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Original article

Effects of Low and High Doses of Atrazine on the Structure of the Ovary of Adult Female Albino Rats

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ABSTRACT

Background: Infertility has been increased due to continuous exposure to chemicals such as pesticides and herbicides in our life. Atrazine is one of the most common used herbicide worldwide.
Aim: to show the toxic effect of atrazine on histological structure of ovaries of adult female albino rats.
Methods: 40 female adult rats were divided into 4 groups; control A group, control B group, Low dose treated group (administrated 6.5 mg atrazine/kg/day) for 30 days and High dose treated group (administrated 65 mg atrazine/kg/day) for 14 days. Atrazine was dissolved in distilled water and was administrated by oral gavage. Body weight was measured at start and at end of the study. Rats were sacrificed. Their ovaries were collected and processed to be examined histopathologically.
Results: There was significant decrease in final body weight of treated groups as compared to control groups. Ovaries of exposed groups showed destructive changes as atretic follicles and vacuolated corpora lutea. Decreased body weight and vascular changes were obvious in high dose treated more than low dose treated group. Area percentage of fibrosis significantly increased in treated groups as compared to control group. This increase was more obvious in low dose treated group for long period as compared to high dose treated group for short period.
Conclusion: Atrazine has destructive effects on the ovarian structure of adult female albino rats which were more prominent in high doses for short period except for fibrosis which was more prominent in low doses for long period.
Keywords: atrazine, histopathological, ovaries, adult rats.

INTRODUCTION

Infertility is a serious medical issue that affects around 9% of couples worldwide [(1)]. Instead of treating the cause of infertility, the main focus has always been on in-vitro fertilization, which is expensive [(2)]. The most prevalent cause of infertility in women is ovulatory problems. They are responsible for about 20–25 percent of all cases of infertility [(3)]. Lifestyle and endocrine disrupting chemical exposure have recently been identified as risk factors for female infertility [(4)].

The World Health Organization (WHO) defines endocrine disrupting chemicals (EDCs) as exogenous compounds that induce adverse health effects and alter endocrine system function(s) in an intact organism, its strain, or (sub)populations [(5, 6)].

Pesticides, personal care products, plasticizers, water and food after agricultural application, and canned foods are all examples of EDCs [(7)].

Every day, human, domestic animals, and wildlife are exposed to a variety of EDCs through ingestion, inhalation, and skin contact. Continuous exposure to EDCs has adverse implications on endocrine health [(8)].

Pesticides and herbicides are chemical compounds that are resistant and dangerous to humans. They are commonly employed in agriculture [(9)]. However, it is well recognized that its use has a negative impact on human health [(10)].

Atrazine is a broadly used herbicide and belongs to the chlorotriazine herbicide family. It's often used as a weed killer to help fruits and cereal crops grow better. It is also used to control weeds in industrial and entertainment areas [(11)].
Atrazine is an endocrine disruptive chemical that interferes with normal hormone functioning, such as sperm generation, steroid production, and

insulin resistance, according to numerous studies [12, 13, 14].

The purpose of current study was to explore the alterations induced by atrazine in the structure of ovaries of adult female albino rats.

MATERIAL AND METHODS

Chemicals

1- Atrazine (ATR): obtained from Kafr El Zayat Pesticides & Chemicals CO. in the form of an odorless white powder with a purity of 97 percent. The powder was dissolved in distilled water.

Experimental animals

The experiment was carried out on 40 adult female albino rats (230-250 gm). The animals were obtained from the animal house of Faculty of Medicine, Zagazig University. The animals were housed in a laboratory under supervision. All experimental methods were carried out according to the Institutional Animal Care guidelines and the Ethical Committee of the Faculty of Medicine; Zagazig University; number of approval ***ZU-IACUC/3/F/186/2019***. Vaginal smears were obtained once a day. The phases of estrous cycle were detected microscopically and monitored to confirm the beginning of the experiment was in the proestrous phase [15].

