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Histological and Histochemical Alterations Induced by Phenol Exposure in *Oreochromis aureus* (Steindachner, 1864) Juveniles

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ABSTRACT

The present study inspects the effect of phenol as one of environmental xenobiotic compound on the histology and histocemistry of liver, gills and spleen of *Oreochromis aureus (O.aureus)* juveniles. Fish were subjected to three sub-lethal concentrations of phenol (20%, 40% and 80% of LC_{50}) for seven days. The liver, gills and spleen were studied from the histopathological and histochemical point of sight. Histopathological changes viz degeneration, necrosis, severe hemorrhage pynknosis and distinct cellular proliferation in the prescribed organs of fishes exposed to phenol were observed. Further, detachment of gill epithelium (GE), marked proliferation of gill epithelium (Hyperplasia, H) with distinct shortening of lamellae were recorded. Even though, complete fusion of gill lamellae, enhanced connective tissue disposition and blood permeation were also induced due to phenol exposure. Furthermore, histochemical annotations affirmed reduction of carbohydrate store due to oxidative stress caused by phenol publicity. Marked rise of gills mucoplysaccharides which impedes fish respiration were reported. DNA was also amplified in tested organs after phenol exposure, signifying carcinogenesis of phenol.

Key words: *Oreochromis aureus* , Phenol, Pollution, Histology, Histochemistry

INTRODUCTION

Phenol is one of xenobiotics environmental contaminants that is commonly polluted the aquatic environment. It reaches the aquatic environment with the effluents of variety of chemical industries such as cool refineries, phenol manufacturing, pharmaceuticals and industries of resin paint, dying textile wood petrochemical and pulp mill (Mukherjee *et al.*,1991; Fleeger *et al.*,2003 and Hori *et al.*, 2006 & 2008). Also, it is commonly present as non-specific pesticides, herbicides, bactericides and fungicides (Gupta *et al.*, 1983). Genotoxic, carcinogenic, immunotoxic, hematological and physiological effects were reported for different species exposed to phenol and its derivatives (Assem *et al.*, 1992, Jagetia and Aruna, 1997; Barisiene *et al.*, 2000; Hori *et al.*, 2006; Adel-Hameid, 2007 and Gad and Saad, 2008). They have a high bioaccumulation era along the food chain due to its lipophilicity (Jagetia and Aruna, 1997). Consequently phenol pollution represents a threat against natural environment and also to human health (Hori *et al.*, 2006).

The fish liver is the center of metabolism and detoxification.The hepatosomatic index and liver histology are used as a biomarkers for the environmental pollution (Abdel-Hameid, 2007; Glover *et al.,* 2007 and Simonato *et al.,* 2008). Hepatomegaly, necrosis and degeneration were reported in the liver of fishes exposed to phenol (Abdel-Hameid, 2007). Similar effects were also reported for fishes exposed to other pollutants (Glover *et al.,* 2007; Simonato *et al.,* 2008; Yang *et al.,* 2008 and Barse *et al.,* 2006).

Fish gills are exposed directly to aquatic media. Therefore, it is sensitive to any change of water components. There are three cell component of gill epithelium: pavement or respiratory cells, mucous cells and chloride cells (Laurent and Perry, 1995). The first cell type may be the site of Na^{+} and Ca^{2+} (Perry, 1997). The mucous cells are of two types: the ordinary and rodlet cells (Mattey, 1990). Although, the main function of mucous cells is respiration and osmoregulation but also play a protective role (Tkatcheva *et al.,* 2004). Environmental pollution causes severe alterations of gill histology as detachement of epithelium, hyperplasia, lesions and lamellae fusion. Therefore, fish respiration was oblitered (Tkatcheva *et al.,* 2004: 2007; Hochachka and Somero, 2002 and Miron *et al.,* 2008). Therefore, gill histology are used extensively as indication of environmental pollution.

RNA/ DNA is useful indicator of fish growth (Smith and Buckley, 2003; Mukherjee and Jana, 2007). Also, the quantative determination of DNA in fish organs is used as a tool evaluating genotoxic effect for different stressors (Gad and Saad, 2008).

Tilapia fish are of economical importance as they support the fishing industry of inland and lake water in Egypt (Maghraby *et al.,* 1972). They grow faster and so constitute high bulk of fish production in Egypt. When phenol present in the aquatic environment, fish food consumption, mean weight and fertility are significantly reduced (Saha *et al.,* 1999). Therefore, phenol impedes fish production. The resistance of fish to different stressors is directly related with fish age i.e., juveniles and fry are so sensitive (Costa *et al.,* 2009). The study regarding the effect of phenol on histochemistry is scarce. Therefore, the present undertaken is directed to fulfill the exist lacking.

MATERIALS AND METHODS

1. Fish and experimental design

Blue tilapia (*O. aureus*, Steindachner, 1864) juveniles (Teleostei, *Cichlidae*) measuring 8.59±0.70 cm in total length and weighing 10.11±1.01g were gifted from central laboratory for aquaculture research (CLAR) Abassa, Abouhammad, Sharkya, Egypt. The fish were acclimated for laboratory conditions (water temperature, 23±0.5 °C, dissolved oxygen 6 mg/L, pH 7.7–7.8 and normal photoperiod) for one week in large cement aquaria ($1m^3$). The fishes used in the present study were apparently healthy. Fish feeding and handling practices were done according to standard laboratory conditions. The experiment was conducted at static system in glass aquaria (40 cm height, 70 cm length and 30 cm width). The lethal concentration for 50% of fish for 96-h (96-h LC_{50}) of phenol was found to be 29 mg/L. Fishes were exposed to three sub-lethal concentrations of phenol (20%, 40% and 80% 96-h LC_{50}) for one week. In other tank no phenol was added (control). In every tank 14 fishes were stocked. The aquaria are well aerated and the water of each one was renewed every 12 h, in this period 30% of the phenol is lost due to volatilization (Hori *et al.,* 2006). The phenol levels were adjusted to remain at constant levels.

