which

was reduced to a large extent compared to that in the CG2 group.

Palm pollen extract (Pp) (Ex2): Anatomical examination of the ovaries in female rats treated with palm pollen showed return of the ovary to the same site as that occupied in the control group (CG1), and the disappearance of ovarian cysts sac from the ovary surface (figure 21). When examining the sections of rat ovaries treated with palm pollen extract, we observed return of the ovary to the normal shape and the presence of all types of ovarian follicles at greater numbers compared to the control treated group (CG2), but this number was less than that in the fenugreek treated group. Some cystic sacs were still observed in the cortex (C) compared to the control treated group (CG2); the number of cystic sacs was greater than that in the fenugreek treated group. Atritic follicles (A) appeared, but were fewer in number compared to the control treated group (CG2), and the number was similar to that in the fenugreek treated group. Some bleeding was observed in the cortex (C) and medulla (M) areas, but less than that in the control treated group (CG2) and more than that in the fenugreek treated group (figure 22). The germinal epithelial tissue (Get) featured as longitudinal cells were composed of more than one layer and in a few areas showed a flattened shape. The tunica albuginea (T) showed an increase in collagen fibers and fewer lymphocytes compared to that in the CG2 group; however, the impact of fenugreek on the germinal epithelial tissue (Get) and the tunica albuginea (T) was much better than the impact of palm pollen (Ex2). Primordial follicles (Pd) appeared almost similar to normal and were more in number compared to the number of Pd in the CG2 group; however, compared to the fenugreek treated group, the number of Pd was greater with palm pollen treatment (figure 25). Most of the primary follicles (Pr) showed an almost normal structure. The monolayer (Pr1) appeared in large numbers and more than that in the fenugreek treated group, with follicular cells surrounding the oocytes (Oo), but a limited number of follicles with empty gaps between cells and some decomposed oocytes was observed (figure 24), whereas the (Pr2) appeared in numbers fewer than in the monolayer (Pr1) and some them resembled the normal phenotype, with oocytes surrounded by a zona pellucida (Zp). The follicle cells were observed to be normal and the tunica albicans also appeared similar to the normal outer cover; however, they were fewer in number compared to the fenugreek treated group and more in number compared to the CG2 group. Some of the follicular cells appeared abnormal, including gaps, loss of oocytes and the surrounding zona pellucida, and cavities filled with cumulus cells (figure 25). The secondary follicles (Sn) were almost intact but were dispersed less regularly than those in fenugreek treated animals, where the oocyte cytoplasm was filled with cavities, appeared granulated, and contained a nucleated ovum, surrounded by the zona pellucida (Zp). The corona radiata cells (Cr) were observed and the cumulus oopharous (Co) appeared with the antrum cavity (An). Follicular cells also appeared the approach normalcy, but had very few gaps, and were surrounded by the inner and outer cover (figure 26). Some secondary follicles (Sn) showed an oocyte, but follicle cell proliferation in the follicle cavity indicated formation of an atritic follicle, while in the others, normal structure was observed (figure 26 or 40). The Graafian follicles (Gf) showed normal positioning and some of them showed distorted oocytes (Oo) and the disappearance of zona pellucida. The corona radiata cells (Cr) appeared in a small scattered number, and the tunica albican appeared, but with a thin area. Some cells were still under the influence of the Ev drug, and showed small size, loss of oocytes, and cavities filled with cumulus follicles, which were limited in thickness and cell number (figure 29).

Hormone and cholesterol levels in the control and treated groups

The estrogen levels of the induced PCO group (CG2) were very high (4300.0 pg/ml) compared to those in the normal control group (CG1) (39.33 pg/ml), the fenugreek-treated group (Fr) (Ex1) (648.60 pg/ml), and the palm pollen group (Pp) (1066.0 pg/ml). The progesterone level was 5.18 ng/ml, 2.49 ng/ml, 6.08 ng/ml, and 11.72 ng/ml in the CG1, CG2, the Fr-treated (Ex1), and the Pp-treated (Ex2) groups, respectively. The insulin level in the PCO (CG2) group (0.8 µU/ml) was high compared to that in the CG1 (0.001 µU/ml), and the Fr and Pp treated groups (0.4 µU/ml). Of the other hormones, FSH and LH showed about the same levels (0.1 ng/ml), as did prolactin (0.6 ng/ml) in all four groups

CG1, CG2, FR, and Pp groups. The cholesterol levels were higher in CG1 (68.72 mg/dl), than those in the PCO (CG2) (37.78 mg/dl), but were close to those in the Pp-treated (60.79 mg/dl) and Fr-treated groups (74.52 mg/dl) (Table 1).