The animals were separated into four groups of ten rats, as follows:

- Group I **(Control A group);** rats were fed on normal food and water.
- Group II **(Control B group**); rats were orally gavaged daily by 1 cm distilled water for 30 days.
- Group III **(Low dose treated group);** rats were administrated 6.5 mg atrazine /kg body weight, that represents approximately 1/100 of the lethal dosage used in rats (672 mg/kg) [16] every day for 30 days dissolved in distilled water by oral gavage [15].
- Group IV **(High dose treated group);** rats were administrated 65 mg atrazine/kg body weight; that represents approximately 1/10 of lethal dosage used in rats (672 mg/kg), every day for 14 days dissolved in distilled water by oral gavage [15].

After fourteen days and thirty days of atrazine administration, rats were sacrificed at the first diestrous stage of the estrous cycle [17].

Vaginal Smear Examination:

By dipping sterile cotton swabs into saline or distilled water, they were gently moistened. After putting the cotton swab into the vagina, the tip was gently rotated. The cotton swab tip was gently rolled onto a clean pre-labeled glass slide after being removed. The vaginal smears were inspected under a microscope after drying [18].

Methods:

The initial body weight of rats in various study groups was measured using a digital balance on the first day of the experiment before any supplementation was administered. At the end of the experiment, the body weight was measured again with the same digital balance. By the end of the trial, the animals had been anesthetized with an intra-peritoneal injection of thiopental (50 mg/kg) [19]. To expose the ovary, a midline upper abdominal incision was made, and ovarian tissues were collected (**Fig. 2**) for histological investigations. Furthermore, statistical analysis was carried out; quantitative data were represented as mean ± SD (Standard deviation). In normally distributed data; ANOVA (F-test) test was done to determine the difference between quantitative variables in the different groups.

Histological investigation

For structure analysis, specimens were prepared for light microscopic examination and stained with hematoxylin and eosin (H&E) and Masson trichrome stains [20].

RESULTS

(1)Histological examination: **showed various phases of the estrous cycle:**

Using vaginal swab; the phases of estrous cycle were checked. They were detected as follow:

(i) Proestrous: characterized by round epithelial cells **(Fig.1 A)**.

(ii) Estrous: identified as cornified cells were predominant **(Fig.1 B)**.

(iii) Metestrous: identified with presence of all cell types but leucocytes were predominant **(Fig.1 C)**.

(iv) Diestrous: characterized by presence of leucocytes **(Fig.1 D)**.

2) Gross findings:

Ovaries are paired spherical structures and are situated at the caudal poles of the kidneys and the mature ovaries appear as a mass of follicles (**Fig. 2**).

3) Rat body weight:

Statistical study of the rats' initial body weight at the start of the experiment indicated no statistically significant difference between the studied groups ($p>0.05$). However, there was a very highly significant difference in the final body weight of rats between various studied groups ($p<0.001$). There was a high significant decrease ($p<0.01$) in final body weight in Low dose treated group and a very high significant decrease ($p<0.001$) in High dose treated group in comparison to control groups (**Table 1; Fig 3A)**.

4) Light Microscopic Examination:

H&E stain

In the control A and control B groups, sections of adult albino rats' ovaries stained with hematoxylin and eosin revealed normal ovarian histological structure with simple germinal epithelium and normal follicles at various developmental stages. The follicles were categorized as primordial, primary, preantral and Graafian follicle (antral follicle). Primordial follicles were made up of an oocyte surrounded by a single layer of squamous granulosa cells. Primary follicles consisted of an oocyte and a single layer of cuboidal granulosa cells. Preantral follicles were made up of an oocyte and granulosa cells in several layers (**Fig. 4 A&B)**. Antral follicles had an obviously antral cavity. Mature Graafian follicle was made up of secondary oocyte bounded by distinct zona pellucida, cumulus oophorus and corona radiata, a great antral cavity filled with secretory fluid and numerous layers of granulosa cell. There was also number of normal corpora lutea **(Fig. 4 C&D)**. H&E stained sections of Low dose treated group revealed presence of vacuolated corpora lutea **(Fig. 4 E)** and atretic follicles which had degenerated oocytes with micronuclei formation, cytoplasmic vacuolation, and disarranged vacuolated granulosa cells **(Fig. 4 F)**. High dose treated group revealed presence of atretic follicles and group of corpora lutea; some of them presented vascular changes, while others showed vacuolations **(Fig. 4 G&H)**. Vascular changes were observable with several areas of hemorrhage in High dose treated group than Low dose treated group.

