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## **Histological study of effect of cadmium toxicity on ovarian tissue**

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### **Abstract**

Cadmium is a widespread and dangerous material that leads to different harmful cellular changes up to death. The goal of this study is to detect cadmium damaging effect on ovaries of adult female albino rat . A total of 14 adult female albino rats were divided into two groups, each group was consisted of 7 rats . The control group ( group I) was given only saline orally and the cadmium-treated group (group II) was given CdCl<sub>2</sub> dissolved in saline at a dose of 5 mg/kg for 28days. The animals of two groups were sacrificed at the same time and their right ovaries were rapidly dissected out. The specimens of each group were processed for light microscopic examination. They were stained with hematoxylin and eosin and masson's trichrome stains for evaluating histopathological changes in ovarian structure. Quantitative morphometric, and statistical studies were performed. Histological changes in cadmium-treated group were in the form of irregular outline of the ovary with flat squamous germinal epithelium , massive loss of follicles and many atretic follicles with markedly dilated and congested cortical blood vessels. Masson's trichrome stained sections showed marked deposition of collagen fiber in the cortical stroma.

### **Keywords:**

Ovary, histology, damage.

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## **1. Introduction:**

The ovaries have two interrelated functions: gametogenesis (the production of gametes) and steroidogenesis (the production of steroids). In women, the production of gametes is called oogenesis. Developing gametes are called oocytes; mature gametes are called ova [1].

Both ovulation and ovarian hormone production are controlled by the cyclical release from the anterior pituitary of the gonadotrophic hormones luteinising hormone (LH) and follicle stimulating hormone (FSH). Estrogen and progesterone in turn regulate LH and FSH production by feedback mechanisms. Thus, ovulation is coordinated with preparation of the uterus to receive the fertilized ovum [2].

Cadmium is a toxic metal and one of the most common environmental hazards found in agricultural and industrial areas, especially in the atmosphere. Humans are mostly exposed to cadmium through the intake of contaminated air, food, and water, or inhalation of tobacco smoke [3].

Cd results in chromosomal aberrations, sister chromatid exchange, DNA strands breaks, and DNA- protein crosslinks in cell lines. Cd causes

mutations and chromosomal deletions potentially [4].

Its toxicity involves depletion of reduced glutathione (GSH), binds sulfhydryl groups with protein, and causes enhancing production of ROS such as superoxide ion, hydrogen peroxide, and hydroxyl radicals. Cd also inhibits the activity of antioxidant enzymes, such as catalase, manganese-superoxide dismutase, and copper/zinc-dismutase [5].

Cd affects cell proliferation, differentiation, and apoptosis. These activities interact with DNA repair mechanism, the generation of reaction oxygen species (ROS) and the induction of apoptosis [6].

It is well-known that Cd toxicity induces oxidative stress via the production of free radicals, which are harmful to cells. Free radicals may damage protein, lipid, enzymes and DNA, and therefore must be neutralized by antioxidants before entering cells [7].

Cd is accumulative in ovary, which will lead to the change of ovary morphology, and this kind of change usually happens quicker than that of liver and kidney tissues. Cd may interfere with ovarian endocrine functions, inhibit follicle growth and development, and increase the

chance of follicle atresia; it may inhibit ovulation, resulting in temporary infertility [8]. Cd has also been shown to affect reproductive toxicity either directly targeting glands or indirectly by interfering with the hypothalamus-pituitary-gonadal axis [9].

## **2. Materials and Methods:**

### **2.1 Drugs and chemicals:**

Cadmium (cadmium chloride) particles are obtained from analytical chemistry department, Faculty of Pharmacy, Beni-Suef University, Egypt. The required dose is 5mg /kg [10]. It was weighed using a digital scale, dissolved in saline and given orally by blind ended tube once per day for 28 days for each animal.

### **2.2 Experimental animals:**

Forty female albino rats 5–6 months old, weighing 200–250 g were used in this study. The animals were housed in the Animal House of Faculty of Science, Beni-Suef University. Each group was housed in a separate cage in a constant temperature (22–24 °C) and light-controlled room on an alternating 12:12 h light-dark cycle and had free access to food. Rats were fed a standard commercial pellet diet and were kept for one week before

beginning the experiment for acclimatization. Animals were treated according to animal rights committee.

