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HISTOLOGICAL CHANGES IN CERTAIN TISSUES OF FISH ON AMBIENT AMMONIA STRESS AND POST AMMONIA STATE (RECOVERY)

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ABSTRACT
Ammonia, one of the most extensively used fertilizers enters the aquatic environment through run off and is commonly detected in aquatic habitats. These on retention in soil and water are reported to result in increased concentrations of ammonia. Ammonia is also a byproduct of fish metabolism. But the increase in ammonia concentration in ambient medium is toxic to fish. Long term exposure of animals to low concentration of fertilizer may cause tissue damage. In order to assess the response of the animal to low concentration of ammonia and its recovery potential on removal of ammonia stress at cellular level, histopathological studies were taken into consideration to observe the tissue alteration and the extent of damage. Fish *Oreochromis mossambicus* is taken for the present study. Animals weighing 12 gm and 8 cm long are exposed to 3.26 ppm of ammonia solution for seven and fourteen days. In order to understand the extent of recovery after ammonia stress, they were kept in ammonia free water for 7 and 14 days. Histological study of the tissues was done. Histopathological lesions were observed in fish liver, kidney and gill under ammonia stress. The changes were reduced in fish kept in ammonia free water for 7 and 14 days (recovery).

KEY WORDS: ammonia stress, histology, liver, kidney, gill, recovery.

INTRODUCTION

The environmental pollution due to extensive usage of the pesticides, herbicides and ammonical fertilizers without proper management has far reaching effects on the survival potential of aquatic animals, for some of these toxic chemicals may persist in the environment for longer periods and show damage at histological level (Roy.S et al, (2006), ,Singh,R and Ahirwar,K,(2009)., Vinodhini,R and Narayanan,M,(2009),and Velmurugan etal,(2009) Ammonia, one of the extensively used fertilizers enters the aquatic environment through run off and is commonly detected in aquatic habitats. In order to assess the damage caused at cellular level and extent of recovery the animal is capable of histological studies were taken for the present investigation.

MATERIAL AND METHODS

Fish, *Oreochromis mossambicus* weighing about 12±2 g and 8 ±2 cm long are selected and maintained in the laboratory. Temperature and pH were maintained throughout experimentation. Toxicity tests were conducted using ammonia solution. LC 50 was determined using Finney's method and it is 16.3 mg/L for 48 hours. To understand the impact of low concentration, 3.26 mg/L or $1/5^{th}$ of LC 50 was selected as experimental concentration. Fish were exposed to this concentration for seven and fourteen days. After 7 and 14 days of exposure to test chemical, the fishes were transferred to normal tap water and kept for 7 and 14 days to allow them to recover. Histological studies of the selected tissues namely liver, kidney and gill were studied using the method of Humason (1972).

RESULTS AND DISCUSSION

Liver:The normal liver of fish comprises of a continous mass of hepatic cells arranged in cords. There is no clear division of hepatic cells into lobules. The hepatocytes are large in size and the nucleus is centrally situated. The pancreatic tissue is distinct with a well developed cellularity and a large number of blood sinusoids are found in hepatic mass (Fig.1 to 3).

Ammonia has induced discrete pathological changes in 7 days exposed fish. These changes include moderate cytoplasmic degeneration in hepatocytes, formation of clear vacuoles in hepatocytes, granular degeneration of cellular disarray and rupture in blood vessels and appearance of blood cells amongst hepatocytes. But the severity in pathological changes is evident in 14 days exposed fish liver. The changes include pushing of nucleus to peripheral region, pycnotic nuclei and fragmentation of nuclear material, disarray of hepatocytes (Fig.4 to 6). Similar histological damage in liver on pesticide exposure has been reported by Van Dyke et al, (2007).After 7 and 14 days of post exposure, liver showed regenerative changes in hepatocytes, reduction in vacuole in hepatocytes and also granular regeneration. It indicates that histological evidence also supports recovery studies

Gill: The primary gill lamellae are flat leaf like structures laterally compressed and situated alternatively on either side of inter branchial septum. The primary gill lamella consists of a centrally placed rod like supporting axis with a row of secondary gill lamella present on either side of it.