Masson Trichrome:

Masson Trichrome stained sections of control A and control B groups revealed normally distributed collagen fibers (**Fig. 5 A&B**). Sections of Low dose treated group rats stained with Masson Trichrome revealed a significant deposition of collagen fibers surrounding the blood vessels (**Fig. 5 C**). A moderate increase in collagen fibers was seen in Masson Trichrome stained sections of High dose treated group, primarily surrounding the blood vessels (**Fig. 5 D**).

5) Morphometric study:

Statistical analysis of the morphometric study of area percent of collagen fibers stained by Masson Trichrome revealed a very high significant difference between the different groups ($p<0.001$). There was non-significant difference ($p>0.05$) in area percent of collagen fibers between control A and control B groups. There was a very high significant increase ($p<0.001$) in area percent of collagen fibers in Low dose and High dose treated groups in comparison to control groups. Also, there was a very high significant difference ($p<0.001$) in area percent of collagen fibers between Low dose treated group and High dose treated group **(Table 2; Fig 3 B)**.

Table (1): Statistical comparison of initial and final body weight by ANOVA & Tukey HSD post hoc test among different groups: -

Group Variable	Control A Mean ± SD (Range) N= 10	Control B Mean ± SD (Range) N= 10	Low dose treated group Mean ± SD (Range) N=10	High dose treated group Mean ± SD (Range) N= 10	F	P Value
Initial BW (gm)	238.5 ± 6.687 (230 - 250)	239.0 ± 7.379 (230 - 250)	238 ± 7.149 (230 - 250)	239.5 ± 7.246 (230 - 250)	0.08219	>0.05
Final BW (gm)	256.5 ± 9.144 (245 - 270)	259.0 ± 8.756 (245 - 270)	242.5 ± 7.169^{a**} (230 - 250)	215.0 ± 7.071$^{a***, c***}$ (210 - 230)	62.28	<0.001*

N: number of rats in each group. **BW:** body weight.

- Very high significant (***) when P value < 0.001.
- High significant (**) when P value <0.01.
- Significant (*) when P value <0.05.

a: Comparison in relation to control A.

b: Comparison between High dose treated group and Low dose treated group.

Table (2): Statistical comparison of area% by ANOVA & Tukey HSD post hoc test among different groups: -

Group Variable	Control A Mean± SD (Range) N= 10	Control B Mean± SD (Range) N= 10	Low dose treated group Mean± SD (Range) N=10	High dose treated group Mean± SD (Range) N= 10	f	P value
Area %	0.6155 ± 0.3157 (0.2200 - 0.9990)	0.5606 ± 0.3002 (0.1110 - .8790)	11.07 ± 0.6291^{a***} (10.02 – 12.13)	7.533 ± 0.6682 ^{a***,b***} (6.456 – 8.505)	1062	<0.001

N: number of rats in each group.

- Very high significant (***) when P value < 0.001.
- High significant (**) when P value <0.01.
- Significant (*) when P value <0.05.

a: Comparison in relation to control groups.
b: Comparison between High dose treated group and Low dose treated group.

