

PROTECTIVE EFFECT OF GINGER (ZINGIBER OFFICINAL) HYDRO ALCOHOLIC EXTRACT ON CADMIUM CHLORIDE INDUCED REPRODUCTIVE TOXICITY IN RATS FEMALE

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ABSTRACT

The present study was conducted to explore the protective effects of ginger on cadmium chloride induced reproductive toxicity in rats female. 40 female adult rats 6-8 weeks old weighing (260 -300 gm.) was used for the study. Animals was divided into four (10 rats for each groups) groups: Animals in Groups I:served as control and were given distilled water. Group II :was given cadmium chloride at a dose of 0.008 mg/kg B.w once daily for two months by oral gavage tube .Group :III were given cadmium chloride at a dose of 0.008 mg/kg B.w once daily along with ginger extract 200 mg/kg B.w. through oral gavage tube once daily for two months .Group IV: were given ginger extract 200 mg/kg B.w. through oral gavage tube once daily for two months .At the end of the experiment, blood samples were taken via cardiac puncture for follicular stimulating hormone (FSH), luteinizing hormone (LH) analysis .After dissection of animals ,uterui and ovaries were excised for histopathological examination .Results showed that cadmium chloride cause a significant decrease in the levels of sex hormones , and induce toxicopathological changes in genital organs these changes were ameliorated with extract of ginger. The results of the present study concluded that cadmium chloride -induce toxicopathological changes in uterui and ovaries of rats, which in turn many affected on the reproductive efficiency of animals, these changes were improved after co -giving ginger extract which provide a strong evidence for the beneficial role of antioxidants plants in improving the effect of cadmium chloride toxicity in rats female.

Key words: cadmium chloride, antioxidants, ginger, reproductive toxicity.

التأثير الوقائي للخلاصة الكحولية المائية لنبات الزنجبيل على كلوريد الكاديوم المستحث للسمية التناسلية في إناث الجرذان

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الخلاصة

هدفت الدراسة الحالية الى معرفة التأثير الوقائي لنبات الزنجبيل على السمية التناسلية لاناث الجرذان استخدمت في التجربة 40 جرذ بعمر (6-8) اسابيع واوزان تراوحت بين (260 -300 غم) .قسمت الحيوانات الى اربع مجاميع متساوية (10 جرذان لكل مجموعة) أعطيت المجموعة الاولى ماء مقطر وعدت مجموعة سيطرة ، المجموعة الثانية أعطيت 0.008 ملغم/كغم /من وزن الجسم جرعة واحدة من كلوريد الكاديوم يوميا ولمدة شهرين عن طريق التغذية الانبوبية ، المجموعة الثالثة أعطيت 0.008 ملغم /كغم /من وزن الجسم جرعة واحدة من كلوريد الكاديوم مع 200 ملغم /كغم /من وزن الجسم جرعة واحدة يوميا من خلاصة الزنجبيل الكحولية المائية ولمدة شهرين عن طريق التغذية الانبوبية أما المجموعة الرابعة اعطيت 200 ملغم /كغم / من وزن الجسم جرعة واحدة يوميا من خلاصة الزنجبيل ولمدة شهرين عن طريق التغذية الانبوبية .بعد انتهاء مدة التجربة جمعت عينات الدم من القلب مباشرة لغرض اجراء التحاليل المختبرية الخاصة بالهرمونات التكاثرية وهي الهرمون المنبه للجريب والهرمون الملوتن .بعد ذلك تم اجراء الصفة التشريحية للحيوانات واستأصل الرحم والمبيض لغرض الفحص المرضي النسجي .بينت النتائج ان كلوريد الكاديوم ادى الى نقصان معنوي في مستوى الهرمونات الجنسية و استحداث تغيرات مرضية نسجية في الاعضاء التناسلية وهذه التغيرات قد تحسنت بعد إعطاء الخلاصة الكحولية المائية للزنجبيل .نستنتج من الدراسة الحالية الى ان كلوريد الكاديوم يؤدي الى تغيرات مرضية سمية في ارحام ومبايض الجرذان، مما تؤثر على القدرة الجنسية للحيوانات وان هذه التغيرات قد تحسنت بعد اعطاء الزنجبيل مما يعطي دليلا قويا للدور الفعال النباتات المضادة للاكسدة في تحسين التأثير السمي بكلوريد الكاديوم في اناث الجرذان .

