

Pathological Changes in Genital Organs of Female Albino Mice After Treatment with Pentoxifylline

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Abstract

- Background** Little to our knowledge has been attempting to show the effect of pentoxifylline (PTX) on ovulation or oogenesis. In a few studies of man with asthenospermia oral treatment with PTX produced significant increase in sperm concentration and motility.
- Objective** Study the effect of PTX on ovulation and oogenesis in albino mice.
- Methods** Sixty albino mice randomly divided into 6 equal groups: Group 1 received tap water and considered as a control group. Group 2 treated with 16 mg of PTX/Kg/BW/daily for 2 weeks. Group 3, 4, 5, and 6 treated with same dose for 4, 6, 8, 10 weeks respectively. Post-mortem examination done according to the time of treatment and the reproductive systems were excised and processed for light microscopic examination.
- Result** PTX administration causes an increase in the diameter of the ovary and in the total number of ovarian follicles and their diameters especially Graafian and secondary follicles. In addition, highly significant increase in the numbers of corpus lutea especially in those groups treated for longer time was noticed. Moreover, an increase in the thickness of uterine and oviduct epithelial lining due to hyperplasia and an increase in the diameter of endometrial glands and oviducts was demonstrated.
- Conclusions** We concluded that administration of PTX has a significant effect on the female genital organs especially if given in small doses for 10 weeks. This will definitely influence reproduction and litter size manifested after mating with untreated males.
- Key words** Pentoxifylline, ovary, uterus, fallopian tube

Introduction

Reproductive efficiency is the major factor that affects profitability in many live stock production systems. Inefficient reproduction may be caused by numerous factors; including increased genetic selections for meat or milk production traits, early embryonic and fetal loss, failure to reach puberty at an optimum age or an inability of young females to conceive early in the breeding seasons, environmental stress such as temperature extremes or changes in photoperiod (day and night cycle),

production of sperms with a low potential for fertilization, and limited sex drive^(1,2).

Pentoxifylline (PTX) is a dimethyl xanthine derivative belongs to a group of vasoactive drugs used in humans for the treatment of peripheral and cerebral vascular diseases caused by impairment of the microcirculation⁽³⁾. PTX is used also in the treatment of male infertility in human by enhancing sperm motility both *in vivo*⁽⁴⁾ and *in vitro*⁽⁵⁾, in cases of normozoospermia and asthenozoospermia⁽⁶⁾. It has an inhibitory effect on

Phosphodiesterase that enhances sperm motility by increasing intracellular cAMP⁽⁷⁾.

In veterinary practice, it is used to improve microcirculation and enhance healing of skin lesions in dogs, and for the treatment of endotoxemia, laminitis, navicular disease, and improve motility of the cryopreserved spermatozoa in stallions⁽⁸⁾.

Studies on reproductive performance in rats, mice, and rabbits revealed no evidence of impaired fertility or harm to the fetus due to PTX⁽⁹⁾. In addition, females with endometriosis-associated infertility may get benefit from the use of PTX without significantly affecting embryo development⁽¹⁰⁾.

To the best of our knowledge little studies has been attempted to show the effect of PTX on ovulation or oogenesis. Therefore, the aim of this study is to accomplish this task and study its effect on some parts of the genital organs like ovary, oviduct, uterus, and vagina.

Methods

1- Experimental design:

Sixty mice were divided into 6 equal groups, after labeling them (at ear or tail) and weighing them using mechanical balance as follows:

G₁: treated orally with tap water, which was regarded as a control. G₂: treated orally with 16 mg/B.W/daily for 2 weeks. G₃: treated orally with 16 mg/B.W/daily for 4 weeks. G₄: treated orally with 16 mg /B.W/daily for 6 weeks. G₅: treated orally with 16 mg/B.W/daily for 8 weeks. G₆: treated orally with 16 mg/B.W/daily for 10 weeks.

2- Treatment:

PTX is presented in the form of coated tablets containing 400mg (Aventis USA).

160 mg of the coated tablets were dissolved in 100 ml of tap water to obtain a stock solution from which 0.1 ml was given orally for each 10 gm of the living body weight of the experimental mice. This amount of the solution will provide a dose of 16 mg/kg Bw/day of the drug. The dose was individually adjusted according to the weight of each animal and was given via a fine plastic stomach tube given to

G₂, G₃, G₄, G₅ and G₆. Control group (G₁) was given tap water only.