The secondary gill lamellae also called as respiratory lamella, are highly vascularised and covered with a thin layer of epithelial cells. Blood vessels can be seen extended into each secondary lamella. The blood cells have a single nucleus which is flattened in appearance. The region between two adjacent respiratory lamella is termed as inter lamellar region (Fig.7 to 9).

After 7 days of ammonia exposure, the tips of the primary gill filaments showed considerable bulging (Fig.7 to 9) and degeneration was observed in the epithelial cells of secondary gill filaments. Swelling of secondary gill filaments was meagerly observed in gill of ammonia exposed fish with signs of necrosis in the epithelial cells of secondary gill filaments, besides the occurrence of damage to inter lamellar epithelial cells and bulging of the tips of secondary gill filaments.

After 14 days of ammonia exposure, fish gill showed severe pathological lesions, the changes from normal structure included necrosis in the epithelial cells of secondary gill filament and inter lamellar cells, fusion of secondary gill filaments and separation of respiratory or secondary lamellae from the primary lamellae. (Fig.10 to 12).

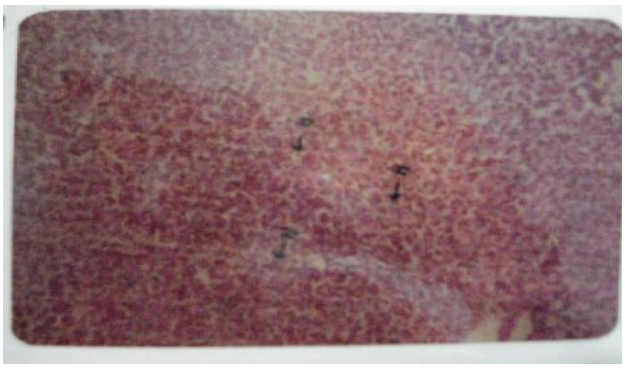

FIGURE1: Normal structure of liver of Oreochomis mossambicus-H & E-100X, H= Hepatocytes

FIGURE :2 Fish exposed to 7 days of ammonia showing moderate cytoplasmic degeneration in hepatocytes(DGH), formation of vacuoles(V);and rupture of blood vessels(RBV) among hepatocytes-200X in liver tissue.

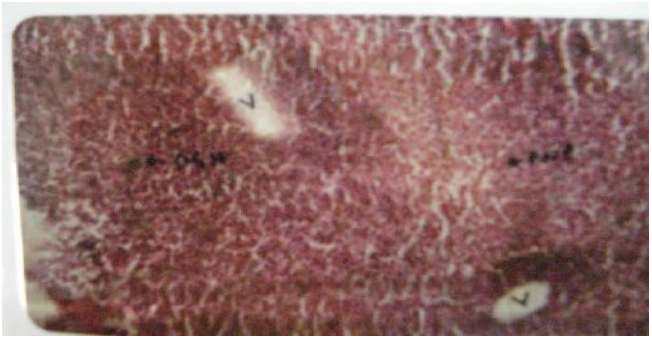

FIGURE 3:. Liver of fish after 7 days during recovery from ammonia exposure(7 days), showed pushing of nucleus to peripheral region (PNP), moderate cytoplasmic degeneration in hepatocytes(DGH) and formation of vacuoles in hepatocytes(V), -200X

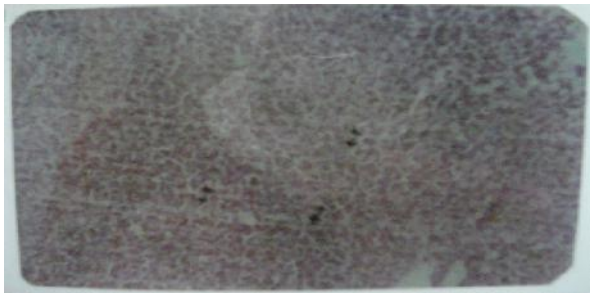

FIGURE 4:.Normal structure of liver of Oreochomis mossambicus-H & E-100X, H= Hepatocytes

