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# Histopathological changes in gills, liver and kidney of fresh water fish, *Tilapia zillii*, exposed to aluminum

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## Abstract

The present study was carried out to study gills, liver and kidney histopathology in the freshwater fish, *Tilapia zillii* which exposed to three levels of aluminum (25, 50, and 100 µg/L) for 96 hour in acidic soft water (pH 6.0). Several histopathological changes were observed in fish organs would serve useful purpose in evaluating the toxic effects of aluminum. Histopathological changes in gills, Liver and kidneys observed microscopically showed increasing degrees of damage in the tissues in correlation with the concentration of aluminum, while gills, liver and kidneys of control groups exhibited a normal architecture.

Key-Words: Aluminum, Histopathology, Gills, Liver, Kidney, Tilapia

## Introduction

Aluminum is the third most abundant element in the earth's crust and makes up approximately 8% of its rocks and minerals. It occurs naturally in soil, water, air, and many foods (CEPA, 2000). Aluminum may enter natural waters via coal strip mining activities, water treatment facilities using aluminum sulphate (alum) as a coagulant for suspended solid particles, industrial wastes and acid rainfall. Thus, when aluminum becomes available to organisms through acidification of surface waters, it is toxic to fish (Driscoll, Baker, Bisigni, & Schofield, 1980).

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish (Van der Oost, Beyer, & Vermeulen, 2003). Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer, Pawet, Schramm, Müller, & Triebskorn, 2001).

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Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta, Rios, Romao, Vianna, & Freiberger, 2003), and serve as warning signs of damage to animal health (Hinton & Laurén, 1990).

Since gills and gastrointestinal tract in fishes considered the main passage for entrance of pollutants to the internal body organs like liver and kidney through the blood (Takashima & Hibiya, 1995). Gills are the first target of waterborne pollutants due to the constant contact with the external environment (Perry & Laurent, 1993).The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Van der Oost *et al.,* 2003) it is also one of the organs most affected by contaminants in the water (Rodrigues & Fanta, 1998).The monitorization of histological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies. The teleostean kidney is one of the first organs to be affected by contaminants in the water (Thophon, Kruatrachue, Upathan, Pokethitiyook, Sahaphong, & Jarikhuan, 2003).

Hence, this study was undertaken to examine the effect of different sublethal aluminum chloride concentrations on histological aspects of gill, liver and kidney of fresh water fish, *Tilapia zillii.*

## Experimental

### Experimental Animals

Adult freshwater fish *Tilapia zillii*, Gervais, 1848 (Family: Cichlidae), were obtained from the

commercial catches of Umhfein Lake (Umalruzam city) on the eastern coast of Libya. On arrival in the laboratory, the fish were placed in large tanks with aerated tap water and were fed with commercially pellets. Fish were acclimatized for 2 weeks under a natural photoperiod and an average temperature of 25°C. The tap water used for the experiment had a pH value of 7±0.1 and a total hardness of 20 mg $CaCO_3$/L and was replaced every 4 days.

**Experimental Design**

A total of 20 adult fish of both sexes were used. The average weight of the fish was 101.4±2·9 g. The experiments were conducted in aerated glass aquariums (120 x 40 x 30 cm) each containing 5 fish in 100 L of contaminated test solution and tap water for the control and allowing one hour for acclimation to laboratory conditions. Aluminum was added as a chloride ($AlCl_3$; $6H_2O$. Riedel- de Haën). The fish were exposed to dissolved aluminum for a period of 96 hour at varied concentrations of 25, 50, and 100 µg/L in acidic water (pH 6.0). The last tank was left untreated as control group.

**Microscopy Examination**

At the end of exposure period, 5 fish were taken from each replicate tank. The gill arches of the fish were excised from both sides. Fish were dissected, the abdominal cavity was operated and the liver and kidney were excised quickly and were fixed in Bouin's solution as a histological fixative for 24 h (Tao, Liu, Dawson, Cao, & Li, 1999). According to Humason (1967), the specimens were processed as usual in the recognized method of dehydration, cleared in xylene and finally embedded in paraffin wax before being sectioned at 5 µm using a rotary microtome (Leica RM 2235 Germany). The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined and photographically enlarged using light microscopy (Hamilton compound photomicroscope).