3- Parameters:

Clinical signs and symptoms and macroscopic and microscopic findings.

A- Clinical signs and symptoms:

Clinical signs were closely observed and continuously recorded along the period of experiment which is 10 weeks, also any change in activity or behavior was noted.

B-Macroscopic and microscopic examination:

At the end of each experimental group, the animals were anesthetized, then the abdomen of each animal was opened and the organs wanted were excised. Then the genital organs were examined for any changes in size, and color then small pieces were kept in 10% neutral buffer formalin for fixation processed routinely in histokinette, cut at 5µm thickness by microtome and stained with haematoxylin and Eosin stain then examine under light microscope⁽¹¹⁾.

Parameters used in studying the tissue section are:⁽¹²⁾

A. Ovaries:

Diameter of the ovary, number of all follicles, number and diameter of Graafian (antral) follicles (GF), number and diameter of submature follicles, and number of corpus luteum (CL).

B. Oviducts:

Diameter of oviduct (ampulla) and thickness of epithelial cell layer.

C. Uterus:

Diameter of endometrial glands and thickness of epithelial layer.

Data from treated and control groups were expressed as Mean± Standard error of Mean (S.E.M) and analyzed using student's t-test Differences between values were considered significant at $p < 0.05$ and highly significant at $p < 0.01$ ⁽¹³⁾.

Results

1- Clinical Signs and Symptoms:-

All treated animals were active and showed an increase appetite for food consumption during

the study period, while the control group showed normal food consumption.

2- Macroscopic findings:-

There were no clear macroscopic changes, except that there were differences in dimensions of the genital organs. The morphological changes in the diameter of ovary were highly significant ($p < 0.01$) increased in G3 and G6 (1386 ± 4.15 , 1412.7 ± 4.23) μm respectively with a significant increase ($p < 0.05$) in G2, G4 and G5 (1306.8 ± 6.53 , 1159.3 ± 4.60 , 1158 ± 4.603) μm respectively compared with that of the control group (1009.8 ± 0.56) μm (Table 5). Gross examination showed an enlargement of the genital organs (Figure 1).

3- Histopathological examination

1- Ovaries:

There were no any inflammatory changes in all examined genital organs. The microscopic examination showed highly significant increase in the diameter of the ovaries ($p < 0.01$) in G3 and G6 (1386 ± 4.15 , 1412.7 ± 4.23) μm , and a significant increase ($p < 0.05$) in G2, G4 and G5 (1306.8 ± 6.53 , 1159.3 ± 4.60 , 1158 ± 4.63) μm respectively compared with that of control (1009.8 ± 8.56) μm (Table 5). The diameter of the primary follicles showed a significant increase ($p < 0.05$) in G2, G3 and G4 (118.56 ± 3.31 , 71.63 ± 3.58 , 74.1 ± 3.70) μm compared with that of the control group (93.86 ± 0.89) μm . The diameter of secondary follicles showed highly significant increase ($p < 0.05$) in G5 and G6 (407.55 ± 4.07 , 427.31 ± 3.84) μm with significant increase ($p < 0.01$) in G2, G3 and G4 (271.7 ± 3.53 , 333.45 ± 3.01 , 365.65 ± 2.19) μm . The diameter of Graaffian follicle was significant in G3, G4, G5 and G6 (363.09 ± 2.54 , 382.85 ± 2.67 , 382.85 ± 3.06) μm (Table 1 and Figures 2-4).

Concerning the total number of follicles, there is a highly significant increase ($p < 0.01$) in G6 (25.0 ± 0.5) and a significant increase ($p < 0.05$) in G2, G3, G4 and G5 (20.0 ± 0.6 , 21.0 ± 0.63 , 23.0 ± 0.46 , 23.0 ± 0.69) respectively compared to the control (18 ± 0.36). The numbers of primary follicles showed a high significant