### **2.3 Experimental design:**

The animals will be divided into two main groups, 7 rats each:

**Group I:** served as control and will be given only saline orally by blind ended tube.

**Group II** (cadmium treated group): each rat will receive 5mg /kg Cd dissolved in saline orally by blind ended tube for 28 days (as CdCl<sub>2</sub>) [10].

On the last day of experiment, final body weight of each animal will be recorded and rats will be sacrificed under anesthesia by ether inhalation and specimen will be taken from right ovary of all experimental animals.

### **2.4 Histological study:**

Specimen was taken from right ovary of all experimental animals, fixed in 10% formal saline for 48 hours, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Paraffin blocks were prepared and 5µm thick sections were subjected to the following studies: hematoxylin and eosin and Masson's trichrome, for evaluating

histopathological changes in ovarian structure. Quantitative morphometric studies for assessment of mean number of atretic follicles and mean area percent of the stained collagen fibers in the ovarian sections of the studied groups were also performed.

### **2.5 Statistical methodology**

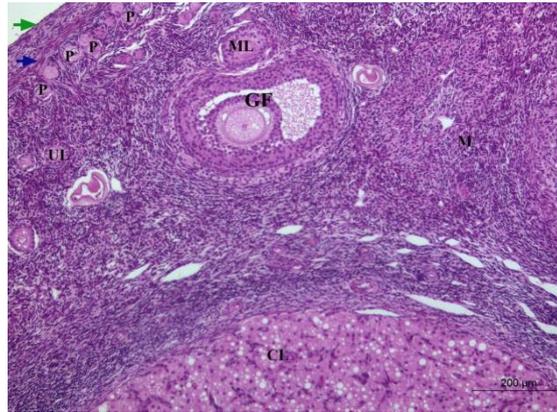
Quantitative data were summarized as means and standard deviations and compared using one-way analysis-of-variance (ANOVA). Any significant ANOVA was followed by post hoc, Tukey test to detect which pairs of groups caused the significant difference. P-values <0.05 were considered statistically significant. Calculations were made on SPSS software version 16.

### **3. Results:**

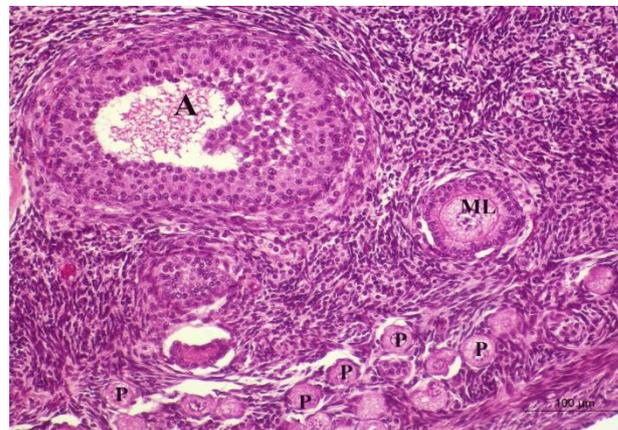
Light microscopic examination of ovarian sections of the control group (group I) shows follicles in different stages of development as primordial follicles, unilaminar primary follicles, multilaminar primary follicles and mature graffian follicle. Corpus luteum is also observed. Some atretic follicles are also observed as the oocyte is degenerated.

On the other hand, Light microscopic examination of ovarian sections of group II (cadmium treated group) shows irregular outline of the ovary with flat squamous germinal epithelium with massive loss of follicles. There are many atretic follicles. Degenerated corpora lutea are also observed. There are also markedly dilated and congested cortical blood vessels.