Introduction

Cadmium (Cd) accumulation within the various tissues of the human and animals has been demonstrated in previous studies (Thompson and Bannigan, 2008; Lin et al , 2010). Cadmium is known to deplete glutathione and protein - bound sulfhydryl groups which results in enhanced production of reactive oxygen species (ROS) such as superoxide (Liu et al , 2001). When amount of free radicals reach more than antioxidant capacity (enzymatic and non-enzymatic), oxidative stress can be occurred and malondialdehyde as sign of oxidative stress and lipid peroxidation increased as their level reach in measurable range in blood. Oxidative stress causes damage to biologic macromolecules (such as nucleic acids, membrane lipids and proteins) and disorder of normal metabolism and physiology especially hormone production and release (Robert and Sindhu , 2009). Furthermore, cadmium can enter to food chain indirectly and effects the female reproductive system in rats (Monsefi, 2013). Cadmium is a toxicant that has a long biological half- life (15-20 years) and accumulate over time within the blood ,kidneys ,livers and reproductive organs (Michale and Jorge ,2004). Different studies have shown that cadmium affects plasma gonadotropin levels (Pasky et al ,1989; Lafuent , and Esquifino, 1999) and ovarian steroidogenesis and thus the granulosa cell function is affected (Priya et al ,2004 ;Sebahat et al 2005). The metal is known to produce oxidative stress in rats ovary by decreasing antioxidant enzymes ,which were associated with delayed puberty and altered steroids and gonadotropin levels (Dailiah et al ., 2013). In addition to abortion which may be consequent upon alteration in ovarian and placental function (Aprioku, 2014).

Ginger is an impotent herbal medicine has antioxidant properties and scavengers oxidative stress (Kota, 2008). Mallikarjuna, (2008) hypothesized that ginger is capable to prevent the adverse effects of oxidative stress on steroidal hormones in female rats. Therefore, the objective of current study was to investigate the effects of daily oral administration of ginger for 45 days on plasma FSH and LH and tissues of uteri and ovaries in rats that treated with

cadmium chloride (CdCl_2) to show the protective effects of ginger .

Materials and methods

Plant collection:

The fresh rhizomes of *Z. officinale* was obtained from local markets in Baghdad. The plants was purchased and dried with room temperature (25°C), shade dried and powdered.

Preparation of hydroalcoholic extract of ginger:

For preparation of hydroalcoholic extract, dried and coarsely powdered rhizome of the plant (400 g) were macerated with 1000 ml of EtOH-H₂O (7:3) for 72 hours. The extracts were filtered through filter paper and evaporated to dryness under reduced pressure at a maximum of 40 °C using a rotary evaporator. The hydroethanolic extract of ginger was stored in small samples at -20°C until use. The extract was dissolved in saline and was then applied (Gomar, 2014).

Dose calculation of cadmium chloride:

The toxic dose of cadmium chloride was calculated according to the Al-Rikabi(2012), while the dose of ginger was calculated according to Subbaiah et al .,(2013).

Experimental design: 40 female adult rats 6-8 weeks of age weighing (260 -300 gm.) were used for the study .Animals was divided into four groups: Animals in Groups I: served as control and was given distilled water. Group II :was given cadmium chloride at a dose of 0.008 mg/kg B.w once daily for two months by oral gavage tube .Group :III was given cadmium chloride at a dose of 0.008 mg/kg B.w once daily along with ginger extract 200 mg/kg B.w through oral gavage tube once daily for two months .Group IV: was given ginger extract 200 mg/kg B.w though oral gavage tube once daily for two months .At the end of the experiment, blood samples were collected via cardiac puncture for follicular stimulating hormone (FSH), lutilizing hormones(LH) analysis .After dissection of animals ,uterui and ovaries were excised for histopathological examination.

Hormonal assay:**Radioimmunoassay:**

The methods of performing radioimmunoassay is as follows:

- A- An antibody that is highly specific for the hormone to be measured is produced.
- B- A small quantity of this antibody is mixed with a quantity of fluid from the animal containing the hormone to be measured and a known amount of radioactive iodinated hormone.
- C- After binding has reached equilibrium, the antibody - hormone complex is separated from the unbound iodinated hormone by a variety of the physicochemical means.
- D- The amount of hormone persist in the plasma can be inferred by comparing with "standard curve " (*Gyton and Hall 2003*).