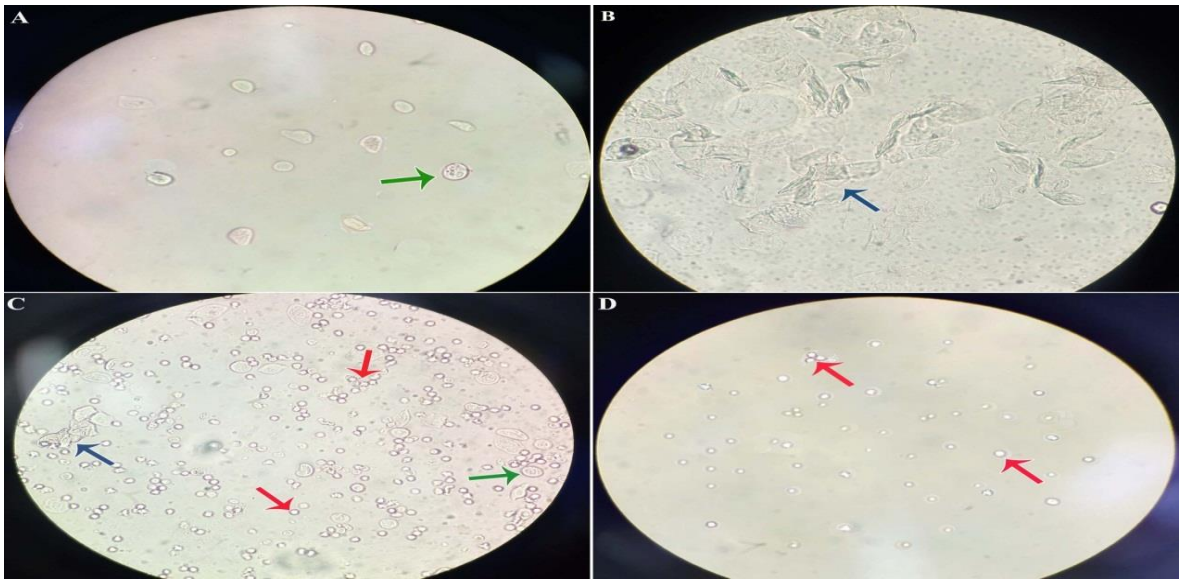

Fig. (1): Vaginal smear (Unstained) of all phases of estrous cycle. A: proestrous phase presenting oval or rounded epithelial cells (green arrow). B: estrous phase presenting cornified epithelial cells (blue arrow). C: metestrous phase presenting various cell types; oval epithelial cells (green arrow), cornified epithelial cells (blue arrow) and predominant leucocytes (red arrows). D: diestrous phase presenting leucocytes (red arrows).

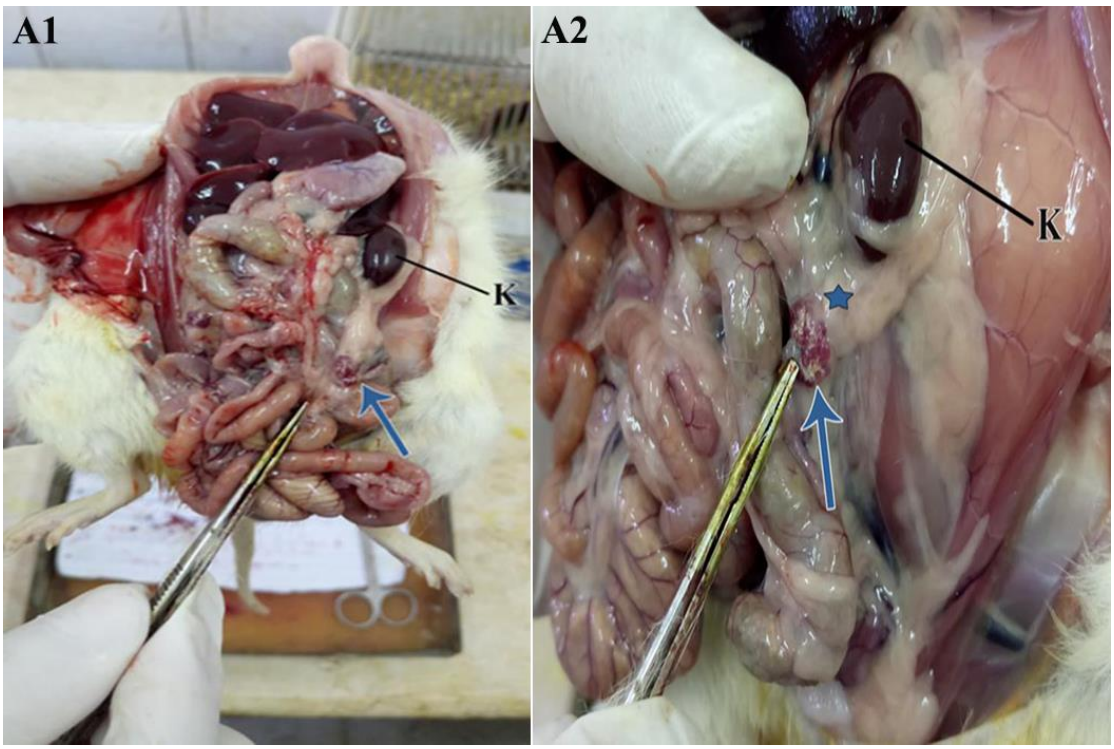

Fig. (2): A1: A photo showing retraction of the ovary (blue arrow) away from the kidney (K). **A2:** A photo showing ovary (blue arrow) with vesicular appearance (mass of follicles). It is embedded in fat (blue star) at caudal pole of the kidney (K).

