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The therapeutic effect of *Silybum marianum* on the Lead Acetate Induced - Reproductive Toxicity in Both Gender Laboratory Rats

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التأثير العلاجي لنبات الكعوب*Silybum marianum* على خلات الرصاص المستحث للسمية التناسلية في كلا الجنسين للجرذان المختبرية

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الخلاصة

الهدف من البحث الحالي هو دراسة التاثير العلاجي للخلاصة الميثانولية لنبات الكعوب على التغيرات المرضية السمية للاعضاء التكاثرية الذكرية والانثوية للجرذان الناجمة عن التسمم بخلات الرصاص. اوضحت النتائج وجود تغيرات مرضية سمية واضحة في انسجة الخصية و البربخ و القناة الاسهرية للذكور وفي ارحام ومبايض الاناث مع حصول افات مهيأة للتسرطن في كل من الرحم والبربخ بعد شهرين جراء التعرض لخلات الرصاص. اما بعد العلاج بالخلاصة الميثانولية للنبات فقد قلت شدة التغيرات المرضية بالتدريج انتهاءً بالوصول الى مرحلة الشفاء.

مفاتيح الكلمات: خلات الرصاص، نبات الكعوب، السمية التناسلية

Summary

The aim of the study was attempted to make knowledge on the therapeutic effect of *Silybum marianum* methanolic extract on both gender rats on reproductive toxicity induced by lead acetate. Results showed toxicopathological changes in testis, epididymis, ductus deferens, uterus and ovaries after two months. Treatment with plant extract the severity of lesions decreased gradually until reaching the stage of resolution.

Key words : lead acetate, *Silybum marianum*, Reproductive toxicity

Introduction

Many industrial chemicals are known to have a negative impact on human reproduction (1), particularly occupational and environmental exposures to heavy metals such as lead (2). Of all of the toxic metals, lead seems to pose the greatest threat to male infertility (3). Experimental animal studies, mainly in rats, have reported that lead is an active element responsible for male reproductive parameter imbalances (4,5). Lead was used in the past to induce an abortion, and severe lead intoxication has been shown to result in infertility and miscarriage, women who just live in lead-polluted areas have also shown a greater risk of miscarriages (6). *Silybum marianum* commonly known as milk thistle an annual or biennial herb belong to the family Aesteraceae . It protects the liver and assists in detoxification process by increasing glutathione supply in the liver. Glutathione is the enzyme primarily involved in the detoxification of toxic heavy metals including lead (7).

There are few data referring to lead impact on reproductive system and the effect of *Silybum marianum* on genetalia. Therefore, the aim of this study is to accomplish this task and study its effect on some criteria of both gender genital organs like testis, epididymis, ductus deferens, uterus and ovary.

Materials and Methods

Plant collection

Silybum marianum was collected from Baghdad at May 2007, and was shed and dried at room temperature. A voucher specimen of the plant was deposited to be identified and authenticated at the Science College of Baghdad University by Dr. Ali AL-Mousaway (Certificated No. 539 in 29/5/2005).

The dried plant was separated into : roots and aerial parts, then the aerial (leaves and barks) parts were ground into powder by coffee electrical grinder (mesh No. 50), and the powdered parts were undergo the primary analysis diagnosis for its component (8).

Preparation of methanolic extract of *Silybum marianum*

According to Harborne and Mabray (9), methanolic extract of plant *Silybum marianum* was prepared as follows:

1) A liquotes of 50 grams of the powdered plant were suspended in 200 ml of 70% methyl alcohol in Erlyn Myer flask and stirred on magnetic stirred over night at 45 ºC.
2) After 24 hrs, the sediments were filtered by gauze and then by filter paper.
3) Steps (1) and (2) were repeated 4-5 times.
4) The pooled extract was evaporated to dryness (45 ºC) under reduced pressure in rotary evaporator.
5) The weight of crude extract resulted from that amount of powdered plant was measured.
6) The crude extract then was kept at -20 ºC until the time of use.