Histopathology :

At the end of the experiment the animals were sacrificed under deep anesthesia, uteri and ovaries were dissected out, fixed with buffered

formalin .The specimens were sectioned (5 μ m thickness) and stained with Hematoxylin and Eosin stain according to the (Luna and Lee,1968).

Statistical analysis:

The one way analysis of variance (ANOVA) was used for analyzing of the study results, and then Duncan's test ($P \leq 0.05$) was detected to compare between groups (*Shiefler,1980*).

Results and discussion:**Hormonal assay:**

The results obtained from the present study showed a significant decrease ($p \leq 0.05$) in FSH (15.33 ± 2.72) and LH (10.00 ± 0.57) hormones (tables 1) in groups II in contrast with control group ,furthermore the study revealed a significant increase ($p \leq 0.05$) in FSH (37.00 ± 1.15) and a significant increase ($p \geq 0.05$) in LH (18.00 ± 0.55) in group III in compared with group II . There is a significant increase ($p \leq 0.05$) in FSH (43.33 ± 1.20) and LH (23.67 ± 0.50) in group IV in comparison with group I and group II.

Table (1): values of FSH and LH levels (mIU/ml) in serum of experimental group's rats

Groups	FSH (Mean \pm SE)	LH (Mean \pm SE)
G _I	40.00 \pm 0.58 AB	21.00 \pm 0.57 AB
G _{II}	15.33 \pm 2.72 C	10.00 \pm 0.53 C
G _{III}	37.00 \pm 1.15 B	18.00 \pm 0.55 B
G _{IV}	43.33 \pm 1.20 A	23.00 \pm 0.50 A

SE is standard error, different capital letters means significant differences between groups.

GI: Control (-ve) animals treated with distilled water.

GII: Animals treated 0.008 mg/kg.bw cadmium chloride.

GIII: Animals treated 0.008 mg/kg.bw CdCl_2 + 200mg/kg.bw ginger extracts

GIV: Animals treated with 200mg/kg.bw ginger extracts.

previous studies have shown that cadmium affect plasma gonadotropin levels (*Lafuente,et al ,1997; Nampoothiri and Gupta 2006 ; Al-Ganami and AL-Lebawi ,2014 ; Younessi and Sadeghi ,2015*) . Nampoothiri and Gupta (2006) demonstrated that both lead and

cadmium cause a significant reduction in gonadotropin binding, which altered steroidogenic enzyme activity of granulosa cells. *Piasek and Laskey (1999)* studied that direct interference of cadmium with hormones production in steroid producing ovary cells. It is

suggested that a predominant mechanism of action of cadmium toxicity is at the level of the hypothalamic - pituitary gonadal axis (*Obianime et al., 2011 ; Lafuente*

Annunciations Food Chem , 2013), or induces oxidative stress in rat ovary by decreasing antioxidant enzymes and altered steroids and gonadotropin levels (*Dailiah et al., 2013*).

The results of the hormonal assay may exhibited that the level of FSH and LH decreased significantly in group II (15.33 ± 2.72 , 10.00 ± 0.57) respectively, these data suggest that cadmium inhibit estrogen release in ovaries and may represent an important mechanism of endocrine disruption (*Zhang et al ., 2008*) similar results were obtained by (*Munga et al ., 2013*).

Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals (*Ali et al ., 2008*).The increase in the levels of FSH , LH in the treated group may explain the important role of ginger as antioxidant agent .The present results are inconsistent with (*Sobhani et al ., 1992*),whom reported that in contrast to males ,cadmium chloride ingestion (10 mg /kg I.p. for 15 days) had no effects on serum FSH and LH concentration in female rats.

Histopathological findings:

Ovaries

Group I: Animals of control groups showing normal histological structure

(fig 1).

Group II: There is increase in numbers of atresia follicles (fig 2) with severe congestion of medullary blood vessels contain inflammatory cells within the Lumina (fig 3). In addition to disorganization of granulosa cells (fig 4) with severe suppression of the ovulation characterized by few and non-developed ovarian follicles with presence of corpus luteum (fig 5).

Group III: Showed large numbers of ovarian follicles in different stages of development (fig 6).

Group IV: Tissue section showed no histopathological changes.

Uteri:

Group I: Animals of control groups showing normal histological structure (fig7).