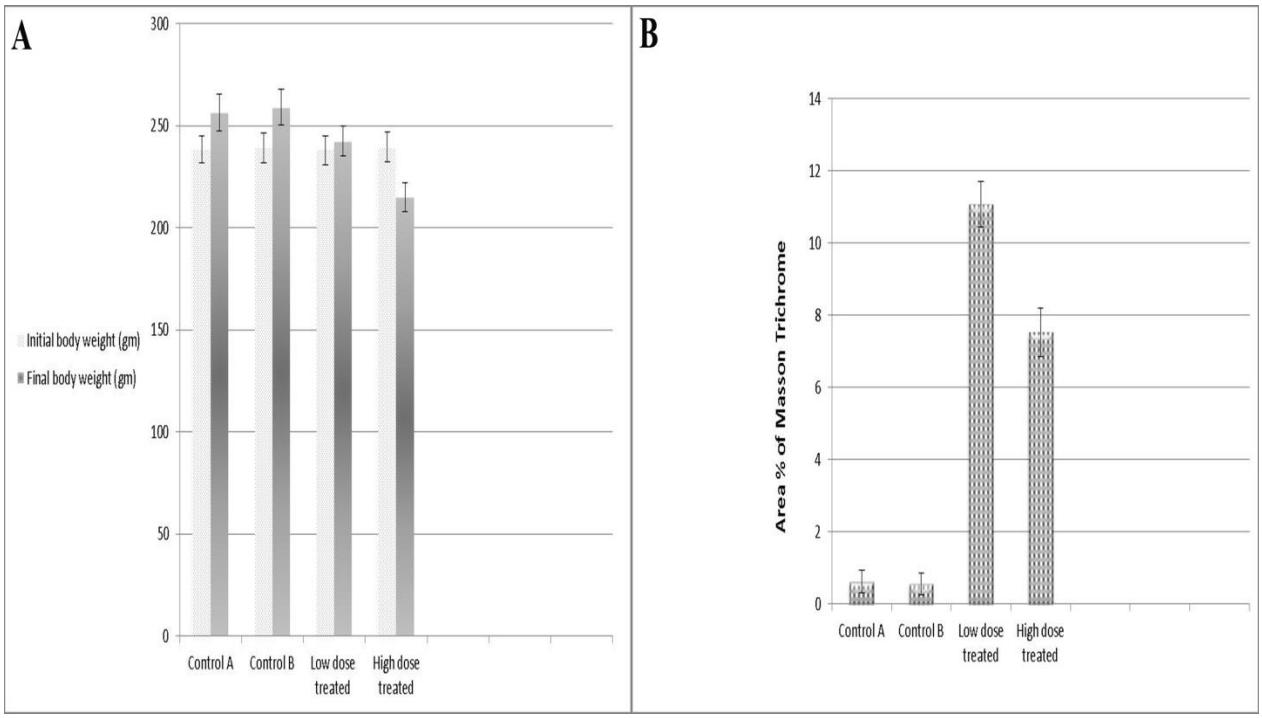

Fig. (3): A: Effect of atrazine on mean ± SD of body weight of different groups. **B:** Area percentage (%) of collagen fibers among the different studied groups.