## Results and Discussion

**Histopathologicl Lesions of Gills**

There are four gill arches on each side of the buccal cavity. Each arch is composed of numerous gill filaments with two rows of secondary lamellae that ran perpendicular to each filament. Secondary gill lamellae were composed cells, which were contractile and separated the capillary channels. One to two erythrocytes were usually observed within each capillary lumen. Chloride cells were identified as large epithelial cells with light cytoplasm, usually present at the base of lamellae. Mucous cells were present in the epithelium of the filament at the base of lamellae, but they lacked the light cytoplasm and were smaller than chloride cells (Peebuaa, Kruatrachuea, Pokethitiyooka, & Kosiyachindaa, 2006).

Histological study of the gills shows a typical structural organization of the lamellae in the untreated fish (Fig.1 A). The treatments with aluminum (Fig. 1 B, C, D, E, F, G, & H) resulted in several forms of histopathological changes such as cellular hypertrophy or hyperplasia in the epithelial layer of primary filaments and fusion of secondary lamellae. Other observations during the experiment include epithelial lifting, interstitial edema and blood congestion in the vascular axis of primary filaments. In addition, a few telangiectasis were also observed at gill lamellae. The examination showed that 100 µg aluminum/liter caused cellular degeneration which result in necrosis of gill epithelial tissues.

The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion (Heath, 1987). It is possible that the damage of the gills could be a direct result of the salts, heavy metals, pesticides, sewage and fertilizers which are conveyed to the water (Temmink, Bouweister, De Jong, & Van Den Berg, 1983). They are directly exposed to poisons occurring in the external environment which often cause pathology in fish (Mallatt, 1985). The gills are among the most vulnerable structures of the teleost fish because of their external location and intimate contact with the water. So, they are liable to damage by any irritant materials whether dissolved or suspended in the water (Roperts, 1978).

The present study demonstrates that aluminum under acidic conditions, i.e. pH.6.0, has acute toxic effect on *Tilapia zillii*. Similar result was also obtained by Dietrich and Schlatter (1989) for rainbow trout, *Salmo gairdneri*, exposed to aluminum in acidic waters for 96 hour. Likewise, consistent results have been reported by Zhang and Li (1997) on several major freshwater fishes at pH 6.0.

The results of both light microscopic and electron microscopic examination demonstrated that at high concentrations, suspended particles in ambient water could be phagocytosed by the epithelial cells. It was speculated that phagocytic uptake of contaminated particles may provide an entry pathway for toxic substances (Martens & Servizi, 1993). With varied affinities for various metals, mucus likely concentrates metals within the gill microenvironment (Kirchner, 1987). It is generally assumed that during the process of gill uptake, metals are adsorbed onto sites in cell walls and cell membranes (Hudson, 1998).Our results suggest that there was a two-step process involved in

the uptake of particulate aluminum by fish gills: (1) adherence of the particles on the gill surface where mucus was attached; and (2) adsorption of aluminum from the particles under conditions of the gill microenvironment.

Karlsson, Runn, Haux, and Forlin (1985) mentioned that, the increase of cellular layers of lamellar epithelium may be due to an increase in the number of mitotic divisions of the lamellar epithelium. Kantham and Richards (1995) suggested that the gill hyperplasia may increase the epithelial thickness so as to retard into the blood stream. Cell proliferation with thickening of gill filament epithelium may lead to the lamellar fusion (Figueiredo-Fernandes, Ferreira-Cardoso, Garcia-Santos, Monteiro, Carrola, Matos, & Fontaínhas-Fernandes, 2007). The fusion and hyperplasia of gill lamellae may be induced by the effect of the toxin which alters glycoprotein in the mucus covering of the cells, thereby affecting the negative charge of the epithelium and favoring adhesion to adjacent lamellae (Ferguson, 1989).