Group II: Animals showed hyperplasia of endometrial epithelial lining with formation of papillary like projection leading to the narrowing of the lumen (fig8).In addition to infiltration of large numbers of eosinophils in all layers of the uterus leading to pressure atrophy of endometrium glands (fig 9),with severe congestion of blood vessels contain inflammatory cells within their Lumina with deposition of hemosiderin pigment in the endometrium (fig 10), and presence of cellular debris within the lumen of uterus(fig 11).

Group 111: There is normal columnar epithelium lining uterine tissue with numerous well developed uterine glands (fig 12).

Group IV: Showed no histopathological changes.

The current study showed that cd cl₂ toxicity leading to histopathological changes in both uteri and ovaries could lead to infertility .However, the information regarding female reproductive toxicity 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 (*Pionon et al ., 1995*).Concerning the ovaries the observation of the present experiment was in agreement with *Wang Ying et al ., (2015)* whom revealed that cadmium affecting the maturation of follicles , the degradation of corpus luteum , the arrangement of the follicles ,and corpus luteum and increased the number of atresia follicles , the arrangement of the follicles .Furthermore *Thompson, and Bannigan , (2008)* concluded that cd associated with failure of progression of oocyte development from primary to secondary stage ,and failure of ovulate .

Histopathological studies on uteri of the treatment group revealed a deleterious effects on the organ . Tissue sections showed hyperplasia of uterine epithelium forming long papillary

projections leading to the narrowing of the lumen .The endometrial hyperplasia was considered preneoplastic lesions by some authors (*Kurman et al .,1985*). In addition to previous lesions there is deposition of hemosiderin pigments which indicate an old ,hemorrhage the presence of congested blood vessels were agreed with (*Salih,2002; Al-Ganami and AL-Lebawi, 2014*) ; *Miko et al ., (1988)* reported that in eosinophilic endometritis ,the degree of inflammation appears to correlated with the extent of previous injury .It is proposed that the probable causative

agent are eosinophil chemotactic substance liberated from the myometrium mast cells and from the degrading blood clot filling the uterine cavity and that agree with the present result. Furthermore *Adegboyega et al .,(2010)* investigate that eosinophils (as a chronic inflammatory cells) can be used as diagnostic markers of chronic endometritis.

The above microscopic alterations were ameliorated by administration of ginger .This confirmed the ability of ginger to improve the functional efficiency of the uterus and ovary (*Labania, 2005 ; Abu Baker ,2013*

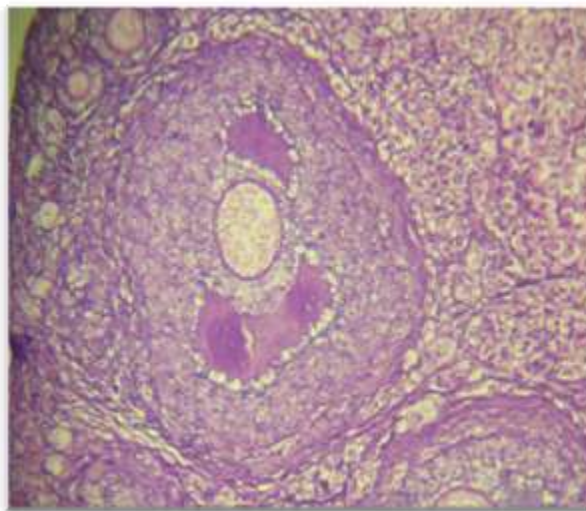


Figure (1): Ovary of rat showing normal histological structures (H&E400 X).

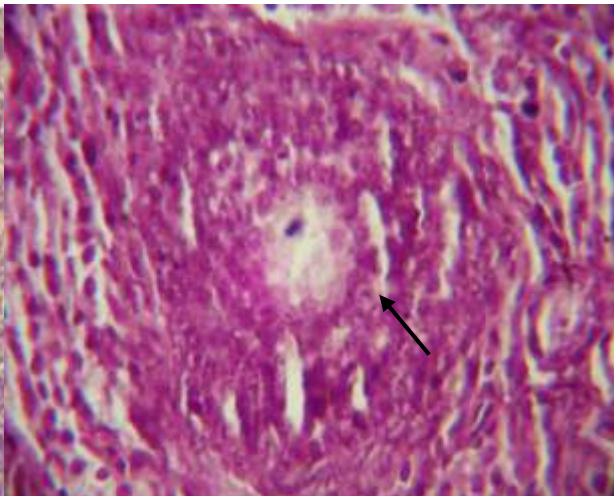


Figure (2): Ovary of rat treated with 0.008 mg /Kg BW/day of cdcl₂ for 45 days showing increase in numbers of atresia follicles . (→) (H& E 400 X).