increase ($p < 0.01$) in G2 (12.0 ± 0.36) and a significant increase in G3, G4, G5, and G6 (8.0 ± 0.24 , 6.0 ± 0.24 , 3.0 ± 0.21 and 8.0 ± 0.56) respectively compared with that of the control (3.0 ± 0.12). The numbers of secondary follicles showed a significant increase ($p < 0.05$) in G₃, G₄, G₅ and G₆ (5 ± 0.15 , 4 ± 0.12 , 4 ± 0.12 , 4 ± 0.16) respectively compared with that of control (3.0 ± 0.12). The numbers of Graaffian follicles showed significant increase ($p < 0.05$) among all treated groups (3.0 ± 0.18 , 5.0 ± 0.15 , 5.0 ± 0.1 , 6.0 ± 0.18 , 6.0 ± 0.12) respectively compared with that of the control (4.0 ± 0.16). The numbers of corpus luteum were highly significant increase ($p < 0.01$) in G5 and G6 (8.0 ± 0.24 , 12.0 ± 0.24) and the increment was higher than that of the control group. G2 and G3 showed significant increase ($p < 0.05$) (3.0 ± 0.18 , 3.0 ± 0.21) compared with that of the control (Table 2 and Figure 5).

2- Uteri and oviducts changes:

All treated groups showed proliferation of the lining epithelium due to hyperplasia leading to increase in the thickness of the epithelial lining, with presence of mitotic figures. The increase changes are highly significant increased ($p < 0.01$) in G3, G4, G5 and G6 (37.05 ± 0.79 , 49.4 ± 0.82 , 61.75 ± 0.92 , 86.45 ± 2.43) respectively, while G2 showed significant increase ($p < 0.05$) in the thickness compared to that of the control (12.35 ± 4.03) μm . The endometrial glands became larger and appear with more numerous colloids in their lumen. The endometrial glands diameters showed highly significant increase ($p < 0.01$) in G3, G4, G5 and G6 (54.34 ± 0.62) μm for each groups, while G2 showed significant increase ($p < 0.05$) (43.225 ± 0.74) μm compared to that of the control (33.11 ± 2.07). (Table 3 and Figures 6-9). Similar changes to uteri were detected in the mucosa of oviducts, the thickness of epithelial lining cells was highly significant increased ($p < 0.01$) in G4, G5 and G6 (123.5 ± 10.8 , 138.32 ± 4.22 and 163.02 ± 2.99) μm respectively, while G2 and G3 showed a significant increase ($p < 0.05$) (86.45 ± 2.44) μm for each as compared with that of the control (59.28 ± 2.81)

µm, also the results showed an increase in the diameters of oviducts (ampulla region) with highly significant increase ($p < 0.01$) in G₃, G₄, G₅, and G₆ (326.7±15.2, 346.5±15.1, 485.1±18.6 and 514.8±21.2) µm respectively, while G₂ showed a significant increase ($p < 0.05$) (277.2±4.66) µm as compared with that

of the control one (247.5±3.33) µm (Table 3 and figure 10).

Other pathological changes were an increased mucus secretion of the cervix, which projects as fine strands into the lumen (Figure 11), and hyperkeratosis of the vagina (Figure 12) especially in the animals treated for 10 wks (G6).

Table 1. Morphological changes in the diameter of the ovary after oral administration of pentoxifylline to mature female mice

Diameter (µm)	Control group	2 weeks treatment	4 weeks treatment	6 weeks treatment	8 weeks treatment	10 weeks treatment
	G1	G2	G3	G4	G5	G6
Diameter of ovary	1009.8±0.56	*1306.8±6.53	**1386±4.15	1159.3±4.60	*1158±4.63	**1412.7±4.23
Diameter of G.F	345.8±0.89	358.15±4.65	*363.09±2.54	*382.85±2.67	*382.85±3.44	*382.85±3.06
Diameter of secondary F.	222.3±3.99	*271.7±3.53	*333.45±3.01	*365.65±2.19	**407.55±4.07	**427.31±3.84
Diameter of primary F.	93.86±0.89	*118.56±3.31	*71.63±3.58	*74.1±3.70	93.86±2.81	93.86±2.81

Values are mean ± standard error (SEM), (n=6 animals/groups), *Significant changes ($p < 0.05$), ** Highly significant changes ($p < 0.01$); G.F: Graafian follicle.

Table 2. Morphological changes in the number of ovarian follicles after oral administration of pentoxifylline to mature female mice

Follicles number	Control group	2 weeks treatment	4 weeks treatment	6 weeks treatment	8 weeks treatment	10 weeks treatment
	G1	G2	G3	G4	G5	G6
No. of all follicles	18±0.36	*20±0.6	*21±0.63	*23±0.46	*23±0.69	**25±0.5
No. of G.F	4±0.16	*3±0.18	*5±0.15	*5±0.1	*6±0.18	*6±0.12
No. of secondary F	3±0.12	3±0.18	*5±0.15	*4±0.12	*4±0.12	*4±0.16
No. of primary F	5±0.1	**12±0.36	*8±0.24	*6±0.24	*3±0.21	*8±0.56
No. of C.L	2±0.14	*3±0.18	2±0.14	*3±0.21	**8±0.24	**12±0.24

Values are mean ± standard error (SEM), (n=6 animals/groups), *Significant changes ($p < 0.05$), ** Highly significant changes ($p < 0.01$); G.F: Graafian follicle, C.L: Corpus luteum.