Discussion

Regarding the body weight and reproductive organ weight, no differences were observed in the controls, induced PCO female rats (CG2), and treated groups, Fr and Pp. This is in agreement with the results of Lee and his coworkers (2003). They did not show any body weight fluctuations in the induced PCO and treated rats. Lalithamma and Changamma (2013) in their studies, also showed no significant changes in the body weight of induced PCO and treated rats, but observed an effect on the ovarian weight and the female reproductive system; owing to the accumulation of fat and increased fat content in the fatty tissues under the influence of estrogen in females. Treatments indicated inhibition of lipase enzyme activity, which is in agreement with the results of our study that the ovary weight was lower in the PCO treated with Ev group compared to that in the control group and both plant extract treated groups, Fr- and Pp-treated. This could be due to the side effect of the induced PCO and Ev treatment, because Ev acts as an antagonist to estrogen hormone receptors in hypothalamus tissues as described by Abdulfatah *et al*. 2009.

The histology of the ovary also revealed more abnormalities in the structure of the induced PCO (CG2) and control (CG1) groups. This is in agreement with the study by Walters *et al*.,2013 who reported the morphological changes that occur in the affected PCO due to the presence of many atritic follicles (A) including the thickness of follicle cells and disappearance of the corpus luteum. Our results show that treatment with fenugreek and palm pollen extracts almost restore normalcy of the induced PCO structure. These results agree with those in the study by Jashni *et al*. 2016, who demonstrated that oral administration of palm pollen extract could improve the induced PCO symptoms, where the average number of primary, preantral, antral, and Graafian follicles, and corpus luteum were reduced in all PCO groups compared to those in the control group. However, the number of follicles and corpus luteum were increased in the palm pollen group compared to those in the PCO (CG2) group. The ovary weight in the plant extract-treated female rats was increased compared to that in the treated PCO (CG2) group probably because the ovarian follicles grow due to the phytoestrogens present in the fenugreek and palm pollen extract, which are similar to natural estrogen. The results of this study also agree with those in the study by Jashni *et al.* 2016 that palm pollen extract can improve tissue symptoms and adjust the levels of sex hormones in polycystic ovary syndrome. Moreover, our study also indicates that treatment with fenugreek improves the structure of the induced PCO. Green tea also increases the reproduction rate in PCO rats through reduction of ovarian cysts and an increase in the appearance of corpus luteum as demonstrated by Ghafurniyan and his coworkers (2015).

In conclusion, this study shows that fenugreek and palm pollen water extracts have a positive effect on ovarian tissue in PCOS. The reduction of PCO side effects due to these herbs could be a result of the chemical compounds contained in them that have a role in influencing the structure and function of ovarian tissue. Therefore, the results of this study suggest possibilities for the use of herbal medicines used in this study, as a safe treatment in PCOS that did not lead to ovarian tissue damage.

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Table (1): The mean body and ovary weight, the mean hormone and cholesterol levels in the blood of control and induces polycystic ovary (PCO) groups of female rats treated with palm pollen and fenugreek extracts.

Mean levels Groups	Mean Body weight difference (gm)	Ovary weight (gm)	Estrogen (pg/ml)	Progesterone (ng /ml)	FSH (IU/ml)	LH (IU/ml)	Prolactin (ng/ ml)	Insulin (µU/ml)	Cholesterol (mg/dl)
Control CG1	19.5	0.53	39.33	5.18	0.1	0.1	0.6	0.00	68.72
PCO CG2	0.5**	0.023**	4300**	2.49**	0.1	0.1	0.6	0.8	37.78*
Fenugreek treated	19.0	0.52	648.6*	6.08	0.1	0.1	0.6	0.40	74.52
Palm pollen treated	17.5	0.43	1066**	11.72	0.1	0.1	0.60	0.40	60.79

*. The mean difference is significant at the 0.05 level compared to the control group (CG).
** The mean difference is significant at the 0.01 level compared to the control group (CG).

Plate I: The ovaries figures of the female rat control group (CG1): (Fig 1-6)

Fig (1): The image of the uterine horn and the ovary of the control group (CG1): showing the location of ovary in the body area.

Fig (2): A cross section micrograph of the ovary in the control group shows: the cortex (C) consisting of primary follicles (Pr), and secondary follicles (Sn) containing the follicle cavity (An) and oocytes (oo), Graafian follicles (Gf), corpus luteum (Cl), and the medulla (M) and atritic follicles (A). (Hematoxline and eosin stains) 40x

Fig (3):An optical micrograph cross section of ovary in the control group shows: epithelial tissue (G)[cubed or oval shape of epithelial cells], theca layer (T) differential thickness, primary follicle (Pd) which contains the primary oocytes (oo) surrounded by a single layer of follicle cells (flat) (hematoxline and eosin stains) 600x.

Fig.(4):A cross section micrograph in the ovary of the control group rats explains: primary follicle (Pr) both monolayer (Pr1)[one layer of cubic cells surrounding the oocytes (oo)], multiple layers (Pr2)[more than one layer (2-3) of follicle cells surrounding. The oocytes (oo) is surrounded by a zone pellucida (Zp) (hematoxline and eosin stains) 400x.

Fig (5):A cross section micrographs in the ovary of control group rats explains: secondary follicle (Sn), oocytes (oo) surrounded by a zone pelluicda (Zp) and corona radiata (Cr) connected to the zone pullucida (Zp).The appearance of antral cavity (An), surrounded by a basement membrane (Bm) followed by the thica layer (Tf) (Hematoxline and eosin stains) 100X, 400X, 600X.