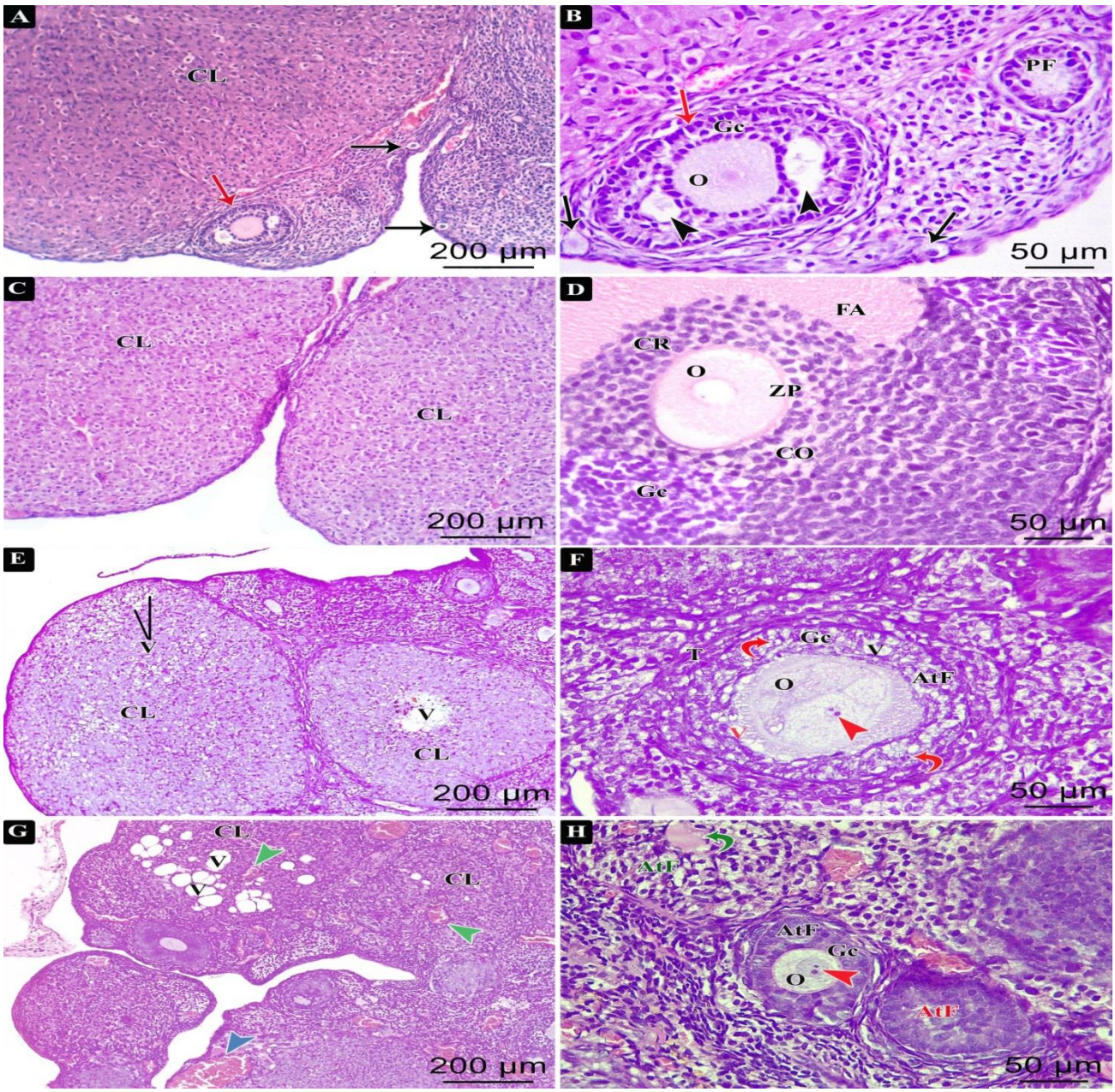

Fig. (4): A photomicrograph of an ovarian section from (A& B) control A group: showing normal corpus luteum (CL), primordial follicles (black arrows), primary follicle (PF) and preantral follicle (red arrow) which is formed of an oocyte (O) and layers of granulosa cells (Gc) with spaces (black arrow heads) in between. (C&D) control B group: showing normal corpus luteum (CL) and a higher magnification of the mature Graafian follicle consisting of an oocyte (O) surrounded by zona pellucida (ZP), corona radiata (CR), follicular antrum (FA) and cumulus oophorus (CO) that connects the oocyte with the rest of the granulosa cells (Gc). (E&F) Low dose treated group: showing vacuolated (V) corpora lutea (CL) and atretic preantral follicle having degenerated oocyte (O) with micronuclei formation (red arrow head) and vacuolated cytoplasm (red V). Granulosa cells (Gc) are disarranged, vacuolated (black V) and some of them have pyknotic nuclei (red curved arrows). There is ill demarcation between granulosa (Gc) and theca cells (T). (G& H) High dose treated group: showing vacuolated (V) corpora lutea (CL) with vascular changes (green arrow heads). Areas of hemorrhage (blue arrow head) are also seen. Also, there are number of atretic follicles (AtF) either with complete loss of oocyte (red AtF) or (green AtF) with remnants of zona pellucida (green curved arrow) and another atretic preantral follicle (black AtF) with degenerated oocyte (O), disarranged granulosa cells (Gc) and micronuclei formation (red arrow heads). (H&E, Bars = 200 & 50 µm).