Table 3. Morphological changes in the uteri and oviducts after treatment with pentoxifylline to mature female mice

(µm)/Diameter	Control group	2 weeks treatment	4 weeks treatment	6 weeks treatment	8 weeks treatment	10 weeks treatment
	G1	G2	G3	G4	G5	G6
Ut.Epi.	12.35±4.03	*24.7±0.52	**37.05±0.79	**49.4±0.82	**61.75±0.92	**86.45±2.43
E.G.	33.11±2.07	*43.225±0.74	**54.34±0.62	**54.34±0.62	**54.34±0.62	**54.34±0.62
OD.Diam	247.5±3.33	*277.2±4.66	**326.7±15.2	**346.5±15.1	**485.1±18.6	**514.8±21.2
OD.epith	59.28±2.81	*86.45±2.44	*86.45±2.43	**123.5±10.8	**138.32±4.22	**163.02±2.99

Values are mean \pm standard error (SEM), (n=6 animals/ groups), *Significant changes ($p < 0.05$), ** Highly significant changes ($p < 0.01$), Ut.Epi.: Uterine epithelia., E.G.: Endometrial gland, OD. Diam: Oviduct diameter, OD.epith: Oviduct epithelia.



Figure 1: Genital system of one animal treated with (16 mg/kg bw/day for 6 wks) shows the enlargement of the uterus (\rightarrow) with thickening of the uterine wall.

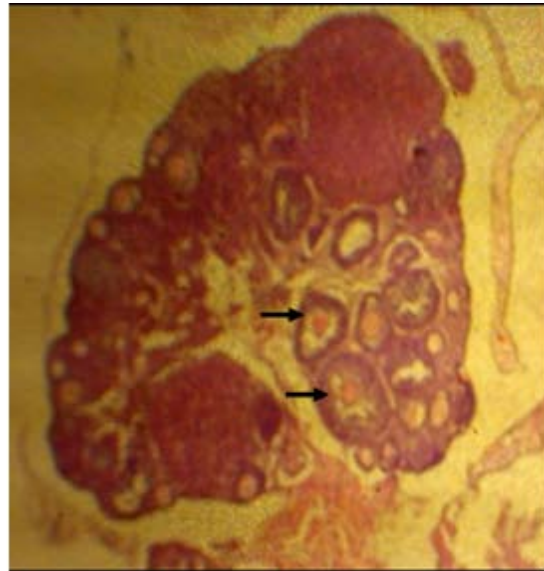


Figure 2: Histopathological section of the ovary from one animal treated with (16 mg/kg bw/day for 2 wks) showed increase in the diameter of the ovary and follicles (\rightarrow) (H&E X 100).

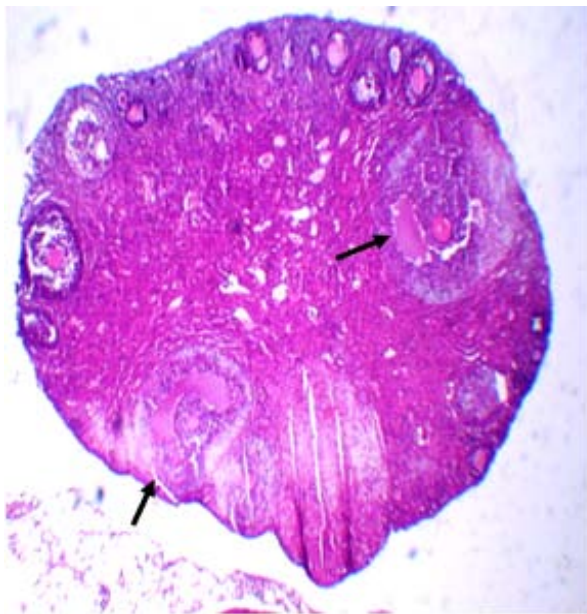


Figure 3: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 4 wks) showed increase in the diameter of the ovary and follicles (\rightarrow) (H&E X 100).