Fig (6):A cross section micrographs in the ovary of control group rats shows :the corpus leutum (Cl) is composed of small groups of granule leutin cells (Gl) (Hematoxline and eosin stains) 200X, 400X.

Plate I : Picture of rat female ovaries normal control group (CG1):

Fig. 1 (CG1) fig.(2) fig, (3)

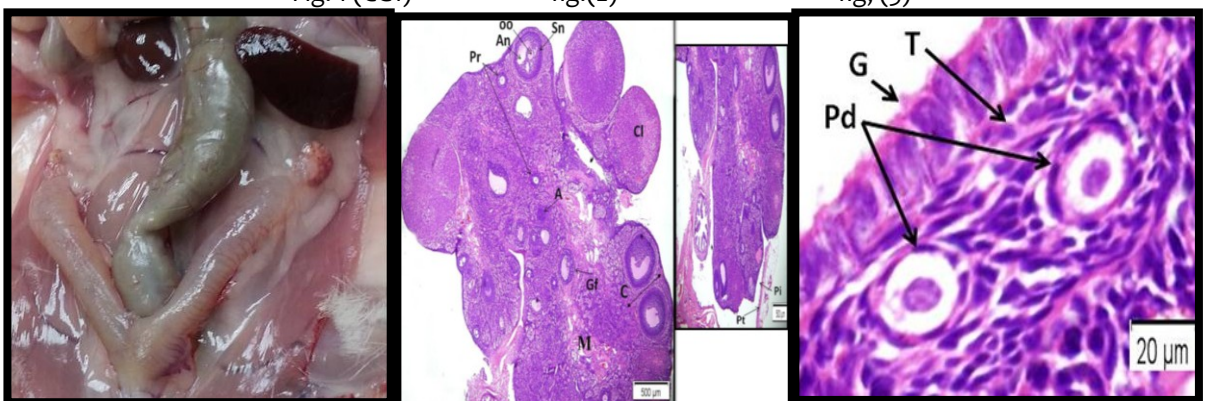


Fig. (4) fig. (5) fig. (6)

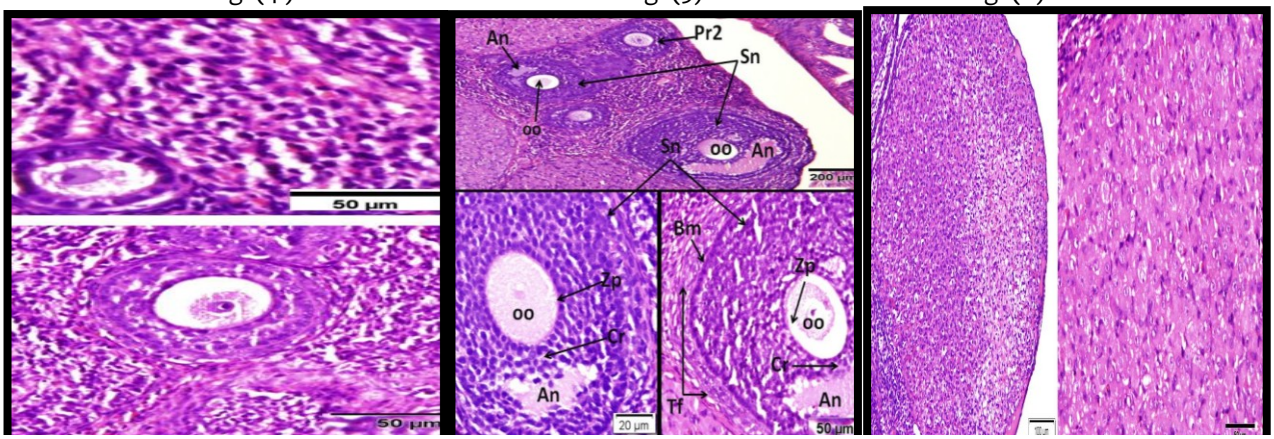


Plate II: The ovaries figures of the female rat treated control group (CG2): (Fig7-17)

Fig (7):A photograph of the dissected female rat of the treated with (EV) (PCO) control group (CG2) illustrate :the reproductive area: the ovarian site higher than its position in the control group. The external appearance of (PCO) ovaries [several small sac and enlarged uterus], clarify the (PCO) ovaries contains many small sacs. (Hematoxline and eosin stains) 200X, 400X.

Fig (8): A cross section micrographs in the ovary of treated group with (EV) PCO (CG2): shows many cystic follicles (*) with a decrease in the number of ovarian follicles. The atritic follicle (A) lot number. The absence of the corpus luteum, and bleeding (B) in the cortex (C) and medulla (M). (Hematoxyline and eosin stain) 400x.

