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Original Article

Histological Changes in Selected Organs of *Oreochromis niloticus* Exposed to Doses of Lead Acetate

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ABSTRACT

This study is carried out to investigate the effects of doubled sublethal concentration of lead acetate on some selected organs of tilapia. 30 Fish were randomly chosen and divided into 3 groups. The first one served as control, the second and third groups were exposed for 3 weeks to 0.4 and 0.7 mg lead acetate/ liter of water respectively. The results of this work clearly indicate that lead has adverse effects on the gills, ovaries, liver and hepatopancreas of tilapia. The severity of lesions caused by lead acetate was positively correlated with the concentration. The gill damage includes hyperplasia of epithelial cells of gill filaments and severe hemorrhage in gill lamellae. Also, lead induces significant atresia in ovaries. The liver showed vacuolar degeneration of hepatocytes and dilatation in hepatic sinusoids. The hepatopancreas showed loss of contact between hepatocytes and pancreaocytes and appearance of apoptotic nuclei.

Keywords: Lead Acetate, Liver, Gills, Liver, *O. niloticus*, Histopathology

INTRODUCTION

Dangerous pollutant that can be absorbed by fish when exposed to elevated levels in an aquatic environment is Lead (Pb). Absorption of lead occurs by different ways through gills and skin or by ingestion of contaminated water and food; .and may lead to high mortality rate or cause many biochemical and histological alterations in survived fish [1]. Two main factors are affect the accumulation of heavy metals in a tissue water concentrations of metals and exposure period, In addition to some other environmental factors such as salinity, pH, hardness and temperature play significant role in metal accumulation [2]. The initial effects of heavy metal pollution is evident only at cellular or tissue levels before significant changes can be identify in fish behavior or external appearance [3, 4, 5, 6]. The fish gill is performing many vital functions such as respiration, osmoregulation, acid–base balance and nitrogenous waste excretion [7]. Histopathological changes were reported in the gills of many fish because of exposure to different toxicants [8, 9, 10, 11]. Moreover, the gonads of teleosts are affected by lead pollutions that in turn affect on reproductive behavior [12]. The liver is a detoxifying organ in fish and is essential for both the metabolism and the excretion of toxic substances in the body [13]. The one of the most sensitive organs in teleosts is liver and frequently showing alterations in histological structure, biochemistry, and physiology following exposure to different types of environmental pollutants [14, 15]. In Egypt, tilapias are the main species of freshwater fishes that inhabit River Nile, irrigation network and drainage canals connected to it. The Nile tilapia, *Oreochromis niloticus* (Pisces: Cichlidae), is an important fish in the ecology of tropical and subtropical region including Egypt and the most popular species of the bony fish in Africa [16, 17, 18]. The aim of this study is to investigate the effects of doubled sublethal concentrations of lead acetate on some selected organs of tilapia.

MATERIALS AND METHODS

Test organisms:

Thirty healthy adult *O. niloticus* specimens (58% female/ 42% male) were collected from the Nile River at Elkhazan bridge in Assuit city (mean standard length ± SD was 14.46 ± 0.22 cm for male & 13.92 ± 0.16 cm for female and the mean body mass ± SD was 96.39 ± 2.19 g for male and 95.52 ± 2.00 g for female). The specimens were selected as test organisms after an acclimation period of 12 weeks in a temperature-controlled aquarium.

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Experimental design:

The fish were divided into three groups of 10 specimens each. The specimens were transferred from the initial acclimation tank to exposure tanks (74 L capacity). Two experimental exposures were executed. The first group acts as a control group. The second and third groups are exposed to 0.4 and 0.7 mg lead acetate /liter of water respectively for 3 weeks. Physicochemical condition of water during experimental period were recorded such as; temperature (mean ±SD = 25.1 °C ± 1°C), dissolved oxygen (mean ± SD = 7 mg/L ±0.19), pH (mean ±SD =7.6 ± 0.13) and photoperiod was a 12:12 light-dark cycle.

Histopathological analysis:

Ovaries, liver samples were taken in addition to the gills, which were excised keeping the filaments and rakers intact, rinsed in seawater, fixed in Bouin's fluid for 24 h and processed for paraffin embedment. Thin sections (7 µm) cut by means of a rotatory microtome were rehydrated and stained with Harris haematoxylin-eosin (H&E) stain (19).