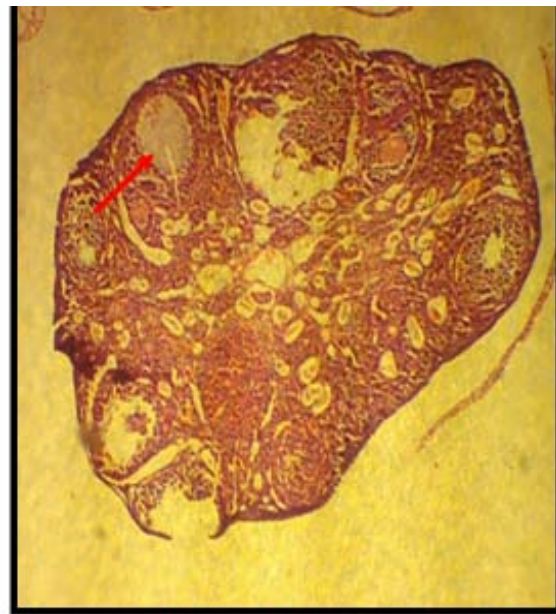


Figure 4: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 6 wks) showed large number of follicles in different stages with congestion of blood vessels (\rightarrow) (H&E X 100).

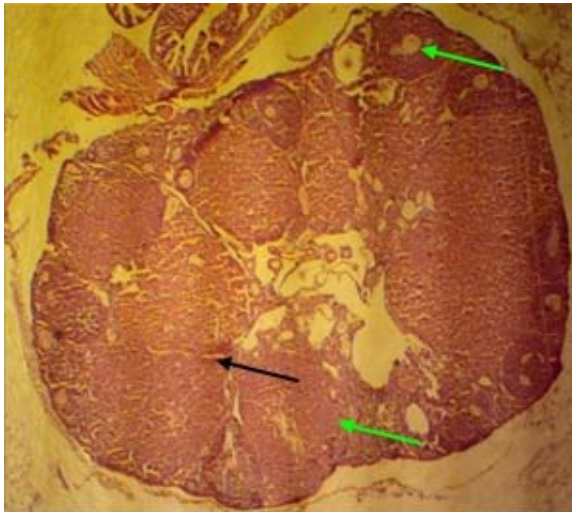


Figure 5: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 6 wks) shows the presence of large number of corpus lutea (→), and the increase in the diameter of the ovary and the number of follicles (→) (H&E X 100).

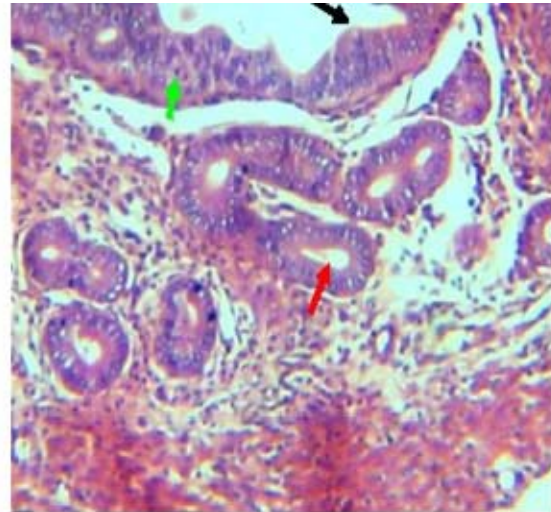


Figure 6: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 6 wks) shows the hyperplastic endometrial lining cells (→) with presence of mitotic figure (→) and the dilated endometrial glands (→) (H &E, X 100).

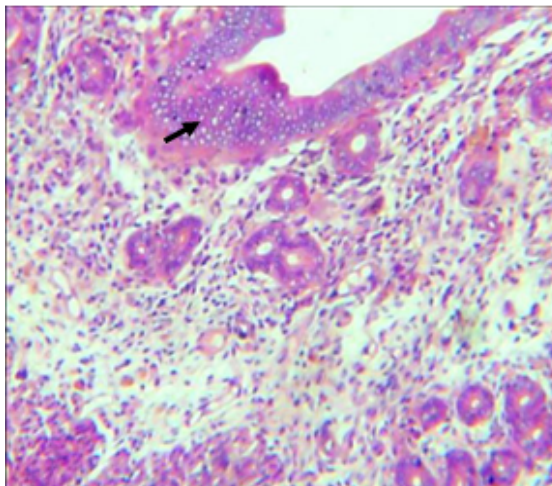


Figure 7: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 8 wks) shows marked thickness of epithelial lining cells of the endometrium also increase number of stromal cells (→) (H&E, X 100).