2. Histological and histochemical preparations

At the end of the experiment after one week the fishes were decapitated and sacrificed. The liver, gills and spleen were carefully isolated out of the fish and washed in saline solution. Small pieces of the prescribed organs were fixed in neutral buffered formalin. Thereafter, the tissue samples were dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. Sections (5 um) were cute using rotary microtome and mounted on slides**.** For general histological structure, tissues sections were routinely stained with haematoxylin–eosin and Masson's trichrome. For histochemical demonstration, paraffin sections were stained with periodic acid Schiff stain (PAS)**,** alcian blue and Feulgen reaction (Bancroft and Stevens, 1990).

RESULTS

Histology

1.1. Liver:

The liver of control fish exhibited the normal histological architecture (Figs 1, 2). Hepatic strands arranged around the central vein which appear devoid from hemorrhage and congestion (Fig., 2). Exposure to 20% (Figs 3, 4) and 40% LC_{50} (Figs 5, 6) phenol induced degeneration, necrosis, severe hemorrhage in central vein. However, exposure to 80% LC_{50} of phenol induces pynknosis, necrosis and distinct cellular proliferation (Figs 7, 8).

Figure (9, 10) showed normal connective tissue (CT) distribution in the liver of control tilapia. Phenol exposure induced slight increase of CT in the liver (Figs 11, 12, 13, 14, 15, 16).

Gills:

Figures (17, 18) showed regular gill structure with distinct primary and secondary lamellae. Fishes exposed to low phenol level (20% LC_{50}) exhibited detachment of gills epithelium (DE) (Figs 19, 20). Furthermore, the exposure to phenol of mid level (40% LC_{50}) induced marked proliferation of gill epithelium (Hyperplasia) with distinct shortening of lamellae (Figs 21, 22). Complete fusion of gills lamellae was recorded for fishes subjected to the highest phenol level (80% LC_{50}) (Figs 23, 24).

Fish raised in phenol free water (control) showed normal CT distribution and density in the gill lamellae (Fig., 25). On contrary, phenol exposure induced marked enhancement of CT profile (Figs, 26, 27, 28).

1.3. Spleen:

Normal spleen structure was accessible in figure (29). Exposure to phenol induced marked degeneration of dose reliant (Figs, 30, 31, 32).

Normal spleen structure with normal CT density was clear in control fish (Fig., 33). On the other hand, CT density was increased in case of fishes raised in phenol polluted water (Figs 34, 35, 36). The uppermost level of phenol induced more CT disposition and blood infiltration.

Histochemistry

2.1. Carbohydrates

2.1.1. Liver:

Periodic acid Schiff reaction in the liver of control *O. aureus* exhibited normal carbohydrate content. It is distributed densely in the hepatocytes around the portal area (Figs 37, 38). Fishes exposed to different phenol levels exhibited marked reduction of carbohydrate in the livers (Figs 39, 40, 41).

Gills:

Exposure to 20% and 40% LC_{50} of phenol induces slight rise of PAS reaction in gill lamellae (Figs 44, 45, 46, 47) over than those of control fish (Figs 42, 43). Marked rise of carbohydrate was observed in gill lamellae of fishes raised in water polluted with the highest phenol level (Figs 48, 49).

Spleen:

The carbohydrate in the spleen of fishes subjected to low (20% LC50) and moderate (40% LC_{50}) phenol was hardly differentiated from those of control group (Figs 50, 51, 52). On the other hand, the highest phenol level induced marked stimulation of PAS reaction (Fig., 53).

Mucoplysaccharides

Gills

The control fishes exhibited normal mucoplysaccharides in the gills (Fig., 54). Phenol exposure induced marked rise of gills mucopolysaccharides, it is directly related with phenol level (Figs 55, 56, 57).

DNA

2.3.1. Liver

Feulgen reaction in the liver of control fish exhibited normal DNA content (Fig., 58). Non visible difference in DNA content of fishes subjected to phenol levels (20%, 40% LC_{50}). Fishes exposed to phenol levels (20%, 40%, 80% LC_{50}) have a moderate rise of DNA content in the liver comparing to the control fish (Figs 59, 60, 61). On the other side, there was no incredible difference between liver DNA content of fishes exposed to different phenol levels.

2.3.2. Spleen:

DNA content was generally increased in the spleen of fishes exposed to diverse phenol levels; it was dose dependent (Figs 62, 63, 64, 65).