The lifting of lamellar epithelium is other histological change observed, probably induced by the incidence of severe edema (Pane, Haque, & Wood, 2004). Some studies revealed that interstitial edema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals (Mallatt, 1985). Edema with lifting of lamellar epithelium could be serve as a mechanism of defense, because separation epithelial of the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream (Arellano, Storch, & Sarasquete, 1999).

Lamellar axis vasodilatation was also found in tilapia exposed to aluminum. Garcia-Santos, Fontaínhas-Fernandes, and Wilson (2006) referred that this lesion can induce changes in pillar cell normal structure, with consequent loss of their support function and probably, and was responsible for the emergence of lamellar aneurysms in fish. In this case, damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, blood congestion or even an aneurysm (Rosety-Rodriguez, Ordoez, Rosety, Rosety, Ribelles, & Carrasco, 2002). Lamellar telangictasis resulted from rupture of pillar cells and capillaries under effect of heavy metals pollution and leads to an accumulation of erythrocytes in the distal portion of the secondary lamellae (Randi, Monserrat, Rodrigue, & Romano, 1996).

Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since; in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants. As a consequence of the increased distance between water and blood, the oxygen uptake is impaired (Fernandes & Mazon, 2003). These histopathological changes of the gills likely resulted in hypoxia, respiratory failure problems with ionic and acid-base balance (Alazemi, Lewis, & Andrews, 1996). Also, the pathological changes in the chloride cells may indicate osmoregulatory dysfunction, which is the main function of the chloride cells (Virtanen, 1986).

A general theme in much aluminum toxicity is an accelerated cell death. Aluminum associations with both extracellular surfaces and intracellular ligands are implicated (Exley & Birchall, 1992). The concomitant iono- and osmoregulatory dysfunction results in accelerated cell necrosis, sloughing and death of the fish (Exley, Chappell, & Birchall, 1991). The available data suggest that aluminum can induce DNA damage by modifying the structure of chromatin through the induction of reactive oxygen species or by damaging lysosomal membranes and liberating DNase (Banasik, Lankoff, Piskulak, Adamowska, Lisowska, & Wojcik, 2005).

**Histopathological Lesions of Liver:**

The liver made up of hepatocytes that not oriented into distinct lobules but arranged in branched laminae two cells thicks, separated by sinusoids. Hepatocytes are polygonal cells with a central spherical nucleus and a densely stained nucleolus (Figueiredo-Fernandes *etal*., 2007). The present study also demonstrates that the liver of control fish exhibits a normal architecture and there were no pathological abnormalities. The hepatocytes present a homogenous cytoplasm and a large central or subcentral spherical nucleus (Fig. 2 A).

The histopathological appearance of liver following exposure to aluminum (Fig. 2 B, C, D, E, F, G, & H) showed important alterations comprise hypertrophy of hepatocyets, nuclear hypertrophy, blood congestion in the central veins, as well as the diffusion of melanomacrophages in the parenchymal tissues of liver. Its revealed that the increase of aluminum concentration causes cytoplasmic vacuolation, cellular degeneration, damage of nuclei, bile stagnation in addition to congestion in the blood sinusoids. The severity of histopathological changes increased with 100 µg /liter, because this dose of aluminum prompt cellular necrosis in the parenchymal tissues and decreasing in the number of hepatocytes nuclei of hepatic tissue.

The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Van der Oost *et*

*al.,* 2003). It is also one of the organs most affected by contaminants in the water (Rodrigues & Fanta, 1998). Several histological alterations were reported in liver of fishes exposed to industrial pollutants (Mukherjee & Bhattacharya, 1975).

Metals can either increase or decrease hepatic enzyme activities and can lead to histopathological hepatic changes, depending on the metal type and concentration, fish species, length of exposure and other factors (Paris-Palacios, Biagianti-Risbourg, & Vernet, 2000). Hepatocytes may thus be expected to be the primary targets of toxic substances, providing an excellent biomarker of aquatic pollution (Braunbeck & Völkl, 1993). The monitorization of histological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies (Figueiredo-Fernandes *etal*., 2007).