Fig (9):A cross section micrographs in the ovary of treated group with (EV) (CG2)describes:The germinal epithelial tissue (G) distorted installation, with gaps in cytoplasm. The epithelial tissue (G) more than replicated layer of cells. Tunica layer (T) show a thin layer and its bloody congestion and fibrosis (a, b), (Hematoxyline and eosin stain) 400x.

Fig (10):A cross section micrographs in the ovary of PCO(CG2) treated group with (EV) shows: primary follicles (Pd) some are still normal intact and some are full decomposition occurred, with blank gaps. (Hematoxyline and eosin stain) 400x.

Fig (11):A cross section micrographs in the ovary of treated group (CG2) with (EV) shows: primary follicles in both type. The monolayer (pr1) some normal fitting and appeared oocytes (oo) and follicle cells, but the disappearance of other as a result of decomposition. The multiple layers (pr2) components and the emergence of cellular debris inside it and lower in the surrounding granule cells in preparation for a cyst inside it (*). Follicle cells multiply or to fill up the cavity to be as atritic follicle, (A). (Hematoxyline and eosin stain) 40x.

Fig (12):A cross section micrographs in the ovary of treated group (CG2) with (EV) shows: the secondary follicle (Sn), oocytes (oo)with improper installation, the disappearance of zone pellucida (Zp) surrounding the (oo), crown radiata cells (Cr) is intact, but with less installation compared to control, cumulus oopharus (Cu) some normal and others cracked. Significant expansion of the follicle cavity. The appearance of the theca layer (Tf), proliferation of follicle cells in regions and areas were few. (Hematoxyline and eosin stain) 400x.

Fig (13):A cross section micrographs in the ovary of treated group (CG2) with (EV) shows: Graafian follicles (Gf), (A) lost its oocytes (oo) and lack of follicle or granular cells (GC) composed ovarian cysts. (B) oocyte appeared (oo) but unconscious natural makeup that the lumen filled with cellular debris with cavity and less thickness of follicle cells (GC) surroundings it. (Hematoxyline and eosin stain) 100x,400x.

Fig (14):A cross section micrographs in the ovary of treated group PCO (CG2) with (EV) shows: Corpus luteum (Cl) consists of granular leutin cells (gl) is an abnormal installation with a bleeding between these cells and the gaps in it. (Hematoxyline and eosin stain) 40x, 200x.

Fig (15): A micrograph of cross section in female rat ovary PCO(CG2) with (EV) drug, shows: (A) germinal epithelial tissue (G) distorted form of the longitudinal layer appeared to install full of gaps and also a multiplication of its cells. (B)With flat in shape. The tunica layer area (T), (a, b) thinner and their integration with a layer of surface epithelium proliferating compared with control group, and the absence in the parts of the tissue. A bloody congestion inside the tunica layer (hematoxyline and eosin) 600x.

Fig (16): A micrograph of cross section in female rat ovary treated with (EV) drug as (PCO) (CG2), shows: the primary follicle (Pd) decomposed oocyte, which formed blank gaps. The primary follicle monolayer (Pr1) lost its follicle cells and the appearance of gaps between the cells and the (oo) irregular wall and lumen is field with cavity.(hematoxyline and eosin) 600x

Fig (17): A micrograph of cross section in female rat ovary treated with (EV) drug as (PCOS) (CG2), shows: The primary follicle multiple layers (Pr2): lost its ovum (oo) and the (Zp) and filling the cavity with empty gaps with the advent of blanks between the granule cells. The other section oocytes appeared (oo), but its distorted structure, lower number and the disappearance of (Zp). The follicle cells surrounding the (oo) are few and gaps formed between them, the disappearance of the theca layer that surrounding the follicle. Also some follicle with disappeared oocyte (oo) and featured the (Zp), but tortuous shape with the absence of the theca layer. The follicle appeared with full lyses of cells and loss all its content. (Hematoxyline and eosin) 600X.

Plate II: Picture of rat female ovaries treated control group (CG2):

Fig. (7) (CG2)

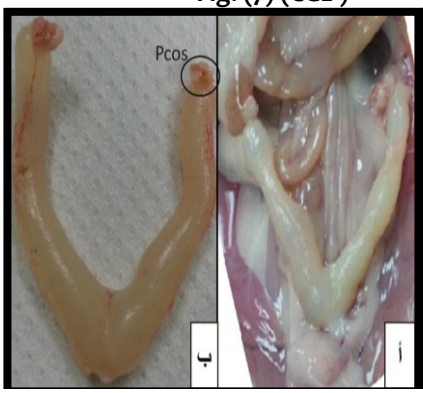
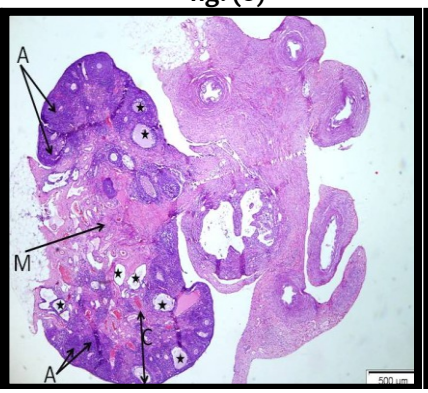


fig. (8)