DISCUSSION

The fish constitutes the major bulk of aquatic habitat. It also, considered a valuable food for human being as it is a source of protein, essential amino acids, essential fatty acids and some trace minerals. For this reason much of attention has been given to aquatic organisms particularly fish (Woo *et al.,* 2009). Recently there is extensive industrialization that is generates pollution especially to aquatic environment (Barakat, 2004). The aquatic pollution is serious problem as it induces hazardous effects of organisms (Vander Oost *et al.,* 2003). Phenol pollution is also one of serious problem as induces toxic effects for fish health (Abdel-Hameid, 2007, Gad and Saad, 2008), therefore the fish production may be possibly reduced. Also, phenol have high bioaccumulation rate alongside the food chain due to its liphopholicity. Hence, phenol talented the toxicity to human being and the other terrestrial animals (Hori *et al.,* 2006).
The present results show that phenol induces ruinous changes in the histological structure of the liver. The most pragmatic histopathological signs reported in the present study were necrosis, and degeneration. This result also was reported for *O. aureus* and *O. niloticus* subjected to phenol (Abdel-hameid, 2007 and Gad and saad, 2008) respectively. Elevated phenol level induces pyknosis and cellular proliferation. The pyknosis reflects the damaging effect of phenol on genetic material. This result agree with those reported by Jagetia and Aruna (1997) and Gad and Saad (2008). Cellular proliferation observed here could possibly explained by the stimulatory effect that is originated from striking deterioration of liver cells. Enhanced fibrosis, i.e., high proliferation of CT, of the examined organs resulted in marked inhibition of the various vital activities. Eminent phenol level induces gill hyperplasia, shortening and even completes fusion of gill lamellae. Accordingly, it faultily affects the fish respiration. Similar fallout was reported as an effect of diverse pollutants (Abdel-Tawwab *et al.,* 2007 and Ismail *et al.,* 2009). Deterioration of splenic capsules due to phenol exposure explains the hazard effect of haemopoiesis. Accordingly, immune response and erythropoiesis showed marked hang-up (Wlasow, 1984, Sitjà-Babadilla *et al.,* 2006).

Enhanced mucopolysaccharides in the gills of fishes exposed to phenol that is reported in the present study represents adaptive response to prevent the entrance of this toxicant. Also, it impedes fish respiration. This phenomenon is previously reported for different pollutants (Miura *et al.,* 2005; Simonato *et al.,* 2008 and Fasulo *et al.,* 2008). Phenol exposure provoked a depletion of the general carbohydrates in the examined tissues, i.e., energy reserve depletion. Dange´ (1986) found that energy was depleted to zero after four days of exposure to phenol. The phenol induced energy depletion was previously reported (Dange´, 1986; Hori *et al.,* 2006 and Abdel-Hameid, 2007).

The amplification of liver DNA for fishes exposed to phenol reported in the present study could possibly explain the beginning of carcinogenesis. This is previously reported for fishes subjected different phenolic compounds (Tsutsui *et al.,* 1997). Many xenobiotics or their reactive metabolites can covalently bind to DNA, forming DNA addition products (DNA adducts). Binding of xenobiotics to DNA is seen as a critical step in the beginning of mutagenesis and carcinogenesis (Millar *et al.,* 1989 and Ali *et al.,* 2009). In a conclusion fishes exposed to phenol pollution showed severe histological and histochemical alterations in addition to cancer.

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LEGEND OF FIGURES

Figures (1,2). Photomicrographs of liver sections of control *O. aureus* junveniles showing normal hepatic structure with normal central vein (CV) devoid from haemorrhage (H&E, 100X and 400 X, respectively).

Figures (3,4,5,6). Photomicrographs of liver sections of *O. aureus* juveniles exposed to 20% and 40 % 96- h LC_{50} of phenol showing degeneration (D), nerosis (N) and severe hemorrhage (H) (H&E, 100X and 400 X, respectively).

Figures (7,8). Photomicrographs of liver sections of *O. aureus* junveniles exposed to 80% 96- h LC_{50} of phenol showing pcknosis (P), distinct proliferation (H&E, 100X and 400 X, respectively).

Figure (9,10). Photomicrograph of liver section of control *O. aureus* juveniles showing normal connective tissue (CT) pattern (Trichrome, 100 X and 400X, respectively).

Figures (11,12,13,14,15,16). Photomicrographs of liver sections of *O. aureus* junveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, respectively showing weak proliferation of connective tissue (CT) (Trichrome, 100 X and 400X, respectively).

Figures (17, 18). Photomicrographs of transverse sections in gills of control *O. aureus* juveniles showing normal structure with distinct primary (PL) and secondary lamellae (SL) (H&E, 100X and 400 X, respectively).

Figures (19,20). Photomicrographs of transverse sections in gills of *O. aureus* junveniles exposed to 20% 96- h LC_{50} of phenol showing detachment of gill epithelium (DE) (H&E, 100X and 400 X, respectively, respectively).

Figures (21, 22). Photomicrographs of transverse sections in gills of *O. aureus* junveniles exposed to 40% 96- h LC_{50} of phenol showing marked proliferation of gill epithelium (PG) (H&E, 100X and 400 X, respectively).

Figures (23, 24). Photomicrographs of transverse sections in gills of *O. aureus* junveniles exposed to 80% 96- h LC_{50} of phenol showing complete fusion of gill lamellae (GL) (H&E, 100X and 400 X, respectively).

Figure (25). Photomicrograph of transverse sections in gills of control *O. aureus* juveniles showing normal connective tissue (CT) (Trichrome, 100 X).

Figures (26, 27, 28). Photomicrographs of transverse sections in gills of *O. aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol , respectively showing marked enhancement of connective tissue (CT) (Tricchrome, 100 X), respectively.

Figure (29). Photomicrographs of spleen section of control *O. aureus* junveniles showing normal structure (H&E, 400 X).

Figures (30, 31, 32). Photomicrographs of spleen sections of *O. aureus* junveniles exposed to 20, 40 and 80% 96- h LC_{50} of phenol, respectively showing marked degeneration (D) (H&E, 400 X, respectively).

Figure (33). Photomicrograph of spleen section of control *O. aureus* juveniles reveals normal connective tissue (CT) distribution (Trichrome, 400 X).