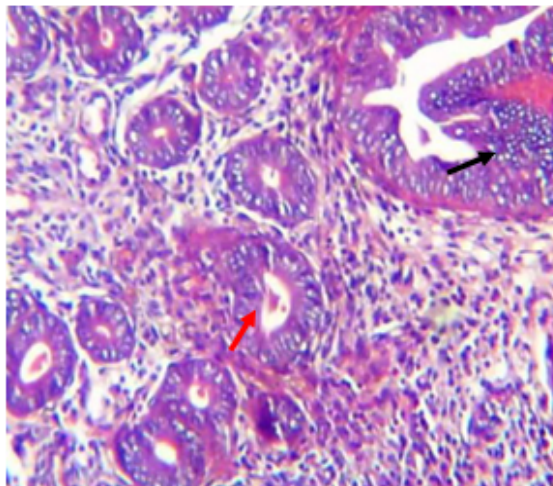


Figure 8: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows marked hyperplasia of epithelial of endometrium with formation of papillary projections toward the lumen of the uterus with marked proliferation of stromal cells (H &E, X 100).

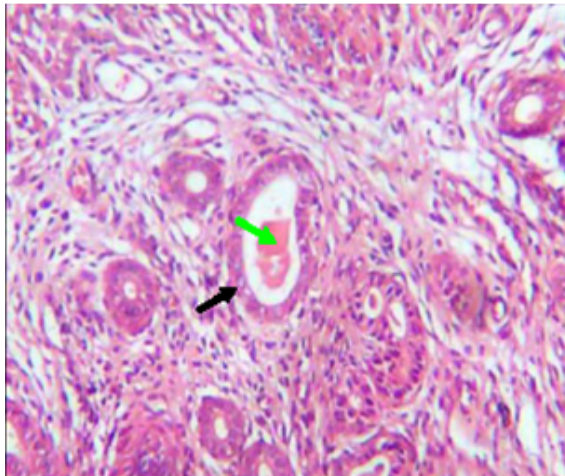


Figure 9: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows proliferation of fibrous connective tissue around the endometrial glands leading to dilatation, many of these glands contain colloids (H &E, X 100).

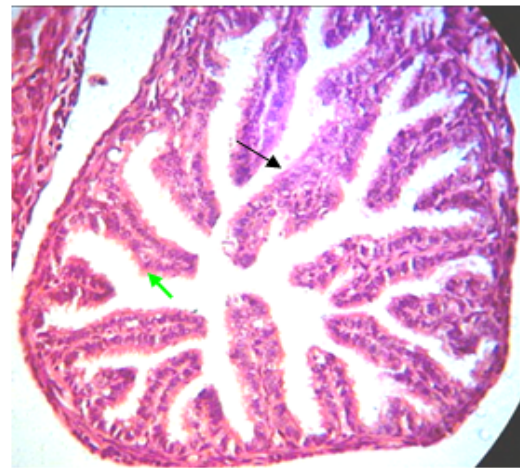


Figure 10: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows marked thickness of epithelial lining mucosa with proliferation of epithelial lining cells forming the branching papillary projections towards the lumen (→) with increase in the number of cilia (→) (H & E. X 40).

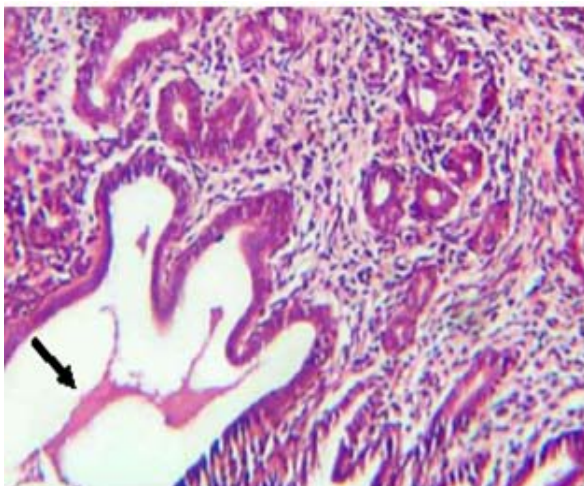


Figure 11: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows the increase in the secretion of mucus seen as fine strands in the lumen (→) (H&E, X 100).