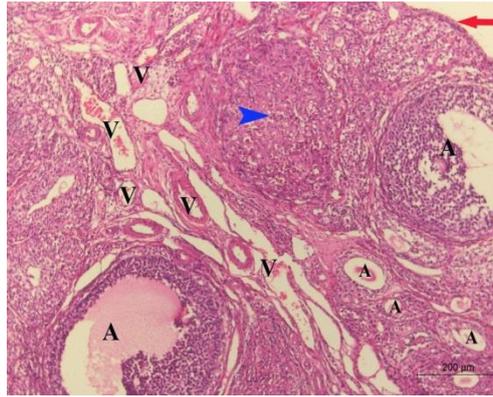
As regard of examination masson trichrome stained sections, ovarian sections of group I (control group) shows minimal deposition of collagen fibers in the ovarian stroma between the cortical follicles, while ovarian sections of group II (cadmium treated group) shows marked deposition of collagen fiber in the cortical stroma between the cortical follicles and extending towards the medulla.



**(Fig.1)** A photomicrograph of group I showing that the surface of the ovary was covered by germinal epithelium ( green arrow) formed of a single layer of cuboidal cells. Beneath the germinal epithelium was a thin layer of dense fibrous connective tissue (tunica albuginea) (blue arrow) . The parenchyma below the tunica albugine is divided into two zones ,outer cortex and inner medulla (M). The cortex contains many follicles at different stages of growth as : primordial follicles (P), unilamellar primary follicles (UL),multilamellar primary follicles(ML) and mature graiffin follicle(GF). Corpus luteum (CL) was also demonstrated. (Hx & E X100)

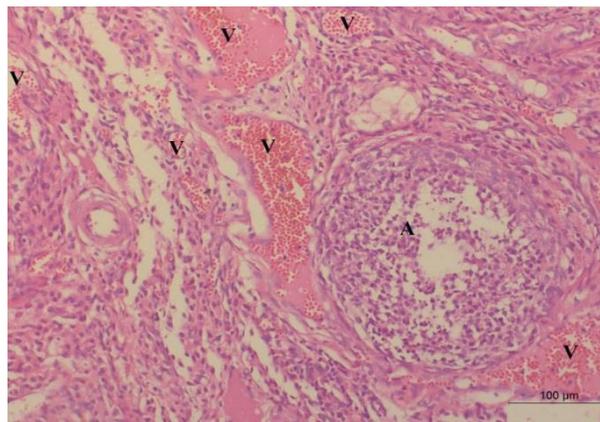


**(Fig.2)** A photomicrograph of group I showing that the cortex contains many follicles at different stages of growth as primordial follicles (P)and multilamellar primary follicles (ML). Atretic follicle (A) was also demonstrated as the ovum is degerated. (Hx & E X200)



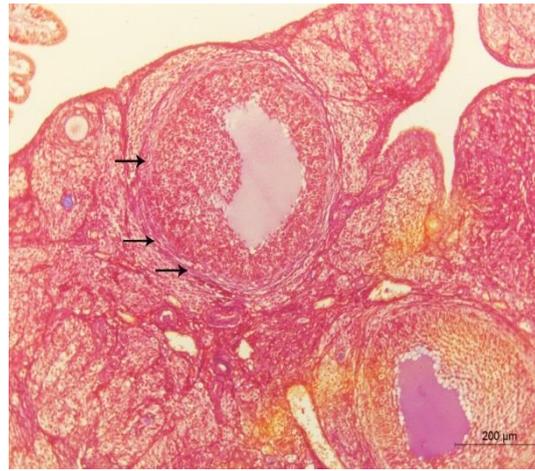
**(Fig.3)** A photomicrograph of group II showing irregular outline of the ovary with flat squamous germinal epithelium ( red arrow ) and many atretic follicles (A). Degenerated corpus luteum(blue arrow head) is also observed. There are dilated and congested cortical blood vessels (V).

(Hx & E X100)

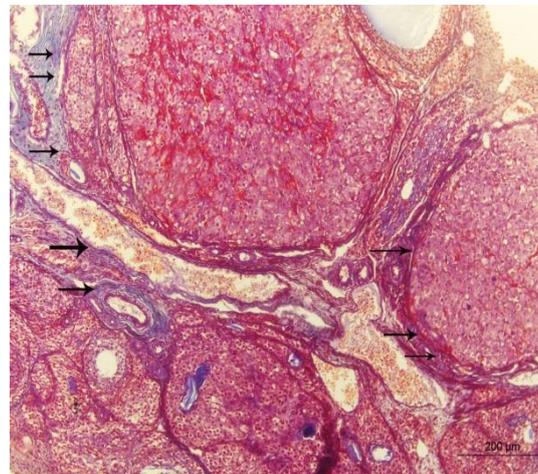


**(Fig.4)** A photomicrograph of group II showing an atretic follicle (A) . There are also markedly dilated and congested cortical blood vessels (V).