Figures (34,35,36). Photomicrographs of spleen sections of *O. aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, respectively showing increased connective (CT) (Trichrome, 400 X), respectively.

Figures (37,38). Photomicrographs of liver sections of control *O. aureus* juveniles showing normal carbohydrate distribution (Black arrow) around portal area and inside the hepatocyte (White arrow) (PAS, 40X, 400X, respectively).

Figures (39, 40, 41). Photomicrographs of liver sections of *O. aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, respectively showing reduced carbohydrate (Arrow) (PAS, 400 X), respectively.

Figures (42,43). Photomicrographs of transverse sections in gills of control *O. aureus* juveniles showing normal carbohydrate (C) in secondary gill filaments (PAS, 100X, 400 X, respectively).

Figures (44, 45, 46, 47, 48, 49). Photomicrographs of transverse sections in gills of *O. aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, respectively showing normal carbohydrate (C) in secondary gill filaments (PAS, 100X, 400 X, respectively).

Figure (50). Photomicrograph of spleen section of control *O. aureus* juveniles reveals normal carbohydarte distribution (PAS, 400 X).

Figures (51,52, 53). Photomicrographs of spleen sections of *O. aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, showing increase of carbohydrate (Arrow) disposition (PAS, 400 X), respectively.

Figure (54). Photomicrograph of transverse section in gills of control *O. aureus* juveniles showing normal mucoploysaccharide (MP) at the base of primary gill filaments (Alcian PAS, 100X).

Figures (55, 56, 57). Photomicrographs of transverse sections in gills of *O. aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, respectively showing marked rise of muc0oplysaccharides stain ability (MP) in primary gill filaments (Alcian PAS, 100X) respectively.

Figure (58). Photomicrograph of liver section of control *O. aureus* juveniles showing normal DNA content (Feulgen, 400X).

Figures (59, 60, 61). Photomicrographs of liver sections of *O. aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, showing rise of DNA content (Arrow) (Feulgen, 400X) respectively.

Figure (62). Photomicrograph of spleen section of control *O. aureus* juveniles showing normal DNA content (Arrow) (Feulgen, 400X).

Figures (63, 64, 65). Photomicrographs of spleen sections of O. *aureus* juveniles exposed to 20, 40, 80% 96- h LC_{50} of phenol, showing rise of DNA content (Arrow) (Feulgen, 400X), respectively.