Hypertrophy is generally characterized by an increase in cellular size. Exposure to compounds that induce proliferation of the endoplasmic reticulum membranes can be regarded as an example of hypertrophy (Hinton & Laurèn, 1990). Some studies revealed that interstitial hepatocytes of Nile tilapia exposed to contaminated sediment showed hydropic swelling (Peebua *etal*., 2006). Figueiredo-Fernandes *et al.* (2007) suggested that increase in the hepatocytes size may be due to the high content of lipids. On the other hand, Braunbeck, Storch, and Bresch (1990) referred that alterations in size and shape of nucleus have often been regarding as signs of increased metabolic activity but may be of pathological origin.

Vacuoles in the cytoplasm of the hepatocytes can contain lipids and glycogen, which are related to the normal metabolic function of the liver (Camargo & Martinez, 2007). The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation (Gingerich, 1982). Pacheco and Santos (2002) described increased vacuolization of the hepatocytes s a signal of degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water. Vacuole formation was considered by Mollendroff (1973) as a cellular defense mechanism against substances injurious to hepatocytes and this mechanism responsible for collecting the injurious elements and preventing them from interfering with the biological activities of these cells.

The bile stagnation and diffusion of melanomacrophages in the parenchymal tissues of liver refer to that this organ undergoes damage in structure and metabolism due to aluminum exposure. According to Hibiya (1982), this condition refers to the stagnation of bile outflow from the hepatocytes, and is recognized by the presence of yellow-brown granules within the cells. This accumulation of bile indicates possible damage to the hepatic metabolism (Fanta *et al.,* 2003). An increase in the density of the melanomacrophage aggregates is generally related to important hepatic lesions (Pacheco & Santos, 2002), such as degenerative and necrotic processes.

Tilapias exposed to aluminum also showed hepatocellular degeneration. Similar results are observed by Hunter, Ross, and Tannahill (1980) who noted that rainbow trout, *Oncorhynchus mykiss,* which survived exposure to 50 µg/L aluminum at pH 8.0 to 9.0 showed extensive necrosis of the liver. Anoxia as a result of gill degeneration being the most common cause of cell damage and destruction (Eder & Gedigk, 1986). Damage will affect oxygen exchange and tissue respiration, culminating in organ and tissue hypoxia, degeneration and necrosis (Taiwo, Olukunle, Ozor, & Oyejobi, 2005). Also, the cellular degeneration and necrosis may be due to accumulative effect of metals in hepatic tissue. Fish commonly accumulate greater concentrations of elements in liver than flesh and gills (Al-Yousuf, El-Shahawi, & Al-Ghais, 2000).

Aluminum interferes with important metabolic processes in the cells (Rosseland, Eldhuset, & Staurnes, 1990). Sanad, El-Nahass, Abdel-Gawad, and Aldeeb (1997) reported that liver cell necrosis may be due to inhibition of synthesis of DNA needed for the growth and maturation of the liver. In addition, contaminants have the capacity of inducing biological disarrangement of the molecular organization of the cell membranes leading to their degradation (Byzkowski, 1976).

**Histopathological Lesions of Kidney**

The kidney was composed of numerous renal corpuscles with well developed glomeruli and a system of tubules. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei and the brush border was reduced or absent. The collecting duct or glomerulus was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border (Peebua *etal*., 2006).

Histological study shows a typical structural organization of the kidneys in the untreated fish (Fig. 3 A). Results of this study demonstrated that concentrations of aluminum produce a variety of histopathological changes in the kidneys of exposed animals (Fig. 3 B, C, D, E, F, G, & H). Gross changes

included irregular diameters of renal tubules, glomerular expansion, renal corpuscle damage, severe degeneration in the tubules cells, in addition to the infiltration of edematous fluid between the tubules, hemorrhage and diffusion the erythrocytes in the interstitial fluid.