(Hx & E X 200)



**(Fig.5)** A photomicrograph of group I showing minimal collagen fibers in the cortical stroma between the cortical follicles(black arrows).  
(Masson's Trichrome X100)



**(Fig.6)** A photomicrograph of group II showing marked deposition of collagen fibers (black arrows) in the cortical stroma between the cortical follicles and extending towards the medulla compared to group I.

(Masson's Trichrome X100)

Table (1): Mean number of atretic follicles ( $\pm$  SD) in the ovarian sections of the studied groups

Group	Mean $\pm$ SD
Group I (control)	1.25 $\pm$ 0.5
Group II(cadmium treated)	8.6 $\pm$ 4.5*

\*Significantly different from the value of the control group at  $P < 0.05$

Table (1) showed significant increase in this value in cadmium treated group (group II) as compared to the control( group I).

Table (2): Mean area percent of collagen fiber content ( $\pm$  SD) in the ovarian sections of the studied groups

Group	Mean $\pm$ SD
Group I (control)	4.04 $\pm$ 1.55
Group II(cadmium treated)	12.91 $\pm$ 3.56*

\*Significantly different from the value of the control group at  $P < 0.05$

Table (2) showed significant increase in this value in cadmium treated group (group II) as compared to the control( group I),

#### 4. Discussion:

Cadmium (Cd) is a heavy metal belonging to the group of the main chemical pollutants of the natural and occupational environment in economically developed countries [11]. It is a highly carcinogenic metal that can cause toxic reactions even in low concentration [12]. Cd ingestion may be accidental, or sometimes even intentional, but can also occur from heavily contaminated dust exposure. It causes desquamation of the intestinal mucosa, resulting in severe and bloody diarrhea, vomiting, Increased salivation, Choking, Abdominal pain, Vertigo and loss of consciousness, Painful spasm of the anal sphincter [13].

The aim of this study was to detect toxic effect of cadmium on histological structure of ovary. Examination of H and E stained sections of the ovaries of group II (

cadmium treated group) showed irregular outline of the ovary with flat squamous germinal epithelium, massive loss of follicles with many atretic follicles, degenerated corpora lutea and markedly dilated and congested cortical blood vessels. These findings were confirmed by the morphometrical and statistical results. There was a significant increase in the mean number of atretic follicles in group II, in comparison with group I.

These findings were the same as the results of [14] who reported that the size of ovaries were reduced with the inhibition of folliculogenesis resulting in diminished numbers of primordial, growing, and tertiary follicles.

In agreement with the results of our study, [15] reported that the ovaries of the CdCl<sub>2</sub> treated rats showed a decrease in number of

follicles, with distorted Graafian follicle. They attributed these changes to Cd accumulation in the the ovaries of rats ,decreased antioxidants [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH)], and raised the concentrations of malondialdehyde (MDA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the uterus and ovaries of these rats.

As regard Masson's trichrome stain, in the present study, the ovaries of rats exposed to Cd (group II) showed marked deposition of collagen fibers in the cortical stroma between the cortical follicles and extending towards the medulla compared to control group. These findings were confirmed by the morphometrical and statistical results; there was a significant increase in the area percentage of trichrome-stained collagen in group II , in comparison with group I.

There were similar findings detected by [16]. They detected fibrosis in the ovarian stroma in Cd treated group as compared to control.

In agreement with results of the current study, [17] detected a noticeable interstitial fibrillar fibrosis with few fields of collagen in all myocardium layers between cardiac cells, which is particularly prominent around the larger blood vessels.

## **5. Conclusion and Recommendations:**

Cadmium is a toxic heavy metal and exposure to it should be avoided. Occupational exposure and work with cadmium or in hobbies involving cadmium exposure such as jewelry making or paints using cadmium should be avoided. Bad habit such as smoking should be forbidden as ciggarets contain cadmium which will be absorbed through lungs. Avoid cadmium contaminated areas and food should be avoided .

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