Plate I

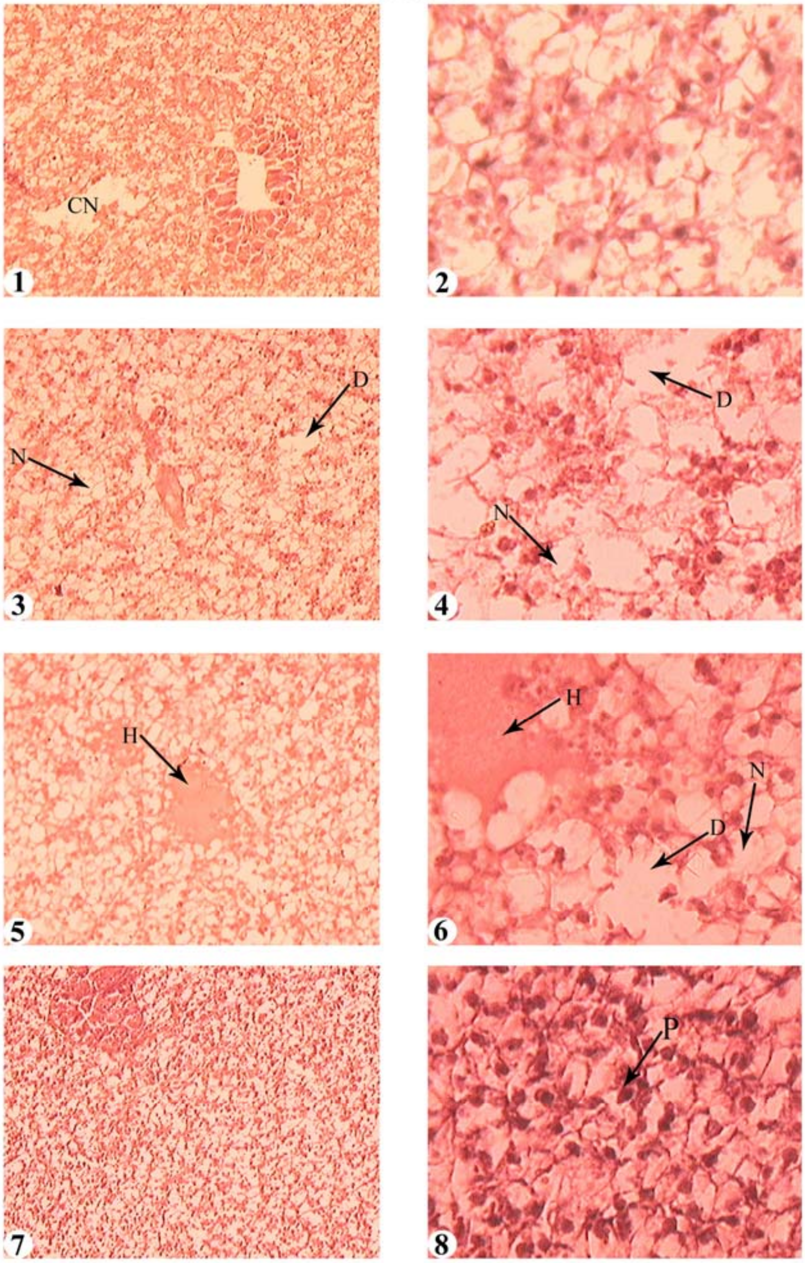


Plate 2

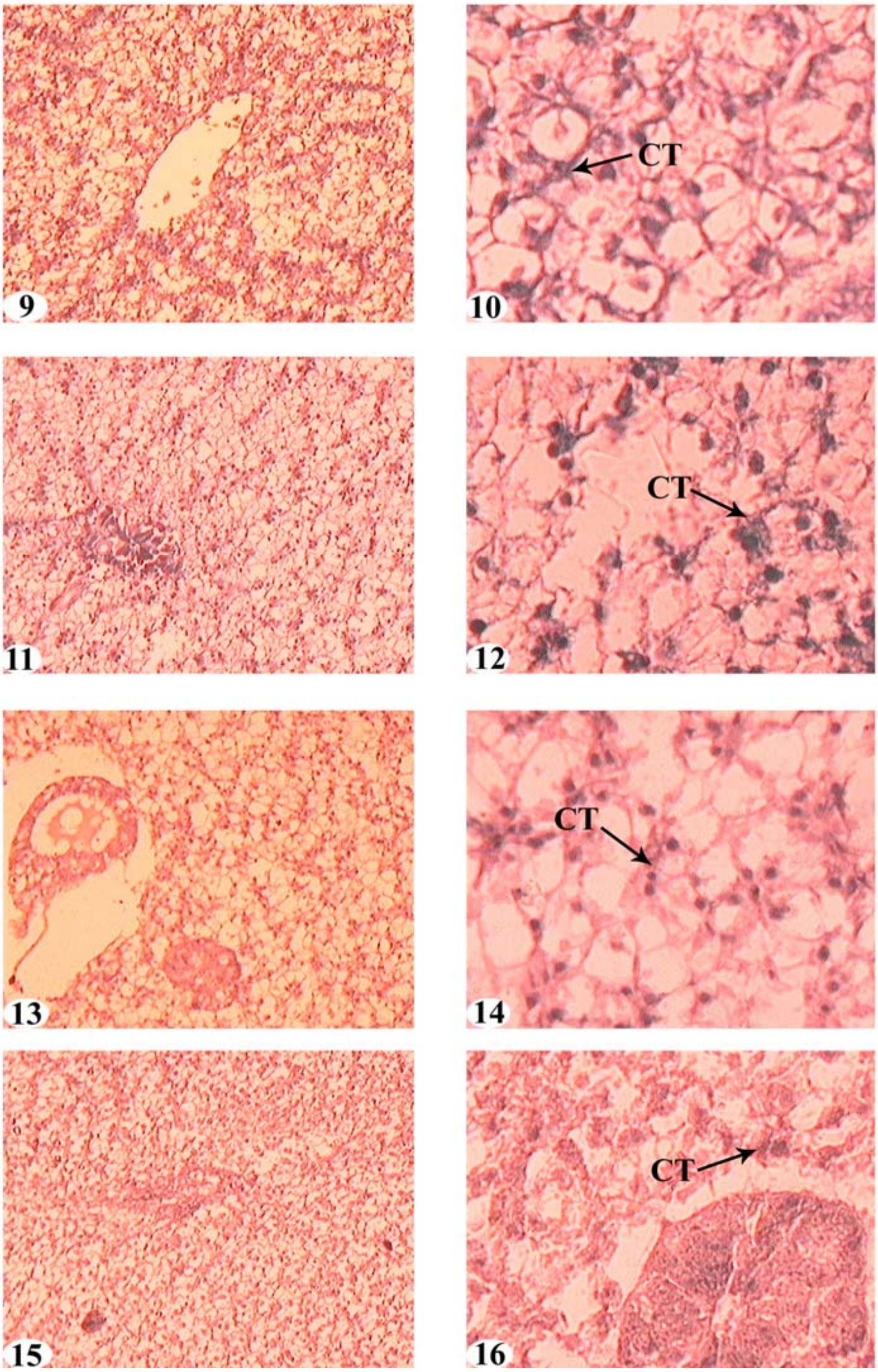