For following experiment, 1 gm of powdered plant extract was dissolved into 10 ml Phosphate Buffer Saline(PBS) (as a solvent), the suspension then filtered and sterilized by using 0.4 mm sterile Millipore filter and kept in deep freeze (-20 ^{o}C) until use.

Experimental design :

Forty male and female rats aged (8 weeks) and weight (45-60 gm) were divided equally into 4 groups (5 males and 5 females for each group).

Group I : Rats served as control (C) and received distilled water for two months.

Group II : Rats served as experimental and received by gavage lead acetate diluted in distilled water at 75 mg/kg. B.W./day for two months.

Group III : Rats served as experimental and received by gavage lead acetate diluted in distilled water at 75 mg/kg. B.W./ day for one month and then received by gavage *Silybum marianum* methanolic extract at 350 mg/kg. B.W./ day for one month.

Group IV : Rats served as control and received distilled water for one month and then received by gavage *Silybum marianum* methanolic extract at 350 mg/kg. B.W./ day for one month.

After one month 2 animals of group II and III were sacrificed under deep anesthesia to detect lead toxicity then at the end of experiment testis, epididymis, ductus deferens, uterus and ovary were dissected out, fixed with buffered formalin. The specimens were sectioned (5 µm thickness) and stained with Hematoxylin and Eosin according to (10).

Dose calculation

The toxic dose of lead acetate was calculated according to (11). Determination of LD_{50} of *Silybum marianum* extract was done by using 10 rats. The procedure employed according to (12).

Results

Determination of LD50 of *Silybum marianum* extract :

Determination of LD_{50} of *Silybum marianum* extract in rats showed that the value was 10000 mg/kg B.W.So the therapeutic dose was 350 mg/kg B.W. The doses which determine the LD_{50} and these which give highly severe pathological changes were lift.

Histopathological Examination

Testis

The light microscopy examination of the testis of control group (group I) had normal structure. The structural components of the testis are the seminiferous tubules and interstitial tissues. The lumen of the seminiferous tubules is filled with sperms (Fig.1).

The morphology of testis of the group II was characterized by vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium, and abnormal distribution of spermatozoa showed in lumina of seminiferous tubules with thickening of basement membrane and formation of spermatid giant cells (Fig.2,3). Other sections showed fibrous thickening of tunica albuginea with total absence of spermatogenesis and spermiogenesis leaving only the appearance of basement membrane (Fig.4). Rats of group III showed nearly normal testicular structure like control (Fig.5). Animals of group IV showed no histopathological changes.

Epididymis

Rats of control (group I), epididymal epithelium enclosed a lumen containing spermatozoa. The interstitial space in between the epididymal tubules was filled with sparse stroma. The pseudostratified epithelium was composed of principal cells with nuclei situated at the base (Fig.6).

In group II animals, showed a decrease in the number of sperms or empty with intraluminal cellular exudates with few sperms (Fig.7). Severe interstitial fibrosis with hyperplasia of tubular epithelial lining forming papillary projections were also seen (Fig.8). Rats of group III showed nearly normal epididymal structure (Fig.9). Animals of group IV showed no histopathological changes.

Ductus deferens

Control (group I). The epithelial lining is surrounded with muscular coats (Fig.10). Group II showed the presence of intraluminal cellular exudate (Fig.11). Group III rats ductus deferens showed vacuolar degenerative changes in their epithelial lining cells (Fig.12). Group IV showed no histopathological changes.

Uterus

Animals of control (group I). Uterine wall consisting of three layers : endometrium, myometrium and perimetrium. The endometrium is lined with simple columnar epithelium and containing the endometrial glands (Fig.13). Group II animals showed hyperplasia of endometrial epithelial lining with formation of papillary like projections (Fig.14). Group III uteri appeared nearly like control (Fig.15). Group IV showed no histopathological changes.