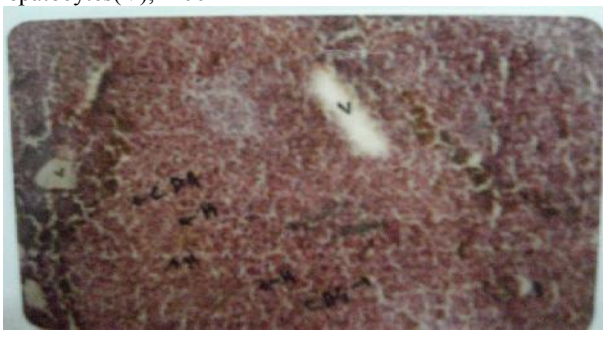

FIGURE 5: Fish exposed to 14 days of ammonia showing cellular degeneration (CDG), formation of vacuoles (V); fragmentation of nuclear materials(FNM),and disarray of hepatocytes(DAH)-200X in liver tissue.

FIGURE 6: Liver of fish after 14 days during recover ammonia exposure(14 days), showed cellular degeneration appearance of blood cells among hepatocytes (BC) and form vacuoles in hepatocytes(V), -200X

FIGURE 7: Normal structure of fish Oreochromis mossambicus gill with secondary gill lamellae (SGL), blood vessel(BV), central axis(CA), and interlamellar region(ILR). (H &E)-200X

FIGURE 8: Fish exposed to 7 days of ammonia showing bulging of tips of primary gill lamellae (BTPL), necrosis in secondary lamellae (NSL), rupture of secondary gill filament (RSGF)., necrosis of interlamellar space (NILS) and hemorrhage in central axis (HCA).

Figure 9: Gill of fish after 7 days during recovery from ammonia exposure(7 days), showing necrosis in secondary lamellae (NSL) and secondary gill lamellae (SGL).

Figure 10: .Normal structure of fish Oreochromis mossambicus secondary gill lamellae(SGL), blood vessel(BV), central axis(C interlamellar region(ILR). (H &E)-200X

FIGURE11: Fish exposed to 7 days of ammonia showing moderate atrophied of secondary gill lamellae (ASGL), fusion of secondary gill filamaents (FSGF), infiltration of leucocytes in the primary axis of the gill lamellae (ILPA) and primary gill filament (PGF). (H &E)-200X

FIGURE 12: Gill of fish after 14 days during recovery from ammonia exposure (14 days), showing slight necrotic changes in epithelial cells of secondary gill filaments (SNESG), necrosis in interlamellar regions (NILR) and hemorrhage in central axis (HCA)-200X.

Necrosis of epithelial cells of the respiratory lamellae resulting in the exudation of leukocytes and hemorrhages can also be observed. Fused respiratory lamellae were observed in the gills of fish, besides the separation of the epithelial layer in secondary gill filaments. The gills of the 14 day exposed fish prominently showed leukocyte infiltration in the primary axis and atrophy of the respiratory lamellae Fig.10 to 12).After 7 and 14 days post exposure (recovery) fish gill showed moderate changes. All degenerative changes seem to be inhibited and regenerative changes can be observed.

The normal architecture of fish gill was found to be altered greatly after 7 and 14 days exposure to sub lethal dose of ammonia. The effect of ammonia on gill structure was more in 14 days than 7 days exposed fish. Recovery was not observed to the same extent as that of the metabolic changes.Gill damage under other conditions have been reported (Wijeyayartne, WMDN and Pathiratna, A., (2006)., Popoola, O.M. and M.O. Olufayo, (2007),Vutukuru ,SS et al,(2007),and Serdar Koca et al,(2008)., Sumonato,JD, (2008).

Kidney:The kidney is made of nephrons and nephron consists of glomerulus and the tubules. The intertubular space is filled by evenly distributed haemopoietic tissue (Plate-V Fig.13 to 15). The cells are parenchymatous in nature, round to polygonal in shape with distinct milieu in the centre. The proximal tubule is cytologically differentiated into an initial proximal tubule and a second proximal tubule. The former is characterized by columnar cells with brush border located along the apices of the cell (Fig.13 to 15).