Plate 3

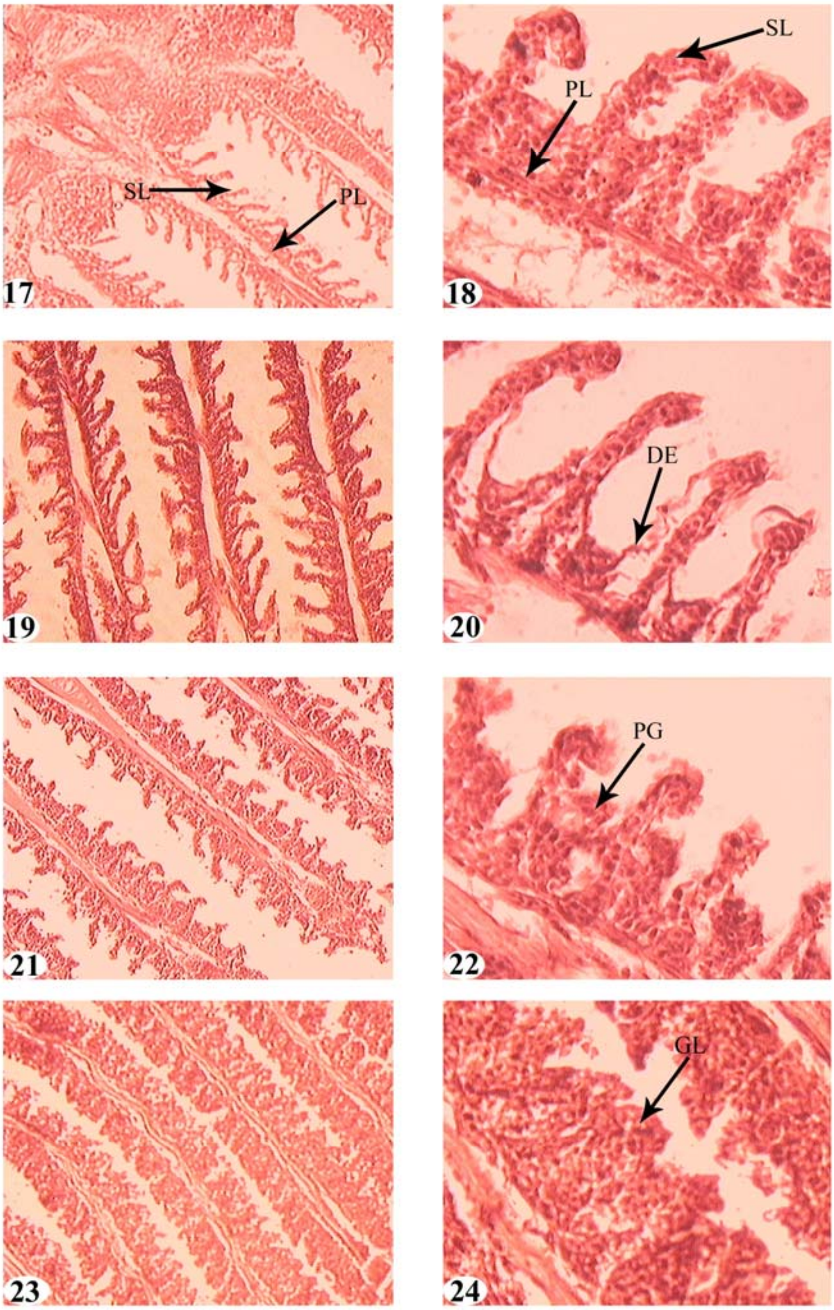
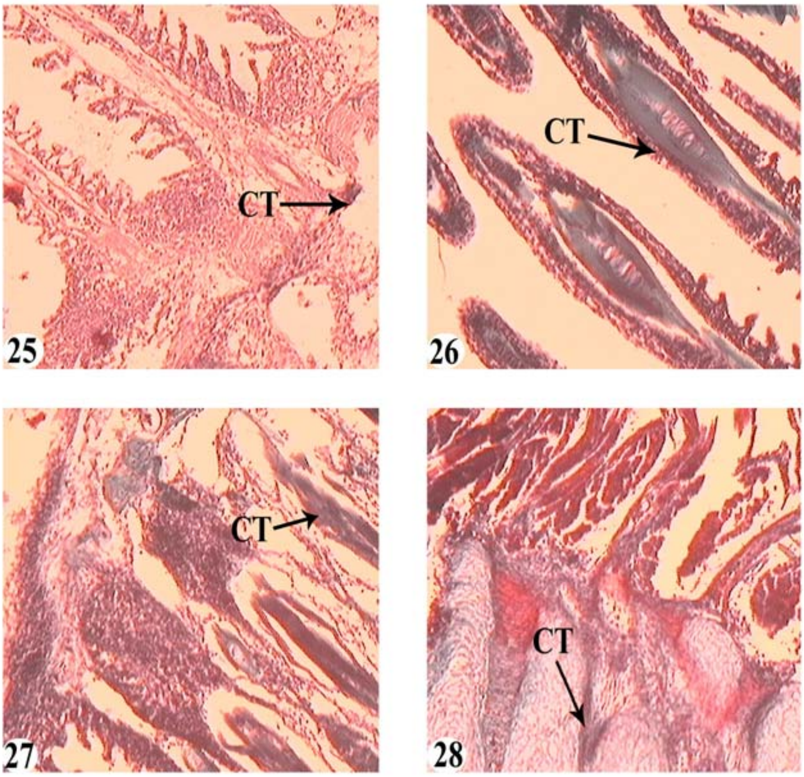