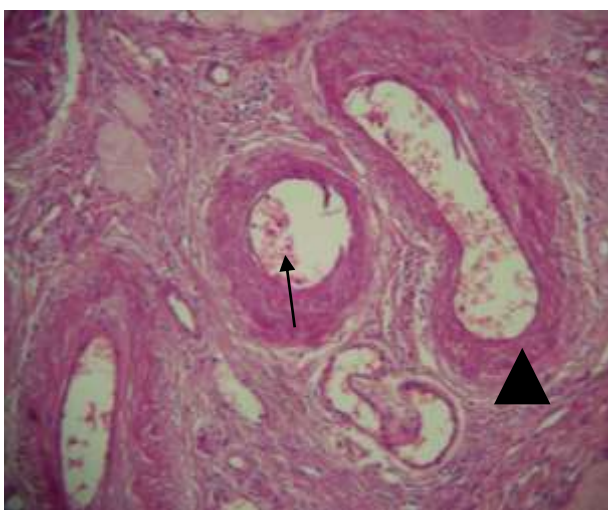


Figure (3): Ovary of rat treated with 0.008 mg /Kg BW/day of cdcl₂ for 45 days showing severe congestion of medullary blood vessels (▲) contain inflammatory cells within their Lumina (→) (H&E100X).

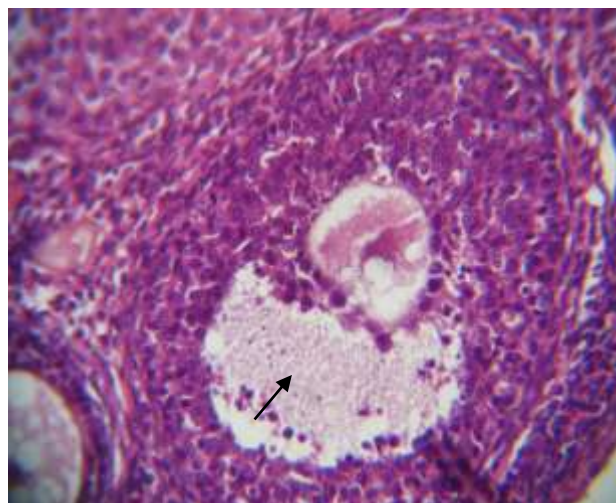


Figure (4): Ovary of rat treated with 0.008 mg /Kg BW/day of cdcl₂ for 45 days showing disorganization of granulosa cells (→) (H&E400X).

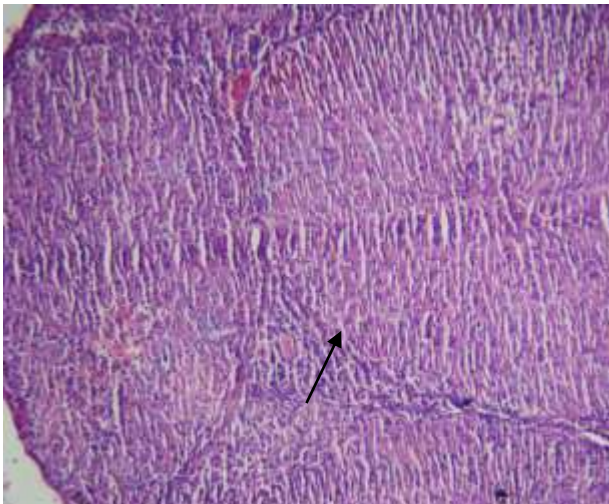


Figure (5): Ovary of rat treated with 0.008 mg /Kg .BW/day of cdcl_2 for 45 days showing corpus luteum (→) (H& E 100X).