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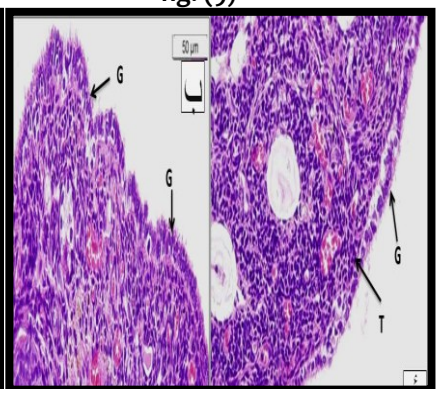
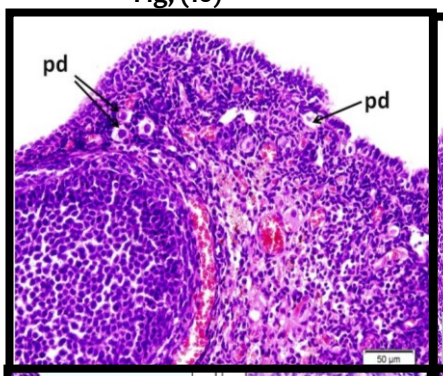


Fig, (10)



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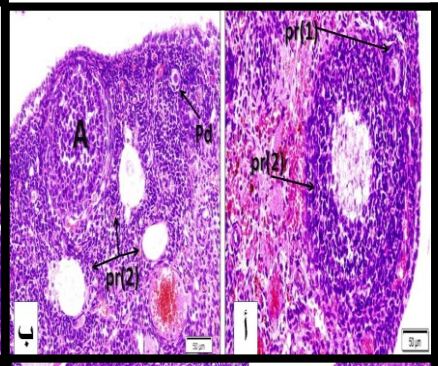
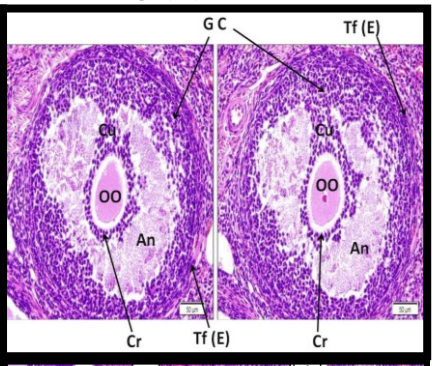


fig. (12)



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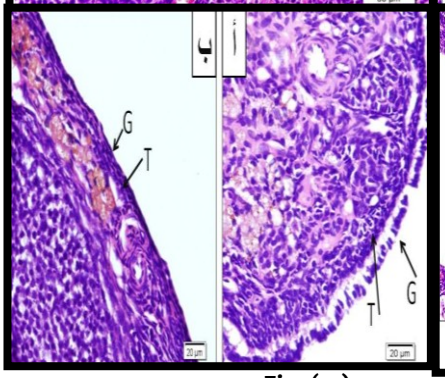

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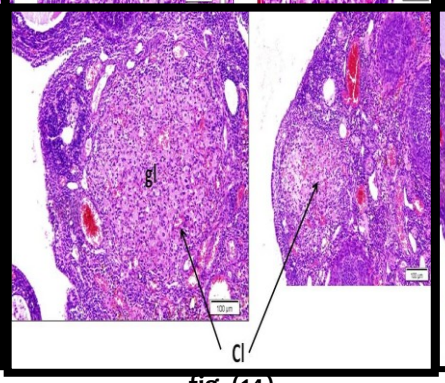

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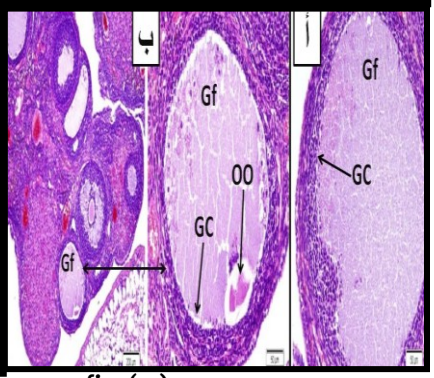

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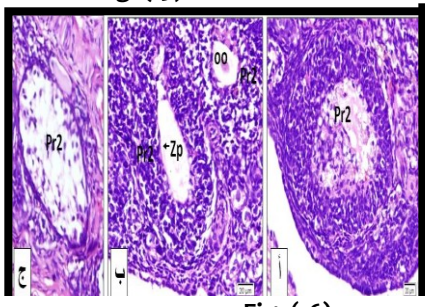

Fig (17)

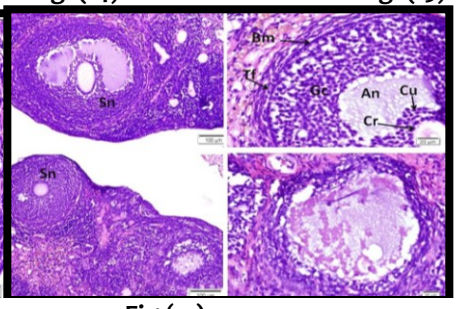

Plate III: The ovaries of female rat group treated with the fenugreek: (Fig 18-24)

Fig (18): A picture of uterine horn and the ovary of the treated female rat group (Ex1) treated with fenugreek, shows: the disappearance of ovarian cysts from the external surface of the ovary (indicated by arrow).