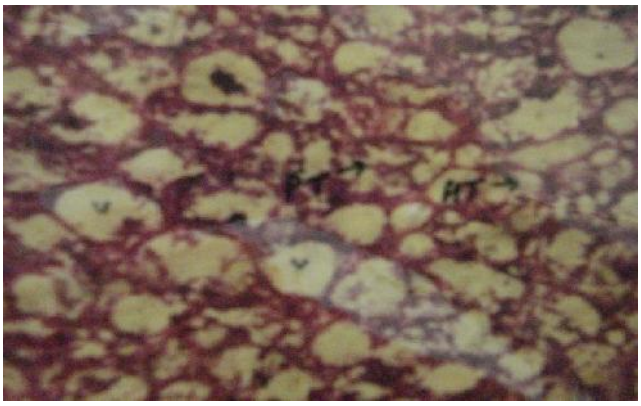

FIGURE13:Normal structure of fish Oreochromis mossambicus kidney with hemopoietic tissue (HT) and Proximal tubules (PT),(H &E)-200X

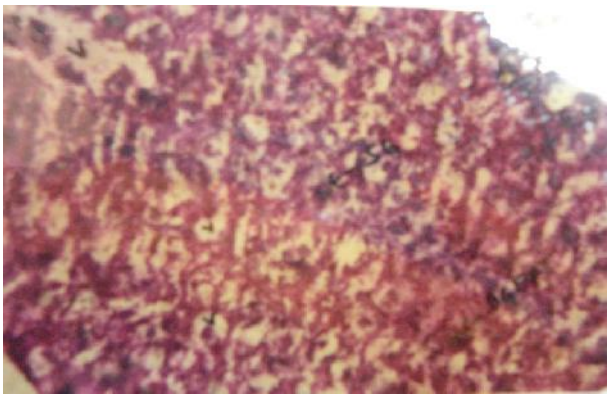

FIGURE14:Fish exposed to 7 days of ammonia showing de changes around proximal tubules (DGEPP), shrunken glome breakage clotting tubules (BCT), formation of vacuoles (V) damage of interstitial cells in kidney tissue –H &E 100X

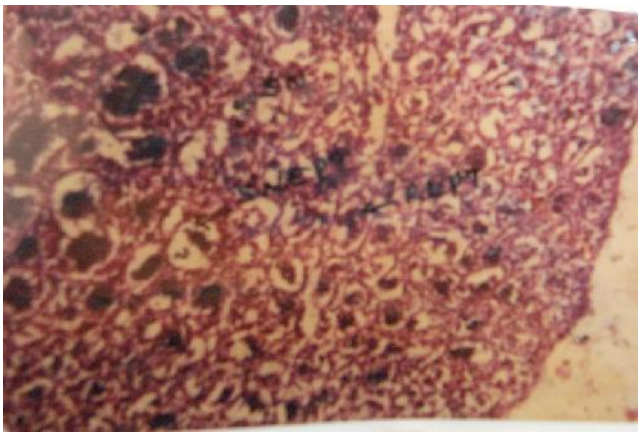

FIGURE 15: Kidney of fish after 7 days during recovery from ammonia exposure (7 days), showing reduced lumen in proximal tubules(RLPT), slight necrosis (SN), in epithelial layer of proximal tubules (SNEPT) and degeneration in haemopoietic tissue (DGH). –H &E 100X.