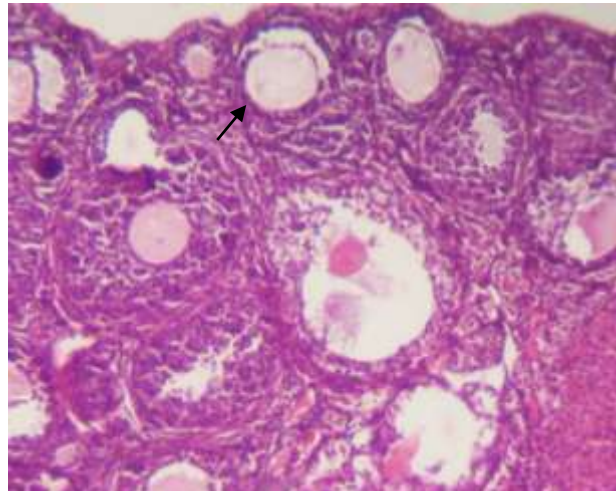


Figure (6): Ovary of rat treated with 0.008 mg /Kg BW/day of cdcl_2 and 200mg/kg .BW ginger extracts for 45 days showing large number of ovarian follicles in different stages of development. (→) (H& E 100 X).

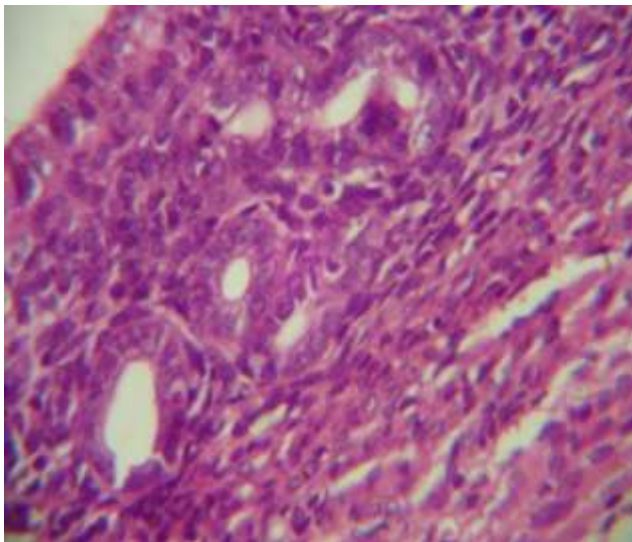


Figure (7): Uterus of rat showing normal histological strictures (H&E400 X).

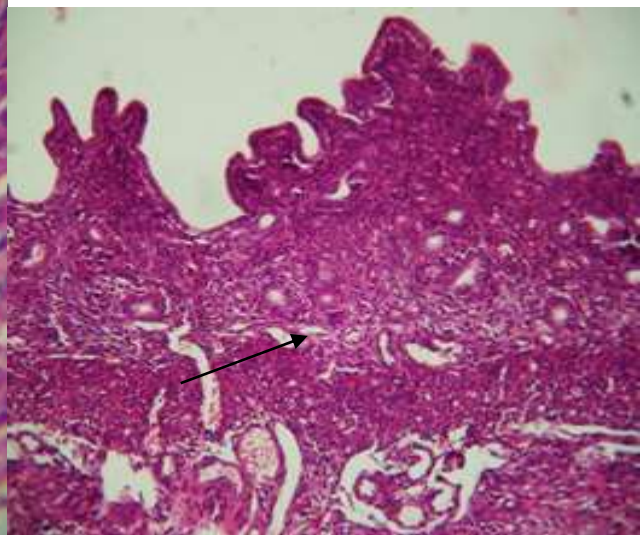


Figure (8): Uterus of rat treated with 0.008 mg /Kg BW/day of cdcl_2 for two months showing hyperplasia with formation of papillary like projection leading to the narrowing of the lumen (→) (H& E 100 X).

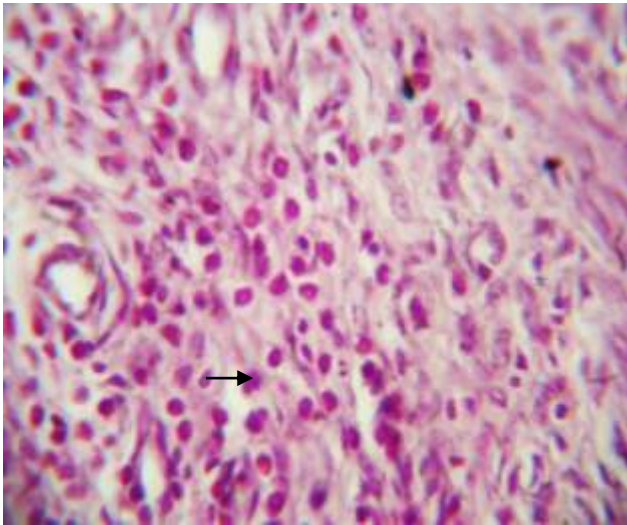


Figure (9): Uterus of rat treated with 0.008 mg /Kg BW/day of $cdcl_2$ for 45 days showing infiltration of large numbers of eosinophilis in the stroma of endometrium () (H& E 400 X).