RESULTS

The exposed fish exhibited a decrease of swimming activity and increase mucus secretion from gills and skin. 4 specimens in 0.7 mg/ L lead acetate exposure group died during the exposure period. One mortality occurred during 0.4 mg exposure. In addition, the results indicated sever alterations in tissues treated with 0.7 mg with comparison with 0.4 mg group, as the severity of lesions caused by lead acetate was positively correlated with the concentration.

Gills histopathological analysis:

General structure of the gills in control fish was typical for teleostean. The gill was consisted of primary and secondary gill lamellae (Fig.1 A). The secondary lamellae were lined by pavement cells PVC (sqaumous epithelial cells). Between secondary lamellae, the primary lamellae is lined by stratified epithelium and mucous cells, while the chloride cells CC (faintly stained acidophilic cells) were scattered in the base of lamellae and in the interlamellar region.

The lesions were observed in the gills included curling in secondary lamellae, lamellar swelling, shorter gill lamellae, complete destruction of gill lamellae, and edema in secondary lamellae and gill filaments and blubbing in gill filaments were observed (Fig.1 B, F). In addition, epithelial lifting which characterized by lifting of the outer layer of lamellar epithelium was observed. Congestion was prominent in primary and secondary gill lamellae (Fig.1 C, D, G, H). Moreover, hyperplasia of the epithelial cells of gill filaments, decrease of interlamellar space, Hypertrophy and hyperplasia of pavement and chloride cells (Fig.1 D). In secondary lamellae, impaired capillary circulation because of congestion of blood vessels of primary lamellae and occasionally free erythrocytes were observed (Fig.1 H).

Ovary histopathological analysis:

Normal histology of the ovary of tilapia reveals that it is surrounded by an ovarian wall which is differentiated into an outer thin peritoneum, a thicker tunica albuginea made up of connective tissue, muscle fibers and blood capillaries. The innermost layer is the germinal epithelium, which joins with the tunica albuginea at several places and projects into the central lumen, the ovocoel, in the form of finger like projections called ovigerous lamellae that are consisted of ovarian follicles (Fig.2 E).

The groups were exposed to higher concentrations of lead acetate showed increased number of atretic follicles. The Pb was more effective on relatively older oocytes (Fig.2 A). The ovary was contained many immature follicles accompanied by a slight decrease of early vitellogenic stages. (Fig.2 B). Moreover, stromal hemorrhage, granulmatous infiltration was prominent. Both pre- and post-vitellodenic follicles underwent wrinkling. Some extent of damage could be seen in the architecture of tunica albuginea and inner germinal epithelium as they became loose. Oogonia were destroyed (Fig.2 A, C, D).

Liver histopathological analysis

The liver of control fish is covered by thin fibrous capsule and a single layer of mesothelial cells. The hepatic parenchyma is not arranged into distinct lobules, the biliary channels and vascular elements did not exhibit the classical triads and theses structures seemed to be randomly dispersed throughout the parenchyma. Within the parenchyma the hepatocytes spread out as irregular cords around the central vein and arranged in two cellular layers surrounded by sinusoids with the bile canalculi located intracellularly (Fig.3 A). The most alterations of liver after lead exposure were disarrangement of hepatic cords, nuclear pyknosis, cytoplasmic vacuolation and moderate melano-macrophages aggregation (Fig.3 B, D). In addition, hemosiderosis, intravascular haemolysis in hepatoportal blood vessels, dilation and congestion in sinusoids and venules and cellular degeneration (Fig.3 B, C, D, E, F).

Hepatopancreas histopathological analysis

The pancreocytes of control fish were seen as islets in an acinar pattern surrounding a branch of the pyramidal broad based cells that rested on a basal lamina. The nucleus of acinar cells located towards the base of the cells and the cytoplasm towards the lumen contain eosinophilic zymogen granules (Fig. 3A). Damage of

hepatopancreas were characterized by loss of contact between hepatocytes and pancreocytes, atrophy and appearance of apptotic nuclei (Fig3 B, C).