Ovary

Group I showed normal structure with the presence of primary and secondary follicles and corpus luteum (Fig.16). Group II ovaries showed no corpus luteum, only few follicles and occasional graafian follicles (Fig17.). Group III the ovaries appeared nearly like control (Fig.18). The animals of group IV showed no histopathological changes.

Discussion

Determination of LD_{50}

The value of LD_{50} of *Silybum marianum* extract was in agreement with that found by (13) who stated that oral Ld_{50} of the *Silybum marianum* extract was 10000 mg/kg B.W.

Histopathology

In the present study, male rats treated with lead acetate exhibited disordered arrangement of germ cells, a decrease spermatogenic cell layer in the seminiferous tubules. These findings support the results from other reports which indicated that lead altered testis histology resulting in structural defects in spermatids and sperm mice, rat, and rabbits (14,15). Various studies suggest an interaction of heavy metal like lead with hypothalamic-pituitary-testosterone (HPT) axis controlling spermatogenesis rather than the direct exposure to high levels of blood lead, due to the protection of testicular cells by blood-testis barrier, considering the wide spectrum effect of lead at different concentrations on reproductive hormones and the priority of hormones for growth development (16,17). Similarly experimental studies in rats have shown that the effects of lead involve multiple sites on male reproductive hormones although the most important part of these disorders probably occurs in the HPT.

Furthermore, depending on lead exposure levels and duration, signals within and between the rat's hypothalamus and pituitary glands appear to be disrupted by lead (18). On the other hand due to imbalance in HPT hormonal axis induced by lead exposure, pituitary cells release inappropriate levels of LH and change the steroid negative feedback loop (19). Another issue in lead's reproductive toxicity might be related to the excessive generation of reactive oxygen species (ROS), an issue which has been paid more attention recently. ROS inhibits the production of sulfhydryl antioxidants has a role in pathological processes in female physiological reproductive functions such as folliculogenesis, oocyte maturation, steroidogenesis, corpus luteal function and luteolysis, lead induces oxidative stress and promotes the generation of hydrogen peroxide (20, 21). The presence of spermatid giant cells within the lumen of seminiferous tubules was due to degenerative changes of spermatogonia. (22) Stated that spermatid degeneration and giant cell formation were observed after spermatocyte degeneration. Spermatid degeneration appeared to be secondary change resulting from disrupted sertoli– to germ cell association. The fibrous thickening of tunica albugenia and interstitial tissue of epididymis. Recent studies have identifies macrophages as critical regulators of fibrosis. Like myofibroblasts, these cells are derived from either resident tissue populations, or from bone marrow immigrants. Studies now suggest the pathogenesis of fibrosis is tightly regulated by distinct macrophage populations that exert unique functional activities throughout the initiation, maintenance, and resolution phases of fibrosis (23).

The results were coincident with the study conducted by (24), they stated that epididymal damage including epithelial degeneration and interstitial tissue caused by lead. Epididymal change may be due to an important contributory factor in infertility due to lead toxicity (25).

Concerning female genetalia the present results revealed that lead acetate toxicity lead to histopathological changes in both uteri and ovaries could lead to infertility. However, the information regarding female reproductive toxicology is less than the one regarding males due to the gametogenesis differences and the access ability of the germinal cells and also because of the revolving nature of female breeding function (26). Furthermore, studies on the effects of lead on female genetalia in lead-exposed women and experimental animal models reported that lead accumulation in granulosa cells of the ovary causing delays in growth and pubertal development and reduced fertility in females (27). Endometrial and epididymal hyperplasia was considered preneoplastic lesions by some authors (28).

Silybum marianum was considered a general tonic for reproductive organs (29). Results of the present study showed that male and female genital organs treated with plant extract exhibits normal structures. This explain the role of *Silybum marianum* to have a membrane-stabilizing activity that prevents toxins from getting into the cells, perhaps by competing for the receptors, or through antioxidant action and free radical scavenging to increase glutathione levels and activate superoxide dismutase (SOD) and glutathione peroxidase (30). It also stimulate the synthesis of ribosomal RNA, an important step in cell regeneration, and inhibits lipoperoxidation and associated cell damage in some experimental models (31). Considering the antioxidant effect and estrogen receptor activity, it is suggested that *Silybum marianum* and its components can affect folliculogenesis, oocyte maturation, granulosa cell apoptosis and endometrial thickness (32, 33).