Fig (19): Across section micrograph in the ovary of female rat group treated with the fenugreek, shows: the return of the ovary close to normal shape and appearance of several ovarian follicle types, the primary follicle (Pr) secondary follicle (Sn) and Graafin follicle (Gf). Lack of ovarian cysts (*) low rate of atritic follicle (A).Lack of haemorrhage in the cortex(C) medulla (M) compared with the (EV) treated control group (CG2) (hematoxyline and eosin stain) 40x, 100x

Fig (20): Across section micrograph in the ovary of female rat treated group with the fenugreek, illustrating: the germinal epithelial tissue (G) in most areas cubed or elongated regular shape, while in very few areas appeared as more than one layer and contain gaps (b). the tunic cover (T) installed about normal. The primary follicle (Pd) contains (oo) surrounded by follicle cells, some of them is out the oocytes, but with proper installation follicle cells structured (hematoxyline eosin stains) 600x

Fig (21):A cross section micrograph in female rat ovary treated with the fenugreek shows: primary follicle monolayer (Pr1) with follicle cells contain oocytes (oo) and some ones out of oocytes (oo) and atritic follicle (A). (hematoxyline eosin stains) 200x,600x.

Fig (22):A cross section micrograph in female rat ovary treated with the fenugreek describes:the primary follicle multiple layers (Pr2) approach to the composition of normal control group, has appeared (oo) with zone pellucida (Zp) and tunic layer (Tf). Very few of them the show spread out the gaps between the cells and the oocytes decayed and follicle lumen filled with cellular debris and disappearance of the (ZP) and the theca layer (Tf) (*). (Hematoxyline eosin stains) 600x.

Fig (23): A micrograph of cross section in the ovary of female rat of the treated group with fenugreek, shows: the secondary follicle (Sn) somewhat close to the normal control group (CG1), the follicle cells normal structure and the oocytes (oo) containing a nucleus with enclosed zone pellucida (Zp). The appearance of corona radiated (Cr), cumulus (Cu), antrum cavity (An) and the theca (Tf). In a very view follicles out of the oocyte and the (Zp) the antrum lumen area filed with cellular debris (*) (hematoxyline and eosin) 200x, 600x.

Fig (24):A cross section micrograph in the ovary of female rat treated with fenugreek, describes: Graafian follicle (Gf) appeared distorted cells installation filed with follicle cell and the oocytes (oo) abnormally appeared with zone pellucida (Zp). Herringbone wall and cells crown radiator (Cr) very view with abnormaly installed, the theca layer (Tf) very thin compared to normal group, the disappearance of ovarian cumulus, and the lumen filed with scattered follicle cells (hematoxyline eosin stains) 600x.

Fig (25):A cross section micrograph in female rat ovary treated with fenugreek describes: a significant decrease of bloody congestion within the medulla (M) a comparison to the treated (EV) control group (CG2). (hematoxyline and eosin stains) 600x.

Plate III: Picture of rat female ovaries Fenugreek (FR) treated group (Ex1):