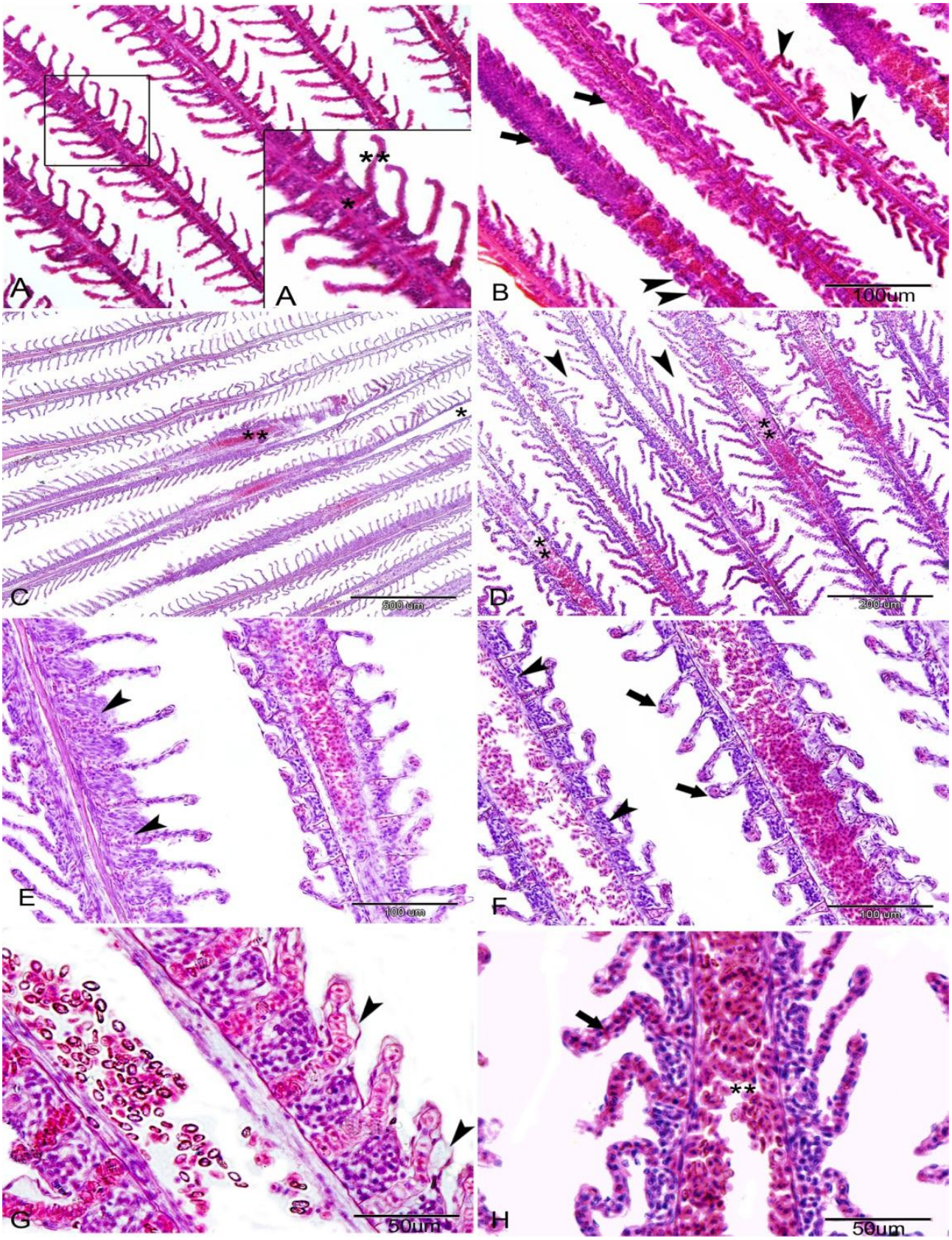

Figure (1): Showing affections observed in the gills[(A) Normal gill tissue with primary (*) and secondary gill lamellae (**). (B) Curling in secondary lamellae (arrow head), shorter gill lamellae (two arrow heads) and complete destruction of gill lamellae (arrows). (C) Congestion was prominent in gill lamellae (**) and ballooning dilatation of lamellae (*). (D) Decrease of interlamellar space (arrow heads). (E) Hypertrophy and hyperplasia of the epithelial cells of gills (arrow heads). (F) Hypertrophy and hyperplasia of the epithelial cells of gills (arrow heads) and bulbing in gill filament (two arrows). (G) Lifting of the outer layer of lamellar epithelium (arrow heads). (H) Hemorrhage was prominent in primary (**) and secondary (arrows) gill lamellae. (C) Bar = 500µm. (B) Bar = 100 µm. (A & D) Bar = 200 µm. (E, F) Bar = 100 µm. (G, H) Bar =50 µm.].