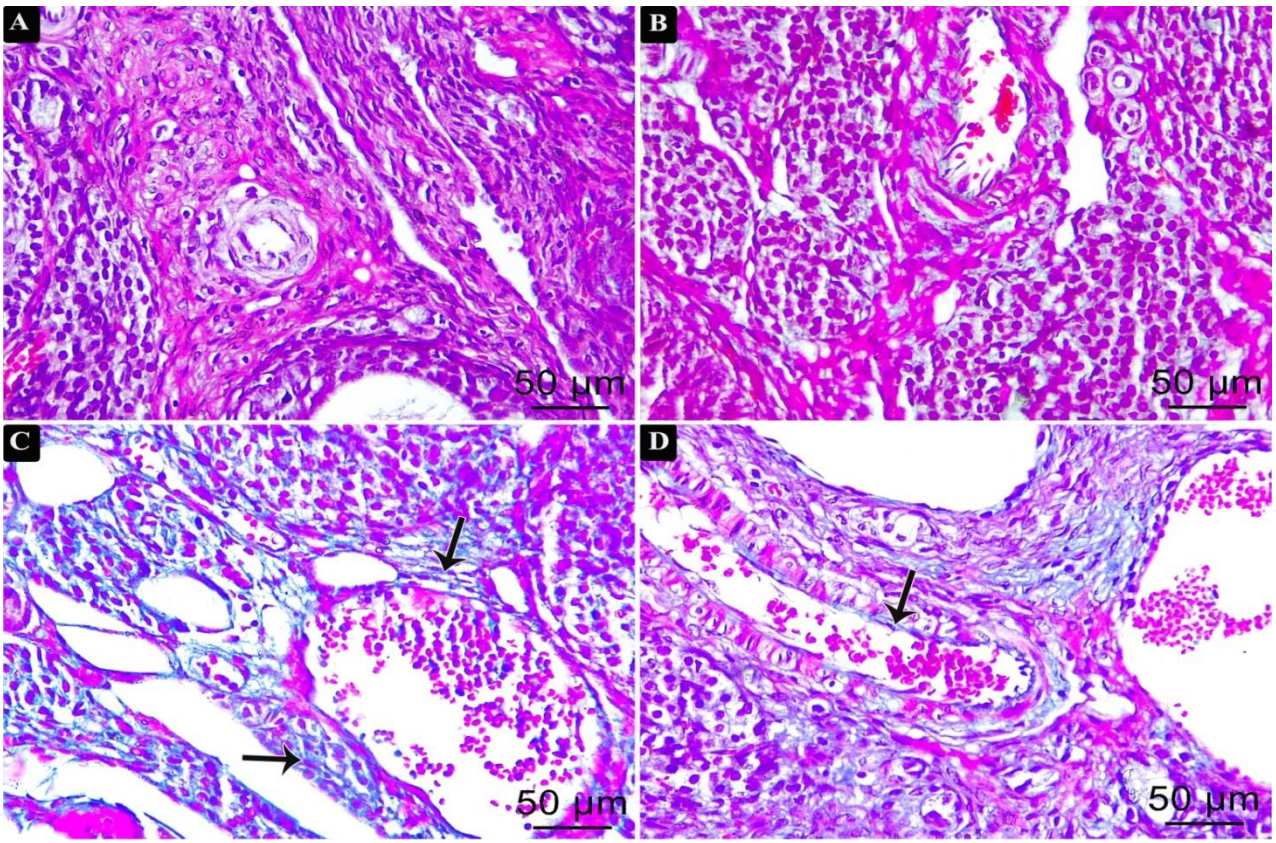

Fig. (5): Photomicrographs of ovarian sections of different groups: (A) control A group, (B) control B group, (C) Low dose treated group showing an extensive deposition of collagen fibers, (D) High dose treated group showing a moderate increase in collagen fibers. (Masson trichrome, Bar = 50 µm).