Plate 4



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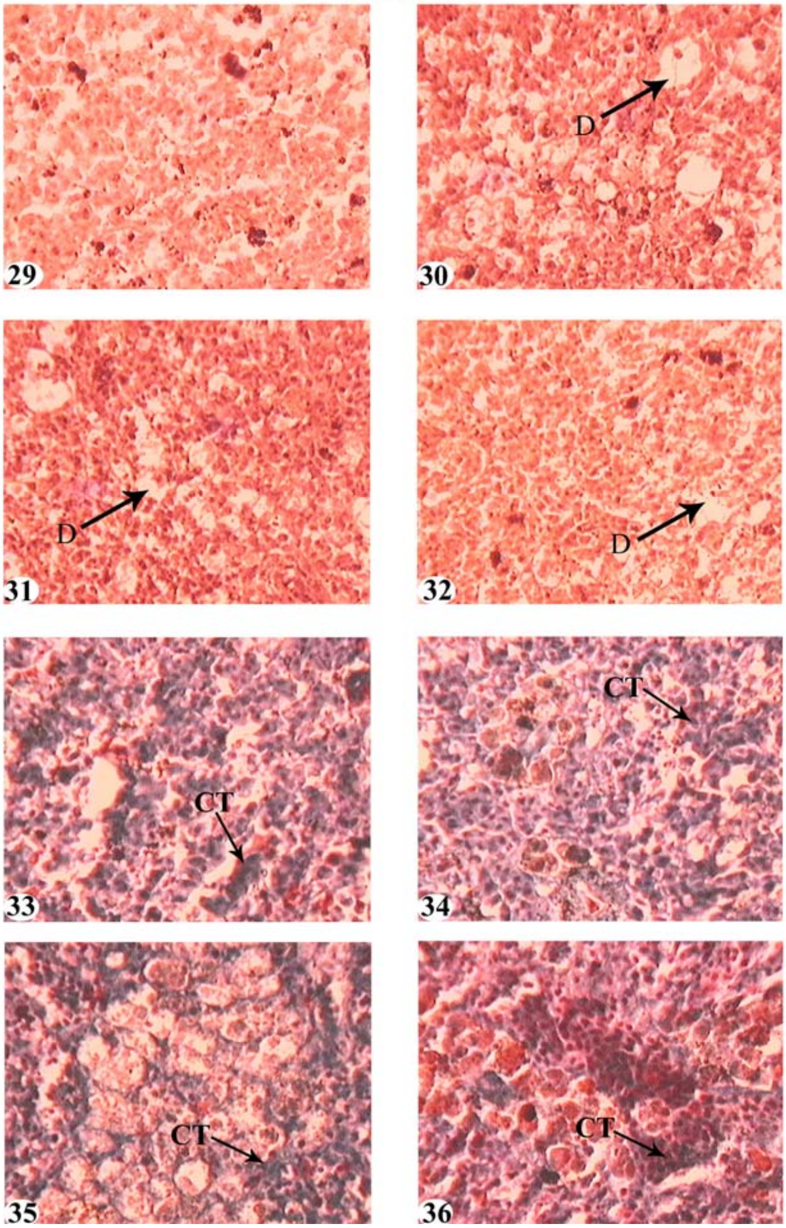