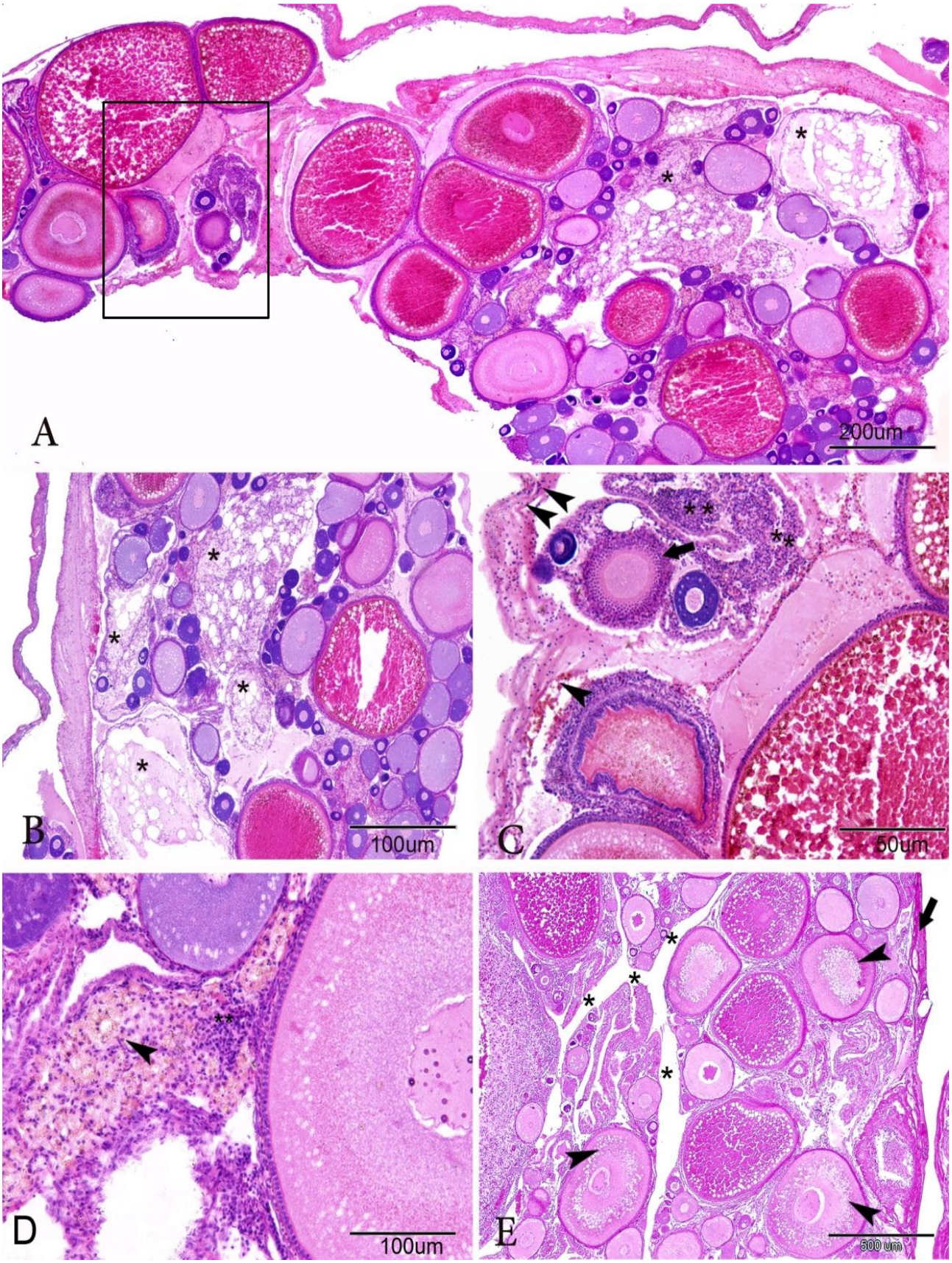

Figure (2): [(A, B, C, D) demonstrating the lesions were observed in ovaries after lead exposure. (A) An overview shows that the increased number of atretic follicles (*). (B) Higher magnification shows many atretic follicles (*). (C) Higher magnification of the outlined area within A shows some extent of damage can be seen in the architecture of tunica albuginea (two arrow heads) as they become loose, stromal hemorrhage (arrow head), lymphocytic infiltration(**) and alteration in germinal epithelium (arrow). (D) Increased amount of stroma (arrow head) and lymphocytic infiltration (**) (E) Paraffin section of normal ovary were stained with Hematoxylin- eosin demonstrating the ovocoel (*) and µm ovarian follicles (arrow heads) and tunica albuginea (arrow). (A) Bar = 200µm. (B, D) Bar =100µm. (C) Bar = 50 µm. (E) Bar = 500 µm.].