The teleostean kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.,* 2003). Because the important role of kidney in the excretion of harmful materials, the present study proved occurrence of several histological alterations in the kidney resulting from aluminum toxicity and reflect participation of kidney in excrete aluminum element from body.

The increasing in the size of urinary tubules that observed in this study refers to hydropic swelling. This lesion was very similar to those observed in Nile tilapia *Oreochromis niloticus* exposed to contaminated sediments (Peebua *etal*., 2006). This alteration can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm, which takes on the appearance of a net. This initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Takashima & Hibiya, 1995).

Disturbance of living processes at the molecular and subcellular levels of biological organization by xenobiotics can lead to cell injury, resulting in degenerative and neoplastic diseases in target organs (Pacheco & Santos, 2002). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima & Hibiya, 1995). The presence of tubule degeneration, coupled with the absence of necrosis in the kidney in the present study indicates that the kidney suffered damage after exposure to aluminum, but the short period of confinement may have prevented the establishment of necrosis in this organ.

In this work, elevated concentration of aluminum caused shrinkage glomeruli and blood hemorrhage. This lesion may be return to cellular degeneration as well as increasing the amount of edematous fluid in the interstitial substance. Hemorrhages and congestion of the gastrointestinal tract and kidneys were previously recorded by Abdelhamid and El-Ayouty (1991) for catfish, *Clarias lazera*, exposed to aluminum. Rothstein (1959) referred that metals affect permeability of cell membranes and resulting in inhibition of the ion-transporting systems causes disturbances in fluid transport from and into cells. It's believed that aluminum integrate with cell membranes causing a large amount of fluid filtrate, as well as disperse of serum albumin and red blood cells in the interstitial substance due to destruction of capillaries endothelium (Hadi & Alwachi, 1995).

## Conclusion

A wide range of toxic effects of aluminum have been demonstrated in aquatic animals in nature and in experimental animals by several routes of exposure, and under different clinical conditions in humans. In conclusion the present study showed that histopathological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution. The organ and tissue damage in the experimental fish due to the direct toxicity of the aluminum on the gills, liver and kidney. Also, the results showed that the degree of distortion of the tissues was proportional to the concentration of the aluminum.