Fig. (18) **fig. (19)** **fig. (20)**

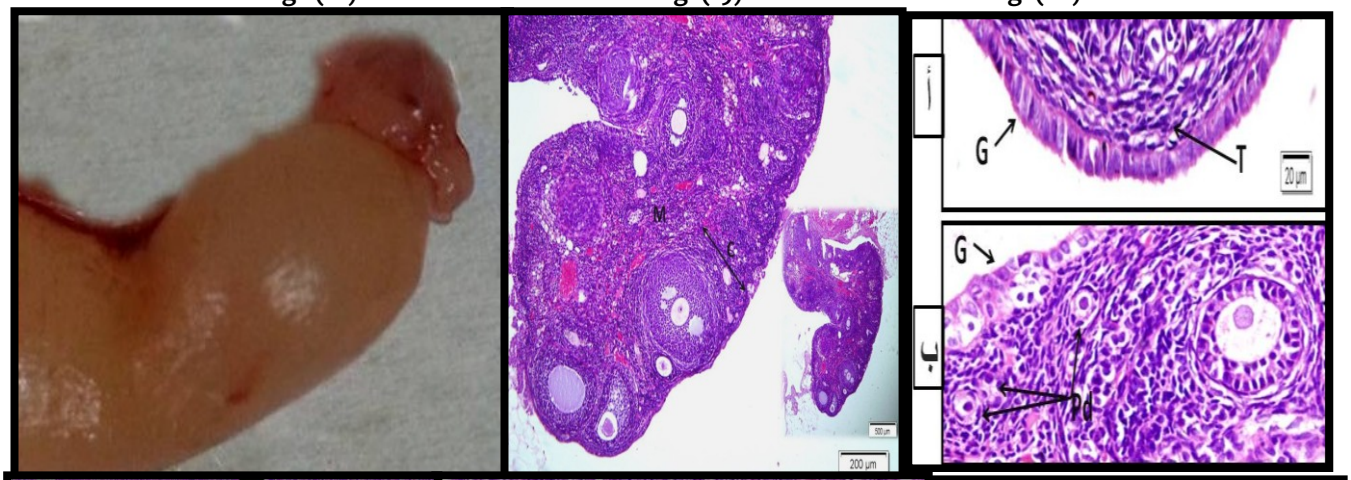

Fig. (21) **fig. (22)** **fig. (23)**

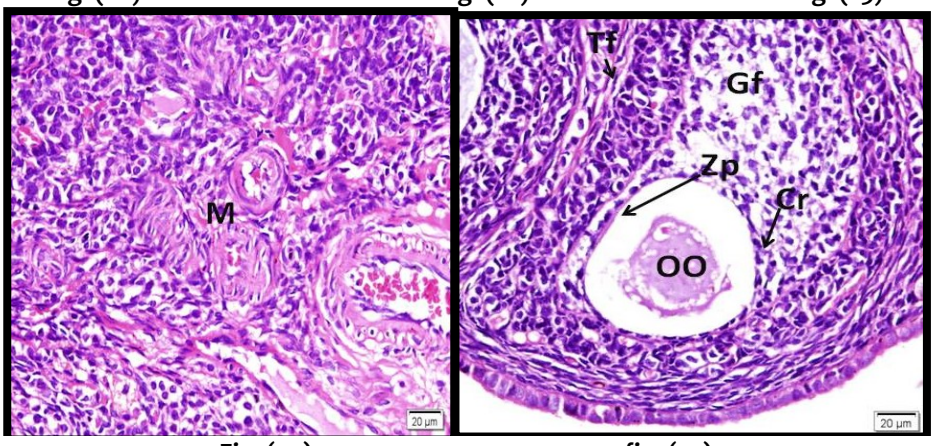

Fig. (24) **fig. (25)**

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Plate IV: The ovaries of the female rat treated group with palm pollen:(Fig 26-32)

Fig. (26): A picture of female rat uterus and ovary of the palm pollen treated group: indicate the disappearance of ovarian cysts on the external surface of the ovary (indicated by arrow).

Fig (27):A cross section micrograph in female rat ovary treated with palm pollen describes: the close return of the ovary into normal shape and appearance of the normal follicles types. There's still some ovarian sacs (*) but very few and got stuck in the cortex (C). Some atritic follicles (A) and there is a haemorrhage in the cortex (C) and medulla (M) with a smaller amount compared to the (EV) drug treated control group (CG2) , (hematoxyline and eosin stains) 40x.

Fig (28):A cross section micrograph in female rat ovary treated with palm pollen describes: the epithelial tissue (G) appeared flat. The tunica cover (T) with a similar thickness. The primary follicle (Pd) with oocytes(oo)appeared surrounded by follicle cells.(hematoxyline eosin stains) 600x

Fig (29):A cross section micrograph in female rat ovary treated with palm pollen describes: the primary follicle monolayer (Pr1) with follicle cells surrounding the ovum (oo) and some others were with decomposed oocytes. (hematoxyline and eosin) 200x, 600x.

Fig (30):A cross section micrograph in female rat ovary treated with palm pollen explains:the primary follicles multiple layers (Pr2) approach the proper installation, the oocytes appeared (oo) surrounded by zone pellucida (Zp) and the theca (Tf.). Some follicles [that are still under the influence of the (EV) drug] is out of the oocytes (oo) zone pellcida (Zp) and the cavity filled with cumulus cells (*). (Hematoxyline and eosin) 200x, 600x.

Fig (31):A cross section micrograph in female rat ovary treated with palm pollen describes :the secondary follicle (Sn) appeared almost intact and oocytes (oo) cytoplasm is full by spaces.The oocyte (oo) appeared with a nucleus and surrounded by zone pellucida (Zp), crona radiate cells (Cr), cumulus cells (Cu) and follicle cavity back (An). Also the follicle cells approach the normal, but having very few gaps, and surrounded by the theca layer (Tf).The secondary follicle (Sn) cells appeared dividing within the lumen of the follicle.(hematoxyline and eosin) 200x.

Fig (32):A cross section micrograph in female rat ovary treated with palm pollen explains: Graafian follicle (Gf) with oocytes (oo) distorted installation and the disappearance of the (Zp), with featured corona radiate cells (Cr) few and scattered with few follicle cells, and theca layer (Tf) is a small thickness. Some other follicles under the influence of the (EV) drug were small in size and lost its oocytes with cavity filled with cumulus cells and limited in thickness and follicle cell number(*). (hematoxyline and eosin) 200x.