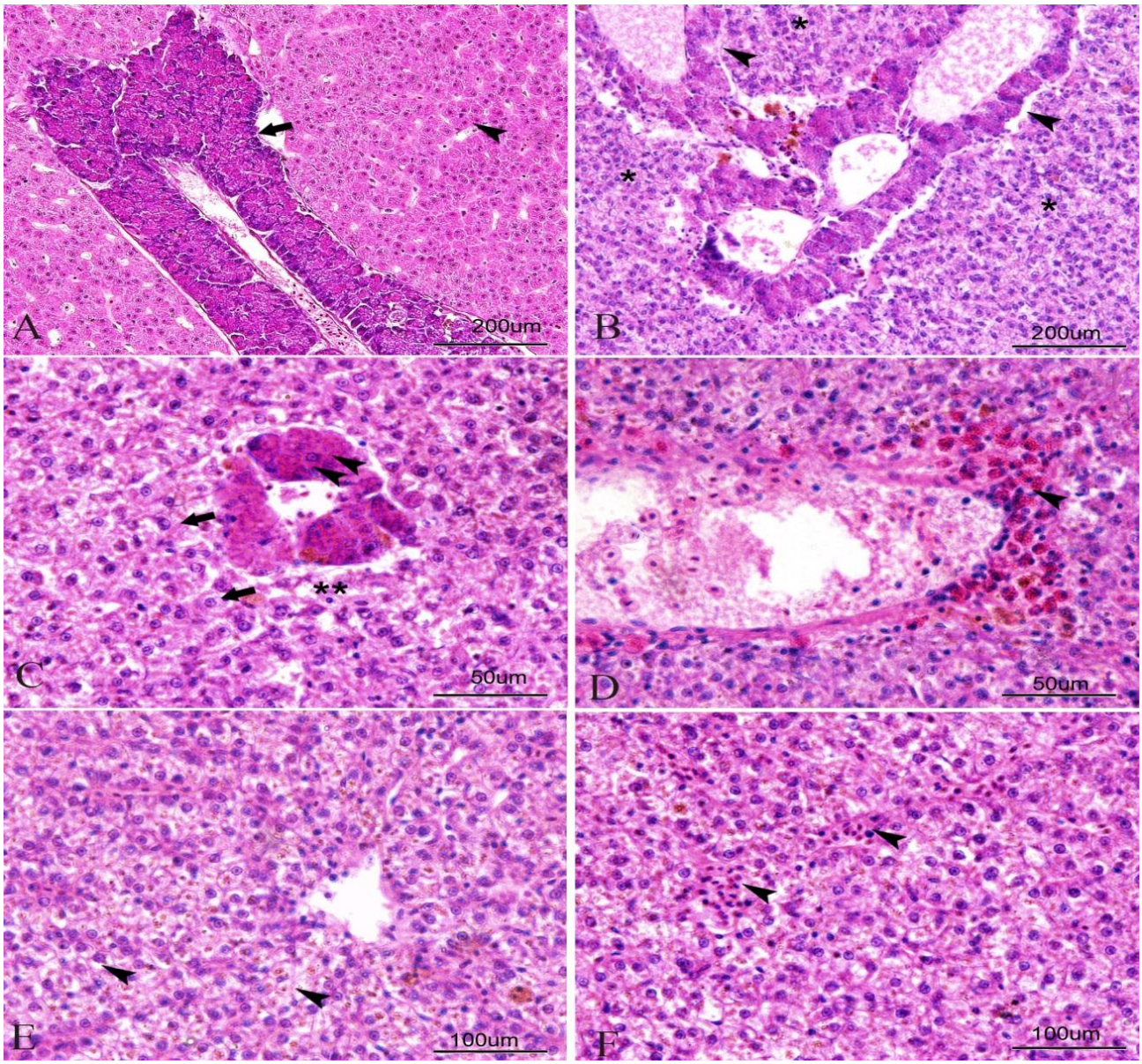

Fig. 3: demonstrating affections of the liver [(A) Normal liver histology in control fish showing normal hepatocytes (arrow head) and pancreas (arrow).(B) Alteration of liver after lead exposure were disarrangement of hepatic cords (*) and damage of hepatopancreas characterized by loss of contact between hepatocyte and pancerocyte (arrow heads). (B,C,D,E,D,F) details of liver with higher magnification showing alteration of liver after lead exposure in (C) and (D) were melano- macrophages aggregation and hepatocyte hypertrophy (arrows), nuclear pyknosis and cellular degeneration(**) , intravascular hemolysis in hepatoportal blood vessels (arrow heads) .Notice the degeneration of pancreocytes (two arrow heads). (E) Cytoplasmic vacuolation in hepatocytes and in (F) dilatation and congestion in sinusoids (arrow heads). (A, B) Bars = 200 µm. (C, D) Bars = 50 µm. (E, F) Bars = 100 µm.].

DISCUSSION

Gills are highly susceptible to toxic chemicals of environmental pollutants, because of direct contact between gills and environmental pollutants .The absorption of toxic chemicals through gills is enhanced by increasing the permeability to water and ions of gill epithelium and by inhibition of ions exchange activity of the chloride cells [20]. The measurements of the effect of aquatic pollutants in marine and freshwater habitats have frequently been tested in gill [21, 22].

Histological study of the gills shows a typical structural organization of the lamella in the untreated fish. However, fish exposed to lead acetate shows several histological alterations, included hypertrophy, lifting of the epithelial linings from the surfaces of secondary lamellae, and at few places, degeneration of lamellar epithelium. Similar observations were reported due to exposure of other freshwater fish to lead [23, 24, 25, 26] and copper [27]. Toxic metals may interfere with vital operations including respiratory chain, compete with essential metals for transport with consequences for the osmotic balance and for active centers of enzymes [28]. Lifting and hyperplasia of lamellar epithelium could be interpreted as defense responses of the fish, as these alterations increase the distance across which waterborne irritants must diffuse to reach the blood stream [29]. The most common cause of cellular degeneration in gill filaments is oxygen deficiency as a result of gill toxicity [30].

Lead (Pb) induced significant atresia in the ovary. The older oocytes were more affected by Pb. These observations coincide with those recorded by earlier researches [31, 32, 33]. These studies suggest a direct action of heavy metals on the gonads. Alteration in hypothalmohypophyseal ovarian function due to Lead accumulation in brain of some fish species resulting in decreased reproductive potential in fish [34].