Plate 6

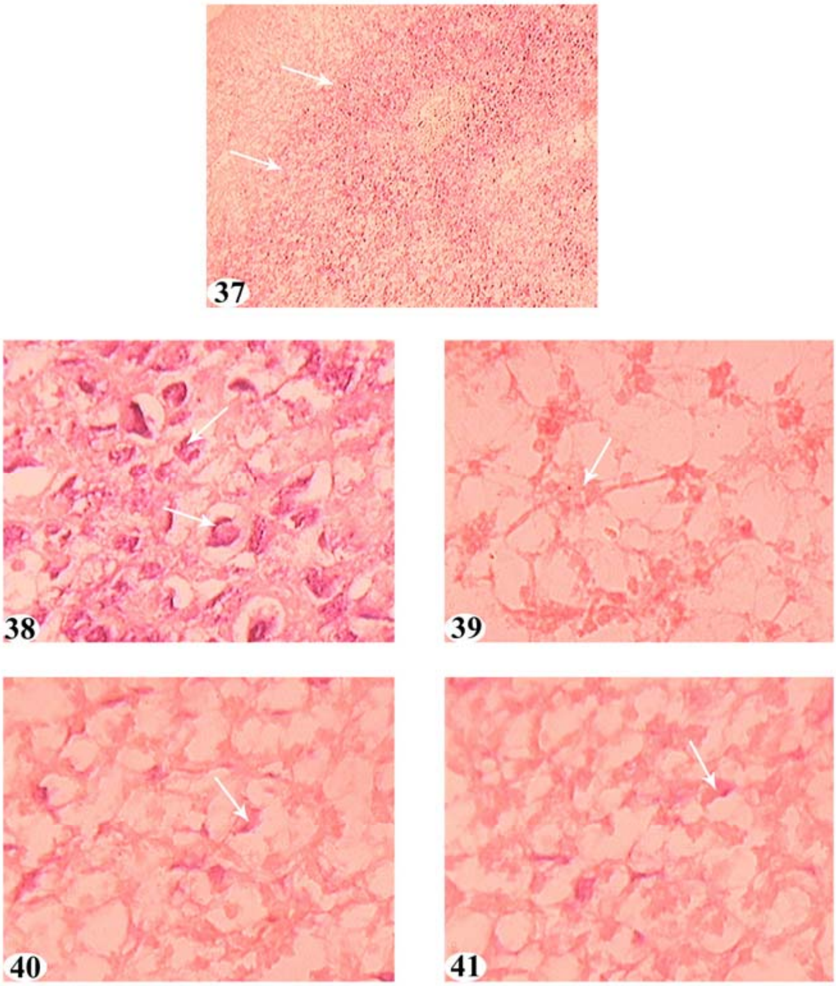

Plate 7

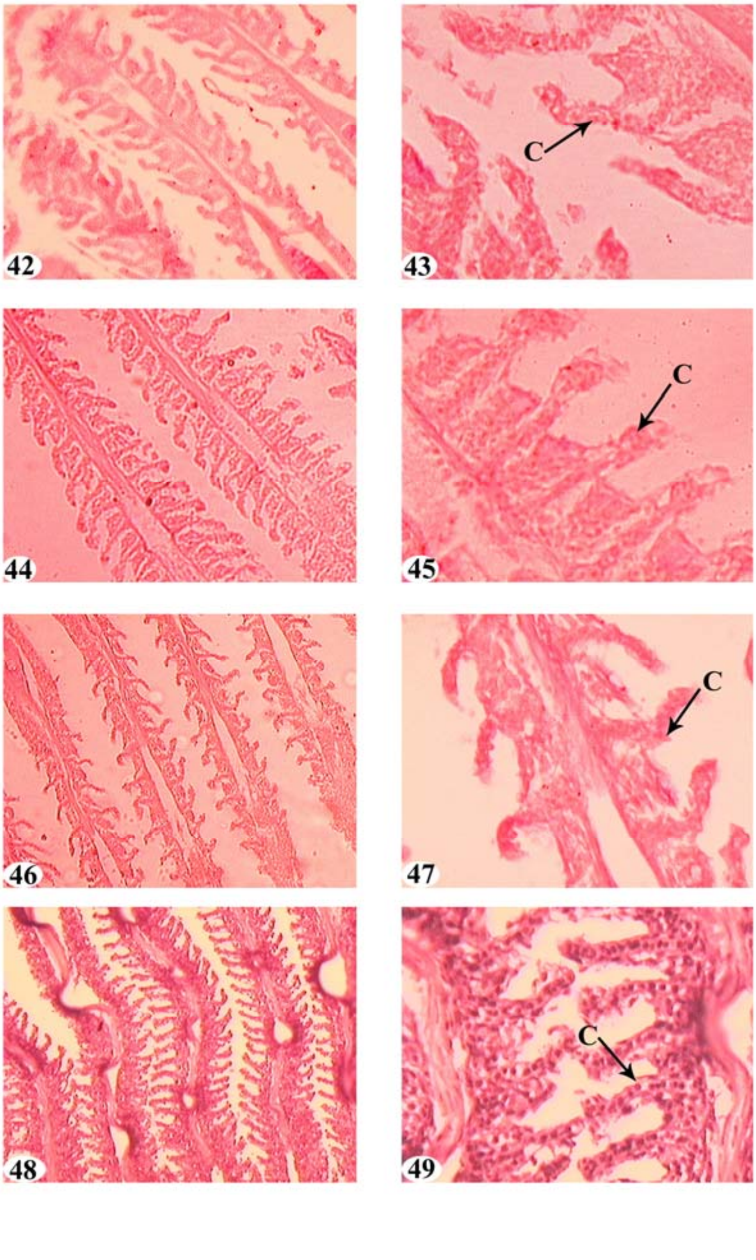

Plate 8

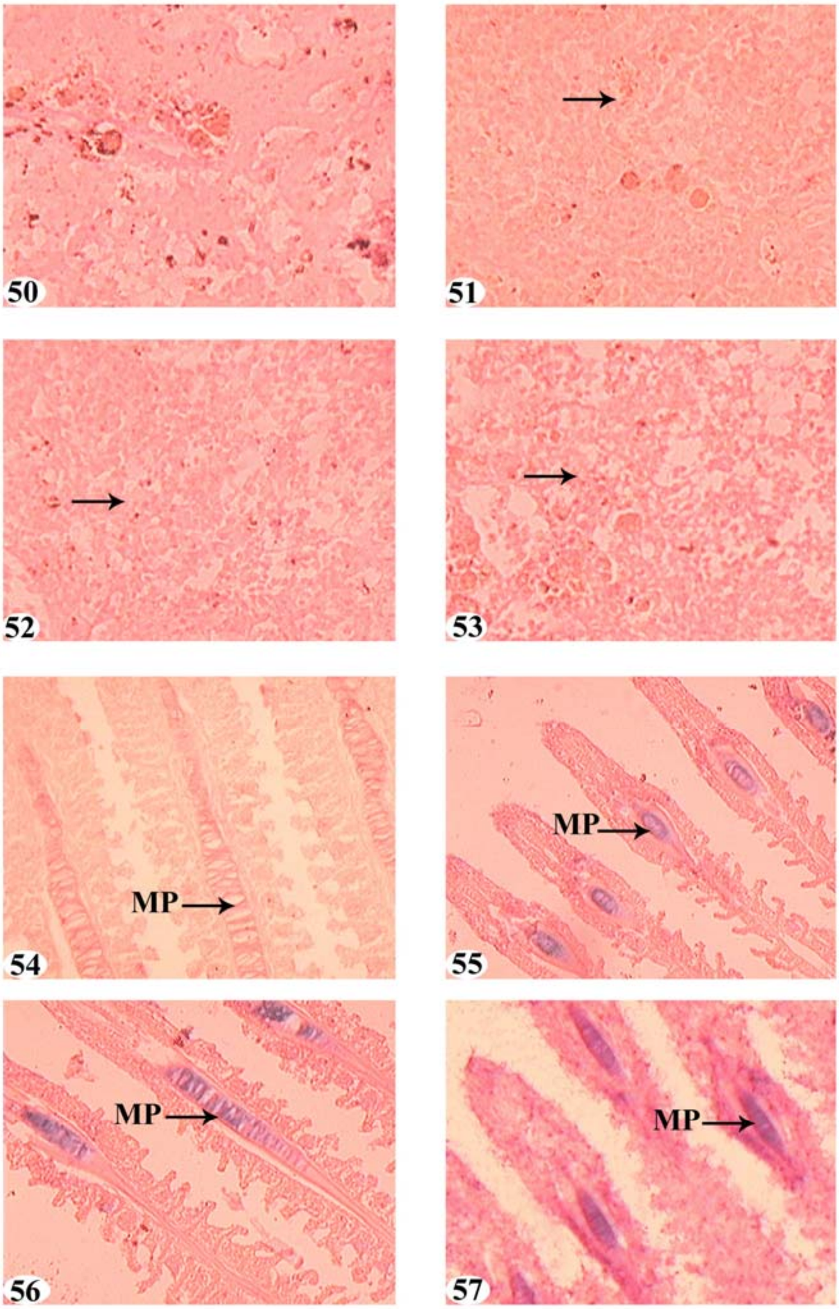

Plate 9