The absence of the cellular exudate from the epididymis and ductus deferens in treated group this is related to the anti-inflammatory activity of *Silybum marianum* (34).

In conclusion, there was a therapeutic effect of *Silybum marianum* on the lead acetate reproductive toxicity in male and female rats.

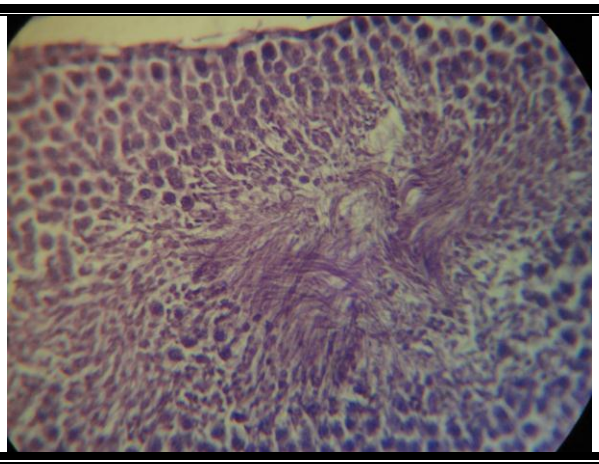


Figure (1): Testis of male rat of control group showing normal structure (H & E x400).

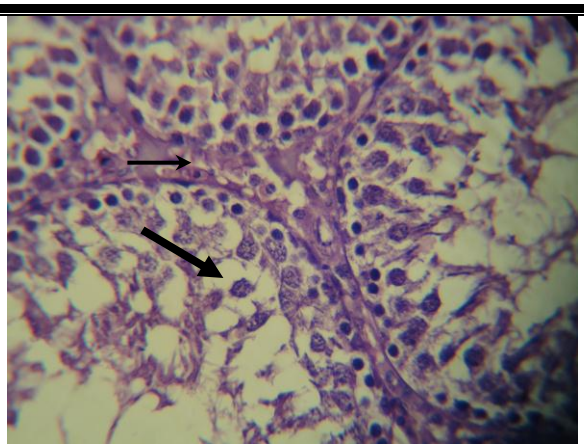


Figure (2): Testis of male rat of group II showing vacuolar degeneration of spermatogenic epithelium (⟶) with thickening of basement membrane and necrosis of Leydig cells (⟶) (H & E x400).

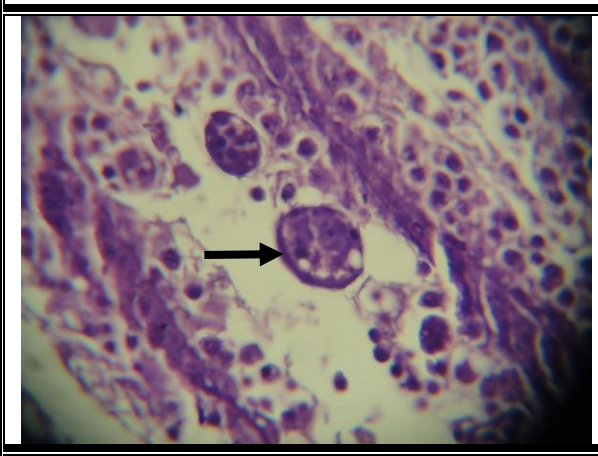


Figure (3): Testis of male rat of group II showing formation of intratubular multinucleated spermatid giant cells (⟶)(H & E x400).