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Plate IV: Picture of rat female ovaries Palm pollen (Pp) treated group (Ex2):

Fig (26) **fig. (27)** **fig. (28)**

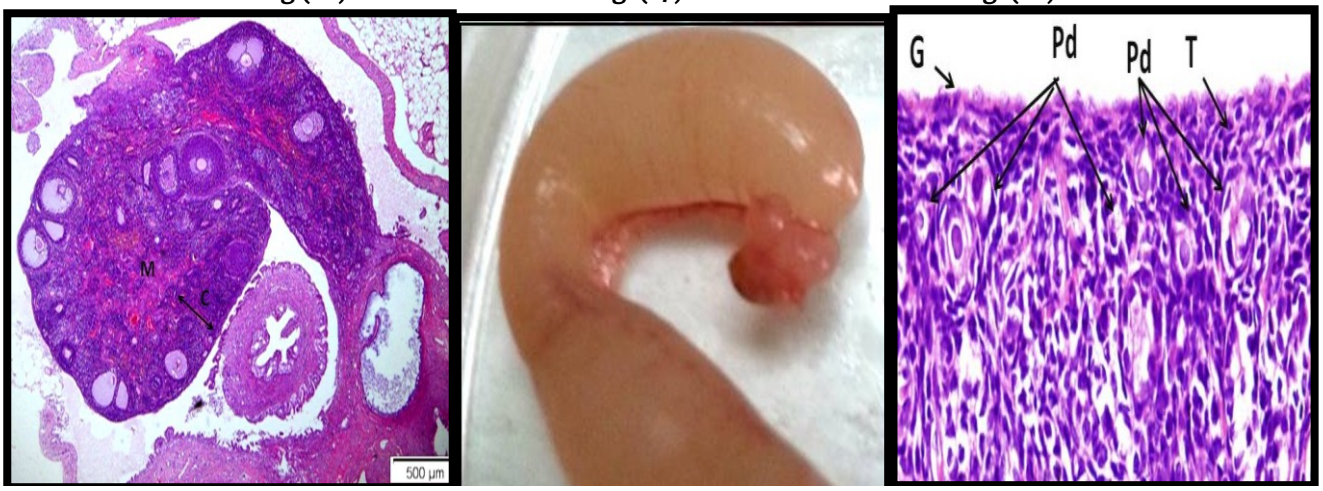
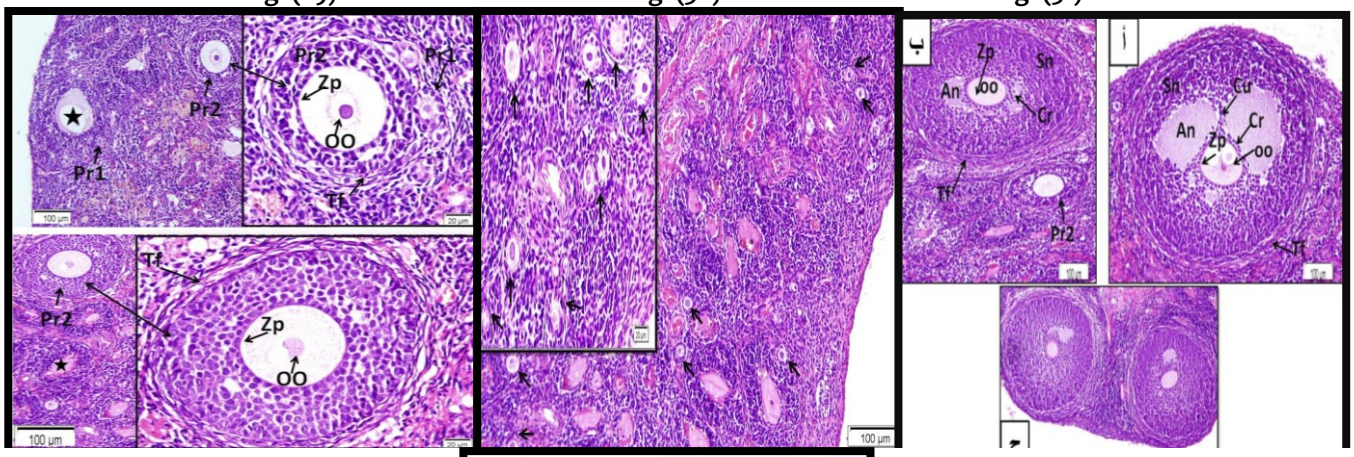


Fig. (29) **fig. (30)** **fig. (31)**



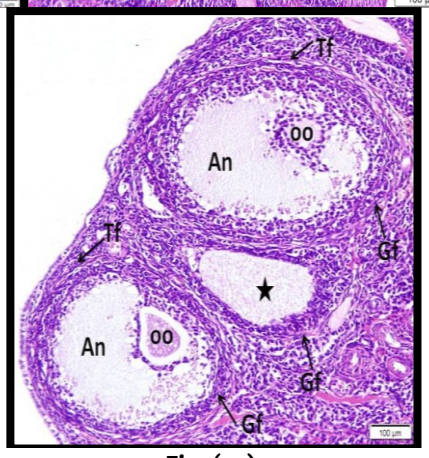


Fig. (32)