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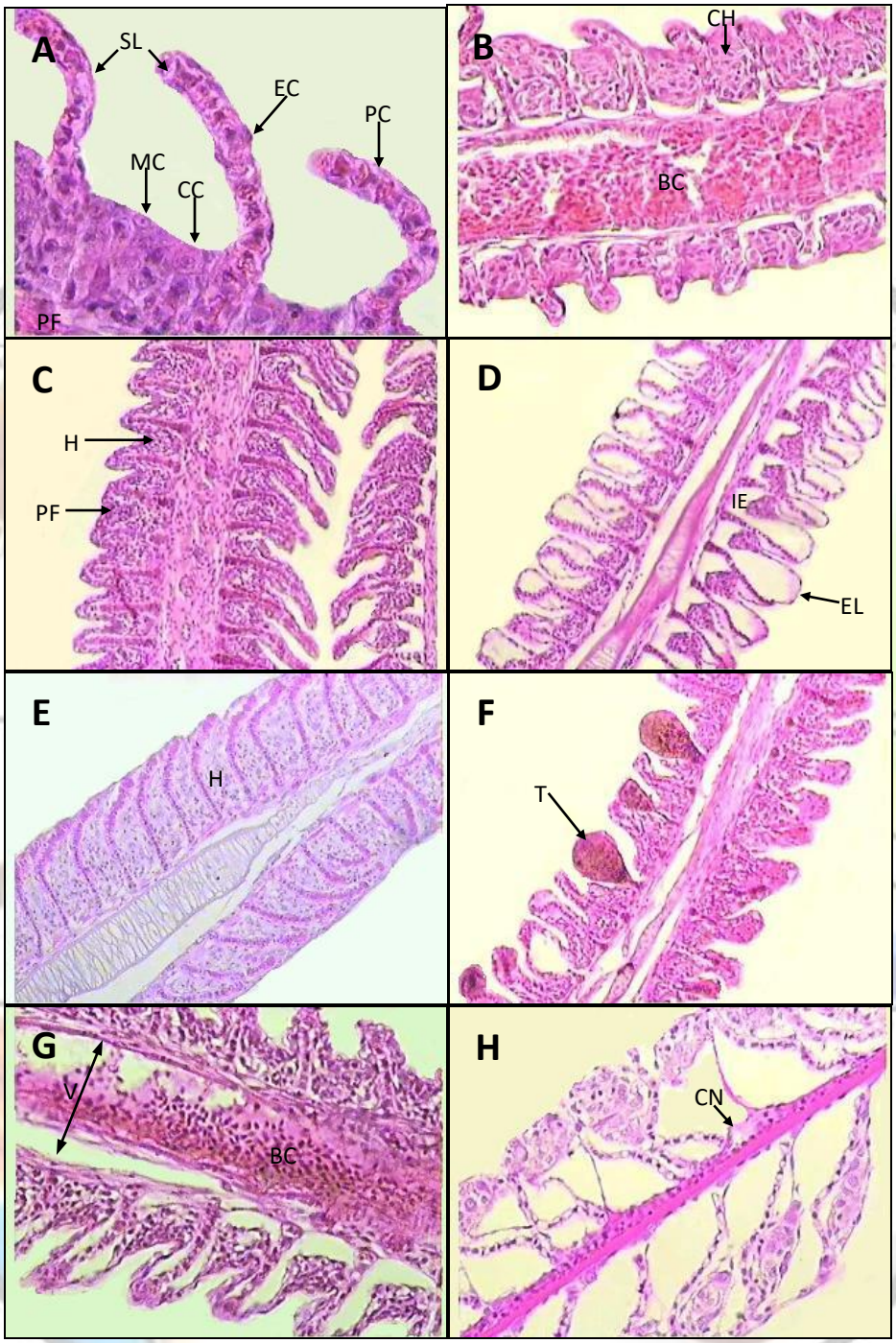


**Fig. 1: Photomicrographs of the gill of *Tilapia zillii.* [A] normal aspect of the gill, showing primary filament (PF), secondary lamellae (SL), mucous cell (MC), chloride cell (CC), pillar cell (PC) and epithelial cell (EC) ; [B] cellular hypertrophy (CH) and blood congestion (BC) ; [C] hyperplasia (H) and partial fusion of some lamellae (PF) ; [D] epithelial lifting (EL) and interstial edema (IE) ; [E] hypertrophy of the lamellar epithelium (H) ; [F] telangiectasis at the tips of secondary lamellae (T) ; [G] vasodilation (V) with blood congestion (BC) ; [H] cellular necrosis and epithelium rupture (CN).**