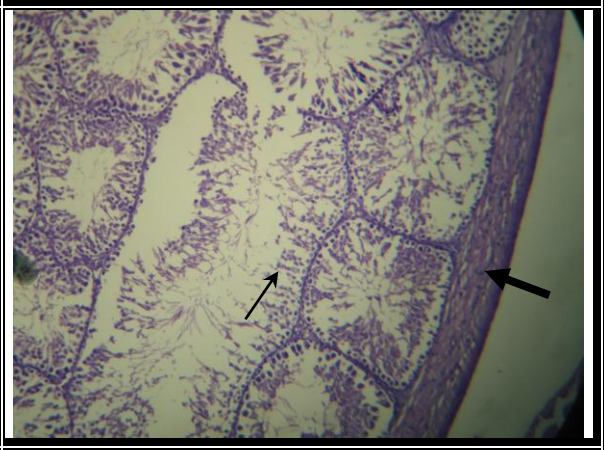
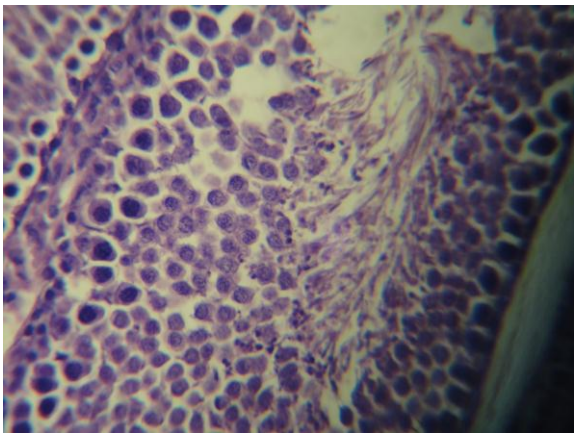


Figure (4): Testis of male rat of group II showing fibrous thickening of tunica albugenia (⟶) seminiferous tubules are lined with tall columnar sertoli cells (⟶) (H & E x400).



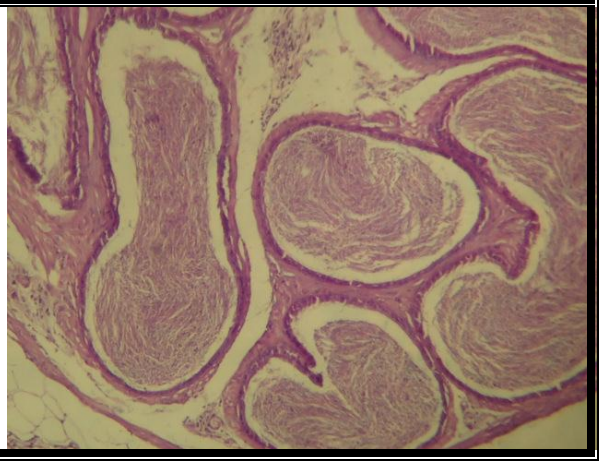


Figure (5): Testis of male rat of group III showing nearly normal structure compared with control (H & E x400).

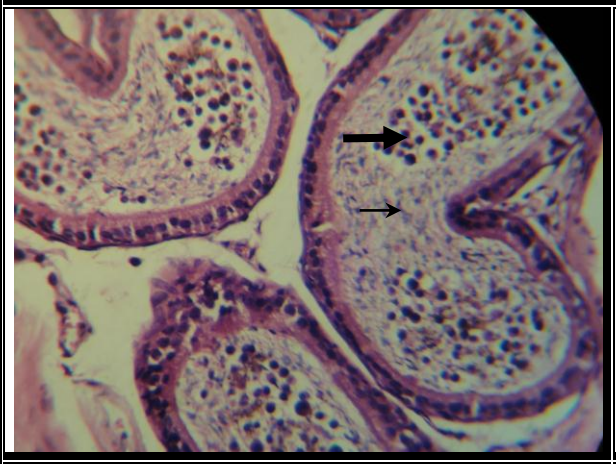


Figure (6): Epididymis of male rat of control group showing normal structure (H & E x400).