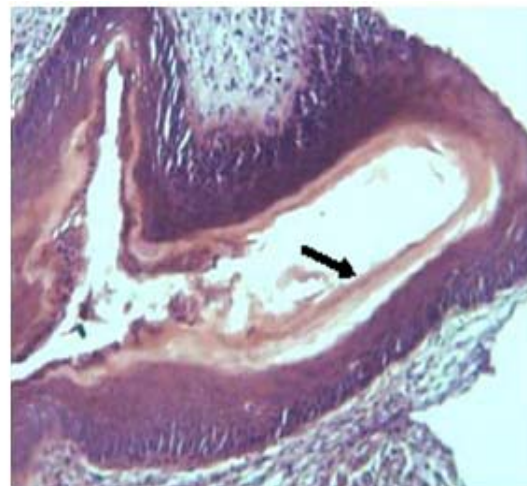


Figure 12: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows the increase amount of keratin (→) (H&E, X 100).

Discussion

The microscopic findings in ovaries revealed a clear increase in the mean of ovarian diameters and an increase in the total number of follicles and in their diameters with acquisition of more number of corpus luteum (C.L) in the treated groups as compared with that of the control group which showed formation of few (C.L)

especially in those groups that were treated for longer duration. These findings are due to increasing levels of estradiol (E2), follicular stimulating hormone (FSH) and luteinizing hormone LH⁽¹⁴⁾. Follicular growth and development depends on the production of E2⁽¹⁵⁾. E2 acts within the follicle as autocrine or

paracrine manner, it promotes the proliferation of granulosa cells and increases their response to FSH, also stimulate the proliferation of theca interna cells, and these events give the follicle progressively greater capacity to produce E2 and makes it increasingly sensitive to FSH as it matures. By these actions, E2 increases its own production, simultaneously, E2 and FSH induce granulosa cells to create receptors for LH, in preantral follicles granulosa cells have few receptors for LH which are not responsive to LH which in contrast, the granulosa cells of preovulatory follicles have abundant LH receptors and consequently have acquired sensitivity to LH⁽¹⁶⁾.

The positive results of uteri due to treatment with PTX consisting of hyperplasia of uterine epithelial lining cells leading to increase in the thickness of uterine epithelial lining cells, and hypertrophy of endometrial glands reflected as an increase in the diameter of endometrial glands. These results reflect the importance of oral treatment of the drug which lead to the elevation of E2⁽¹⁴⁾. Since it is well known that estrogen causes marked cells proliferation in the mucosal cell lining and greatly increases development of the endometrial glands which later aids in providing nutrition to the implanted ovum⁽¹⁷⁾. Although, the increase in the thickness of endometrium improves the pregnancy rate in females having a thin endometrium before treatment⁽¹⁸⁾. The only risk factor in treatment for more than 10 wks is that hyperplasia may lead to neoplasia⁽¹⁹⁾.

Estrogen has important effects on mucus secretion of the cervix, it increases the secretion of mucus, which become abundant, clear, and non-viscous. All these characteristics are most pronounced at ovulation and allow sperms, which are deposited in the vagina, to move easily through the mucus on their way to the uterus and uterine tubes⁽²⁰⁾.

Oviduct observations can be attributed to the increase in E2 level⁽¹⁴⁾. The influence of the steroidal hormones on the fallopian tubes appear to be quite significant, since their

effects on the mucosal lining of fallopian tubes are similar to those seen in the uterine endometrium.

They cause the glandular tissues to proliferate and, they cause an increase in the number of ciliated epithelial cells of the fallopian tubes also, the activity of the cilia is considerably enhanced, these cilia help to propel the fertilized ovum in the direction of the cavity^(16,21), and this is exactly seen in the treated groups compared with that of control group.

The experiment showed the treated groups enter subsequent estrus cycles because of the elevation in the reproductive hormones, which might lead to improvement in folliculogenesis. Estrogens cause the vaginal epithelium to proliferate and to show an increased cornification^(22,23). This was clear in both vaginal smears and tissue sections in addition to the elevation in the LH, which is the physiological signal for ovulation⁽¹⁶⁾.

All previous results revealed to the effect of PTX on different parts of the genital organs, which might reflect itself on reproductions and the number of new generations.

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