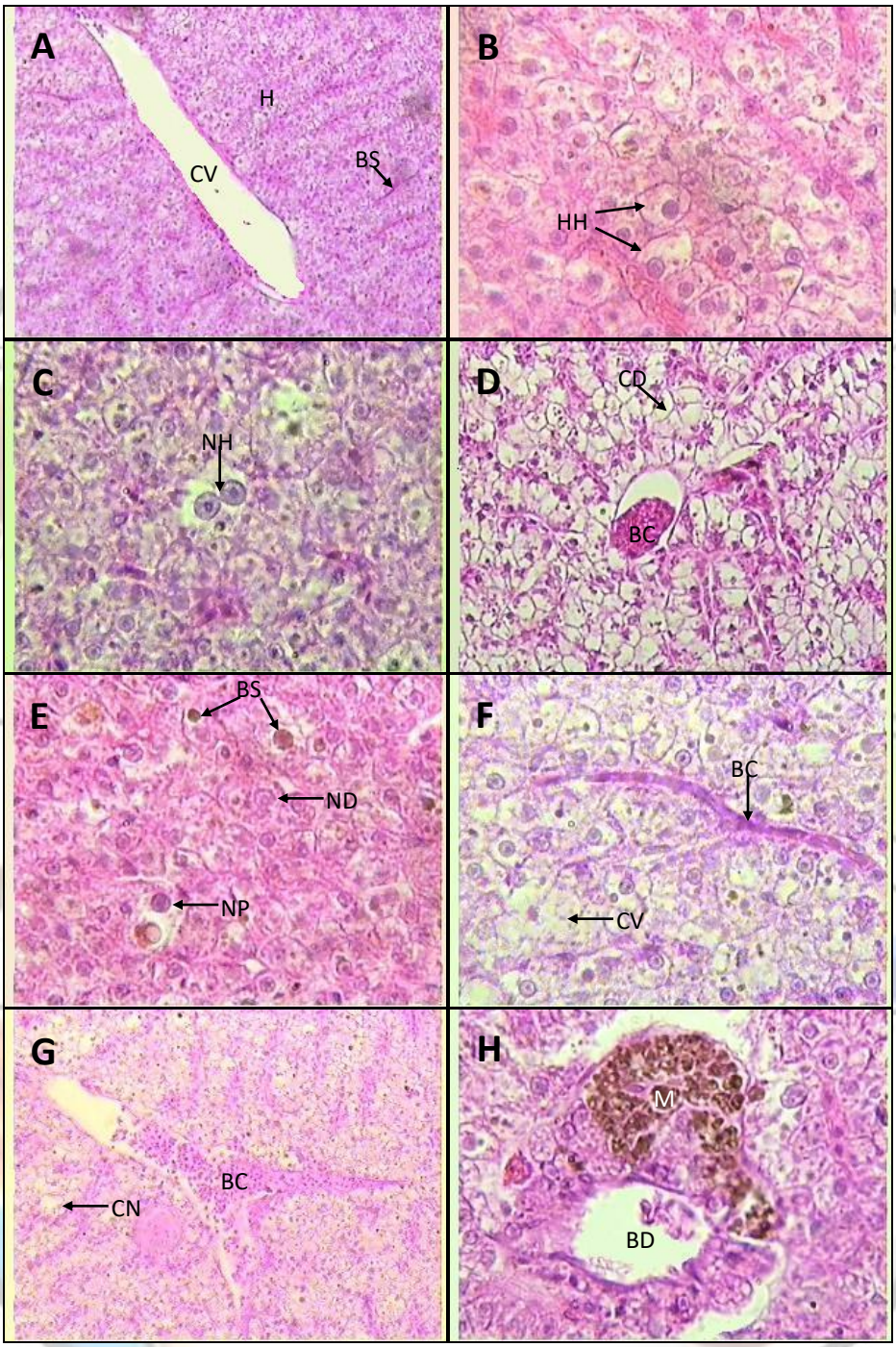


**Fig. 2: Photomicrographs of the liver of *Tilapia zillii.* [A] normal hepatic tissue, showing hepatocytes (H) blood sinusoid (BS) and central vein (CV); [B] hepatocytes hypertrophy (HH); [C] nuclear hypertrophy (NH); [D] cellular degeneration (CD) and blood congestion (BC); [E] nuclear pyknosis (NP) nuclear degeneration (ND) and bile stagnation (BS); [F] cytoplasmic vacuolation (CV) and blood congestion in sinusoids (BC); [G] cellular necrosis (CN) and blood congestion (BC); [H] melanomacrophages aggregate (M) close to a bile duct (BD).**