Mobarak et al. [35] Mentioned that fish liver histology could use as a model for studying the interactions between environmental factors and hepatic structures and functions. Two factors were detecting the harmful effect of metal pollution on fish liver histology, the duration of the exposure and the concentration level of the specific metal.

The present study documents pathologic changes in fish treated with two different doses of lead acetate include degeneration and vacuolation of hepatocytes. Inhibition of protein synthesis, energy depletion and aggregation of microtubules resulting from vacuolation of hepatocytes [36], and this might indicate an imbalance between the rate of synthesis of substances in parenchymal cells and the rate of their release into the circulatory system [37, 30). Damage of hepatopancreas characterized by loss of contact between hepatocytes and pancreocytes and atrophy. Similar results observed by [15]. A number of fish diseases are associated with melano-macrophage aggregates as phagocytic cells [38]. The widespread of this pathologic condition in the present study may be because, with the loss of energy in the detoxification process, increases in metabolic byproducts caused aggregation of more melano-macrophage [21]. These histopathological findings are similar to those mentioned by Liao et al. [22] when exposed medaka *(Oryzias latipes)* to sublethal exposure of methyl mercury chloride, [15] when exposed *Channa punctatus* to arsenic, and when exposed *Oreochromis mossambicus* to cadmium and zinc. Finally, the severity of lesions caused by lead acetate was positively correlated with the concentration and from the present results we can suggest that the cause of mortality in fish during the exposure period may due to combination of all histological alterations in gills, liver and hepatopancreas. Further work should be made on the central nervous system and other organs, which may help in detection of other causes of death [13].

CONCLUSION

30 Fish were divided into 3 groups. The first one served as control, the second and third groups were exposed for 3 weeks to 0.4 and 0.7 mg lead acetate/ liter of water respectively. The severity of lesions caused by lead acetate was positively correlated with the concentration. The gill damage includes hyperplasia of epithelial cells of gill filaments and severe hemorrhage in gill lamellae. Also, lead induces significant atresia in ovaries. The liver showed vacuolar degeneration of hepatocytes. The hepatopancreas showed loss of contact between hepatocytes and pancreaocytes.

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