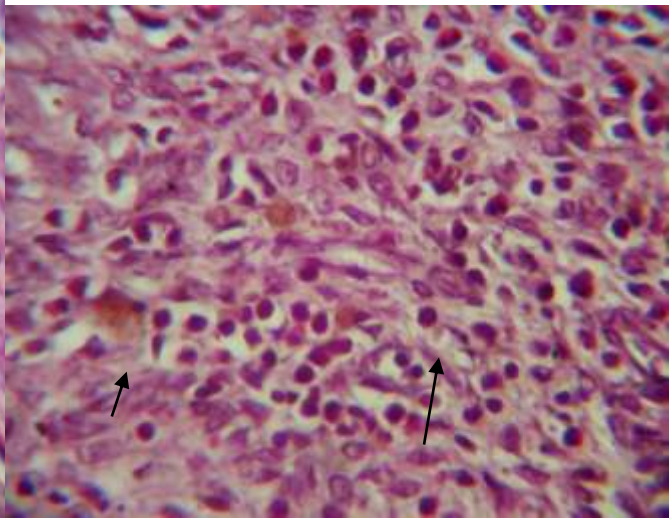


Figure (10): Uterus of rat treated with 0.008 mg /Kg BW/day of $cdcl_2$ for 45 days showing deposition of hemosiderin pigment in the endometrium(→) (H& E 400 X).

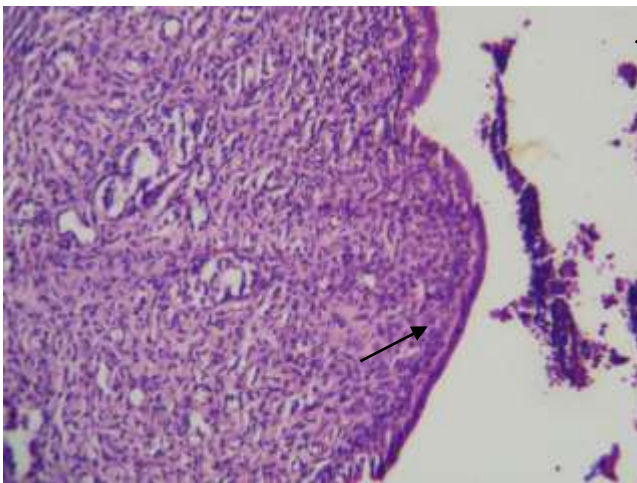


Figure (11): Uterus of rat treated with 0.008 mg /Kg BW/day of $cdcl_2$ for 45 days showing and presence of cellular debris with in the lumen of uterus (→) (H& E 400 X) .

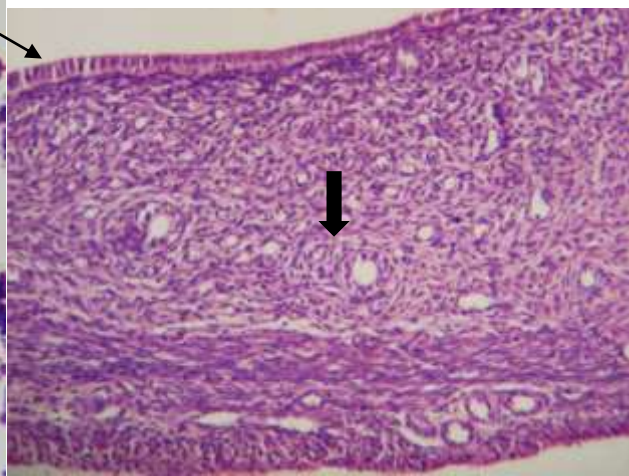


Figure (12): Uterus of rat treated with 0.008 mg /Kg BW/day of $cdcl_2$ +200 mg/kg BW ginger extracts for 45 days showing the normal columnar epithelium lining uterine tissue(→) with numerous well developed uterine glands (➡)

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