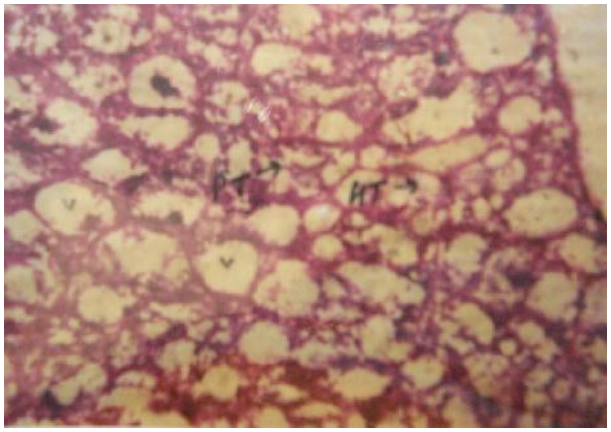

FIGURE 16: Normal structure of fish Oreochromis mossambicus kidney with hemopoietic tissue (HT) and Proximal tubules (PT),(H &E)-200X

Changes in Kidney on zinc exposure have been reported by Pallavi gupta and Srivastava,N,(2006). After 7 days of ammonia exposure, fish kidney showed reduced lumen and moderate degenerative changes around proximal tubule and moderate necrosis in the epithelial lining. Initiation of cellular disarray was evident. The exposed fish kidney also showed clear damage around proximal tubule and haemopoietic tissue. After 7 days recovery,

reduced lumen has increased in size and moderate degenerative changes around proximal tubules are reduced and these changes were coming back to control kidney structure. It is possible that fish was recovering. After 14 days of ammonia exposure, fish kidney showed more pathological conditions (Fig.16 to 18).

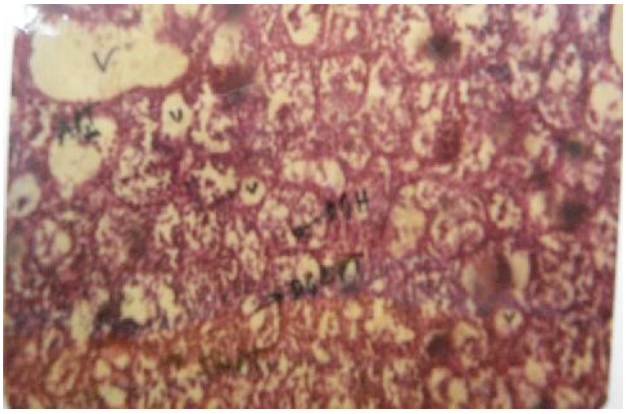

FIGURE 17: Fish exposed to 14 days of ammonia showing degeneration of hemopoietic tissue(DGH) atrophied proximal tubules (APT), severe necrosis in the epithelial cells proximal tubules (SNPT), degenerative changes around proximal tubules (DGEPT) and formation of vacuoles(V)- H &E 100X.

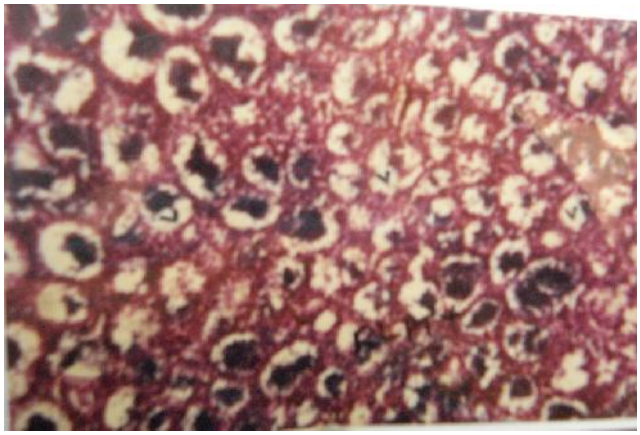

FIGURE18: Kidney of fish after 14days during recovery from ammonia exposure (14 days), showing reduced lumen in proximal tubules(RLPT)and formation of vacuoles(V)–H &E 200X.

These changes include vacuole formation, reduced lumen in the proximal tubules, and shrunken glomeruli. Damage to the interstitial cells was also observed. The intensity of the damage to kidney tubules was more clearly seen in 14 days exposed fish compared to 7 days exposed fish. After 14 days of recovery, fish kidney showed reduced pathological conditions. Shrunken glomeruli have enlarged and also interstitial cells have recovered to normalcy levels. The damaged connective tissues are also slowly regenerating in kidney after 14 days recovery period.

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