DISCUSSION

Atrazine has an effect on reproductive and endocrine characteristics in rats. These effects vary according to the dosage, exposure duration, age of the animals, and the type of rats utilized [21, 22].

In the present study, the final body weight in Low dose treated group was highly significantly decreased when compared with the control groups. This was in contrary with a study by **Zhao et al.** [23] in which atrazine administration at dose of 5 mg/kg/day orally for 28 days to female rats had no significant difference on final body weight compared with the control groups. Also, another study by **Song et al.** [24] showed that there were no obvious changes in the body weight during the 3 months of 10 mg/kg ATR treatment.

On the contrary, there was an increase in final body weight in comparison to the initial body weight during 30 days. This result was in accordance with **Jestadi et al.** study which showed an increased body weights in rats with the administration of atrazine at low concentrations. They explained this increase might be due to increase of insulin resistance and hence led to weight gain. [25]

In the present work, there was a very high statistical significant decrease in the final body weight in High dose treated group when compared with the control group. This result was in accordance with **Akunna et al.** [26] who gave 200 mg/kg/day atrazine orally for 16 days and reported a decrease in the body weight in atrazine treated animals. They observed reduction in the food consumption so there was significant body weight loss.

The present study revealed that atrazine induced variable histopathological changes in the ovarian tissue of adult albino rats. Different follicles of the ovary showed degeneration of variable degrees which produced by atrazine.

Chronic exposure to low-dose of toxicants may cause ‘‘silent’’ damage in the reproductive system. This would indicate that exposure of the organism to low doses of atrazine for a long time could represent a stressful condition to this organism [15].

H&E stained sections of Low dose treated group showed presence of pathological changes of the ovary. One of these changes was

presence of atretic follicles with disorganized vacuolated granulosa cells and micronuclei formation that indicated that DNA damage was present. These results agree with those of **Salem and Nabeeh (27)** who revealed that treatment of female rats by low dose (150 mg atrazine/kg) for 30 days produced atretic follicles with vacuolated granulosa cells and blood congestion in ovarian tissue. Another study by **Juliani et al.** [15] revealed that the rat group treated by low dose (0.75 mg atrazine/kg) for 30 days showed presence of atretic antral follicles. Also, a study by **Zhao et al.** [23] who indicated presence of atretic follicles in tissues of rats' ovaries administrated 5mg atrazine/kg/day for 28 days.

H&E stained sections of High dose treated group showed atretic follicles either with loss of oocyte completely or with presence of remnants of zona pellucida. There were also preantral atretic follicles with micronuclei formation which indicated DNA damage. Most of corpora lutea showed vacuolation and vascular congestion. These findings were in agreement with a study by **Juliani et al.** [15] which showed a great amount of preantral follicles with disorganized layers of granulosa cells and/or oocyte in degeneration process.

Also, **Salem and Nabeeh** [27] revealed that ovaries of female rats treated with 300 mg atrazine/kg (high dose) for 15 days had atretic follicles and congestion of blood vessels in corpora lutea. There was vacuolated corpus luteum and hemorrhage in interstial cells in group treated by high dose (300 mg/kg) for 30 days.

In this study, H&E stained sections of High dose treated group revealed vascular congestion. These findings were in agreement with a study by **Abarikwu** [28] that showed severe congestion with marked interstitial cellular infiltration in the kidney of rats treated with atrazine.

Pro-inflammatory mediators are released during tissue damage, stimulating vasodilation and increasing vascular permeability causing congestion with hemorrhagic foci. In addition, the increase in blood vessel diameter helps to accelerate the arrival of the immune system cells to the damaged tissue [29].

In this study, Masson Trichrome staining of Low dose treated group and High dose treated group showed very high significant increase in area percentage of collagen deposition. This was in the same line with **Khalaf et al.** [30] who stated increase in collagen fibers in ovarian tissue of clozapine and haloperidol treated rats.

These results also agree with previous studies explaining that oxidative stress led to ovarian vessel congestion, follicle degeneration and fibrosis [31, 32].

CONCLUSION

Exposure to Atrazine causes harmful effects on the structure of the ovary either with low dose for long duration or high dose for short duration.

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