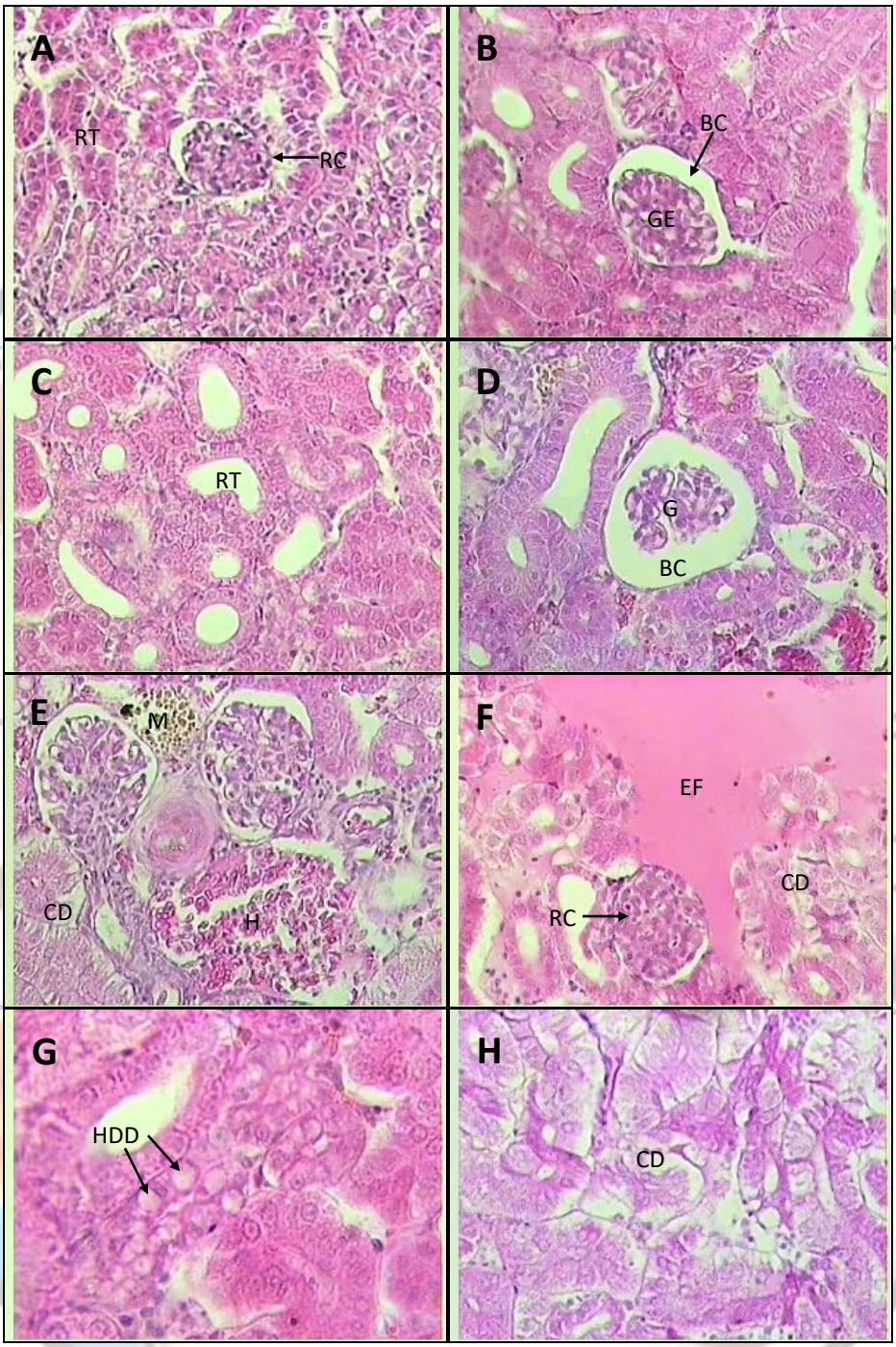


**Fig. 3: Photomicrographs of the kidney of *Tilapia zillii.* [A] normal renal corpuscle showing the glomerulus and the Bowman's space well defined (RC) renal tubules (RT); [B] glomerular expansion (GE) and dilation of Bowman's space (BC); [C] increasing in the diameter of renal tubules (RT); [D] deterioration of glomerulus (G) and dilation of Bowman's space (BC); [E] cellular degeneration (CD), melanomacrophages aggregate (M) with hemorrhage (H); [F] edematous fluid (ED) , shrinkage of renal corpuscle (RC) and cellular degeneration in tubules (CD); [G] hyaline droplet degeneration and deformation in renal tubules architecture (HDD); [H] acute cellular degeneration and occlusion of the tubular lumen (CD).**