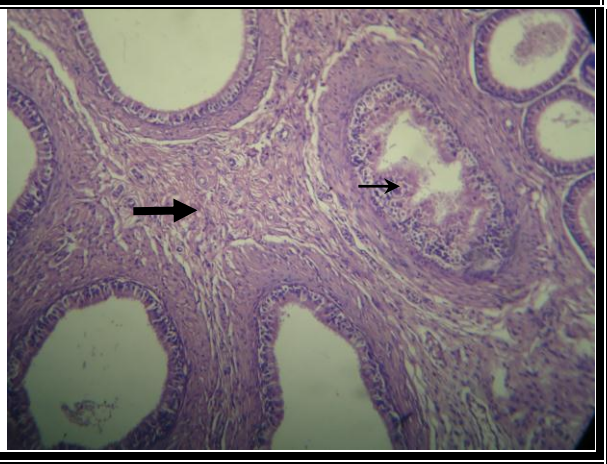
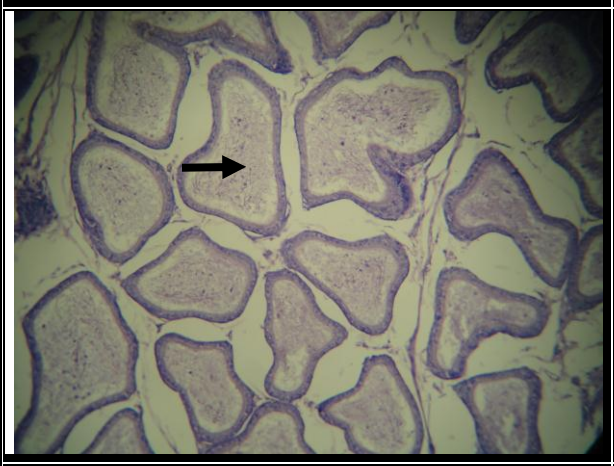


Figure (7): Epididymis of male rat of group II showing intra-luminal cellular exudates (➡) with few sperms (→) (H&E x400).

Figure (8): Epididymis of male rat of group II showing severe interstitial fibrosis (➡) with hyperplasia of tubular epithelial lining forming papillary projections (→) (H & E x400).



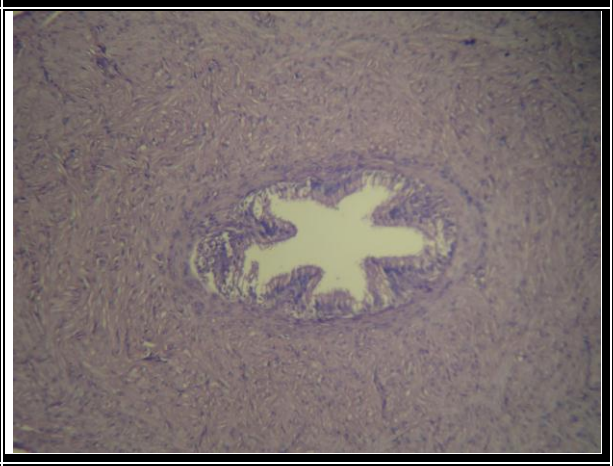


Figure (9): Epididymis of male rat of group III showing nearly normal structure of tubules containing spermatozoa (➡) (H & E x400).

Figure (10): Ductus deferens of male rat of control group showing normal structure (H & E x400).

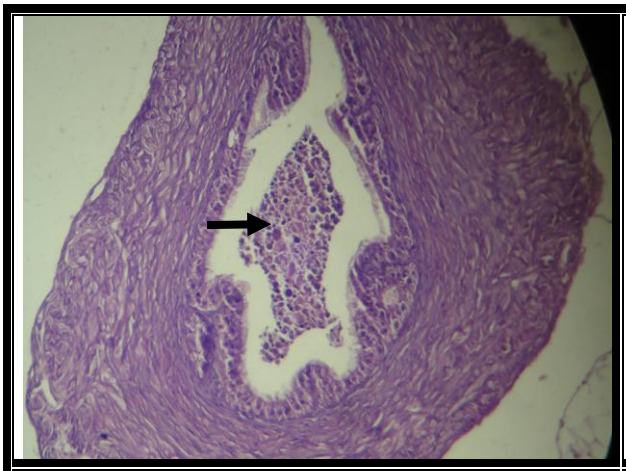


Figure (11): Ductus deferens of male rat of group II showing intra-luminal cellular exudates (⟶) (H & E x400).

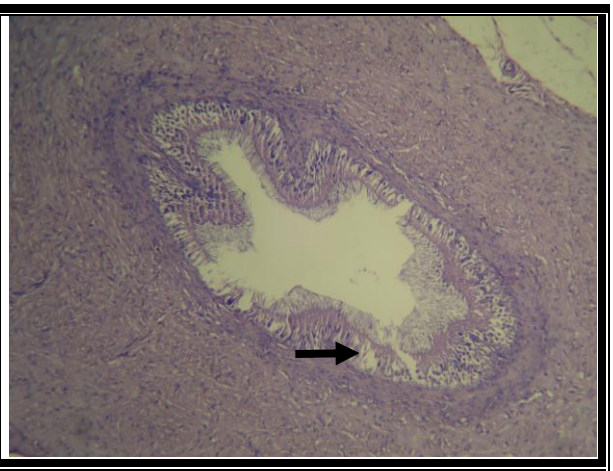


Figure (12): Ductus deferens of male rat of group III showing vacuolation of epithelial lining (⟶) with absence of exudate (H & E x400).

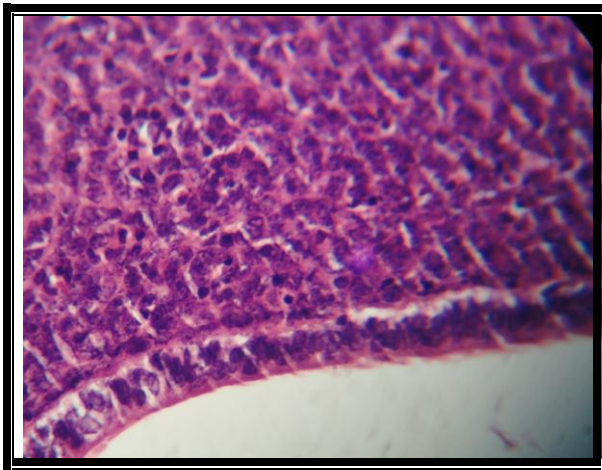


Figure (13): Uterus of female rat of control group showing normal structure (H & E x400).

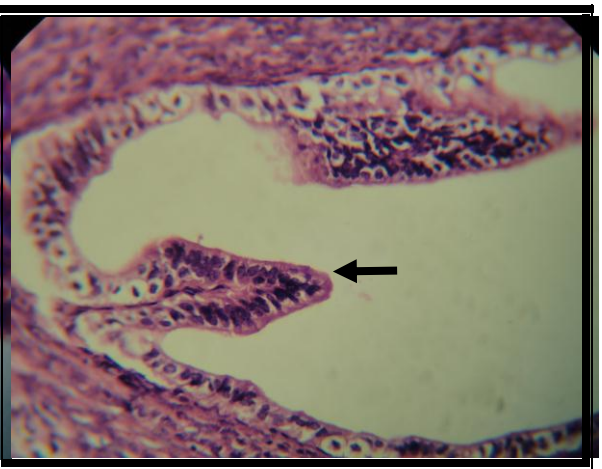


Figure (14): Uterus of female rat of group II showing papillary projections of endometrial lining (⟶) (H & E x400).

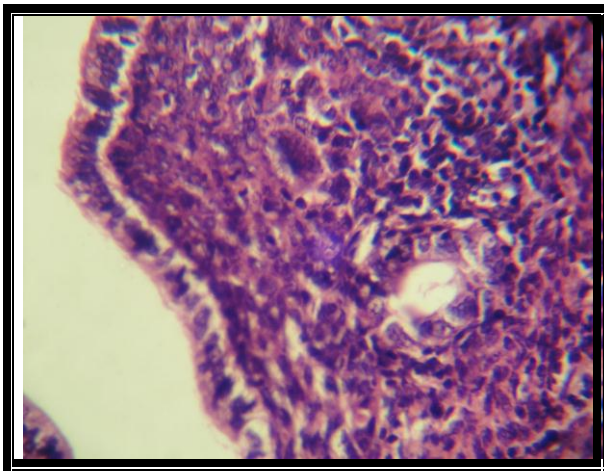


Figure (15): Uterus of female rat of group IIIshowing nearly normal structure as compared with control group (H & E x400).

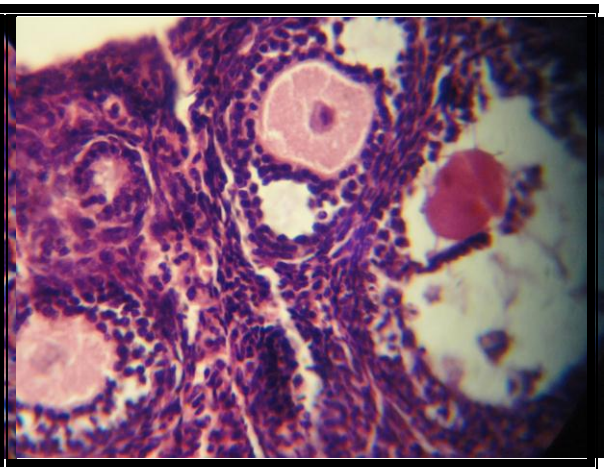


Figure (16): Ovary of female rat of control group showing normal structure (H & E x400).

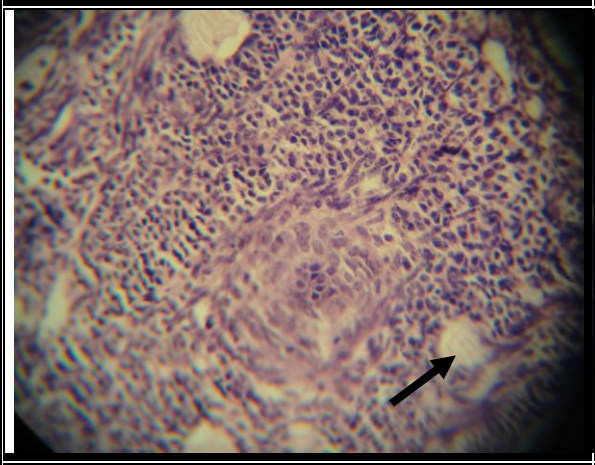


Figure (17): Ovary of female rat of group II showing decrease in numbers of follicles (➞) (H & E x100).

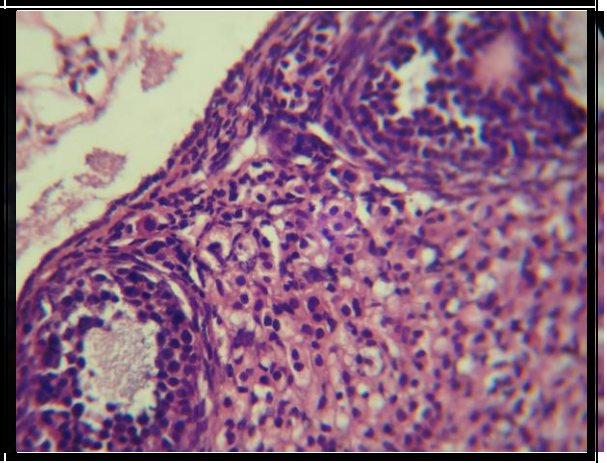


Figure (18): Ovary of female rat of group III showing nearly normal structure as compared with control group (H & E x100).

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