Toxicity Of Ammonium chloride On Fish bihaviour & Histopathology Of Air Breathing Fish *Clarias batrachus* (Linn.)

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ABSTRACT

T*his study includes the histopathological alterations induced by chronic (20 days) exposure of the fish Clarias batrachus to a sublethal concentrations (20.9 ppm) of Ammonium chloride. Test fishes lost their natural colouration and become almost reddish in colour. Loss of equilibrium before death is a symptom shown all the test fish. The present study therefore points towards a major histopathological alteration found in liver, kidney and intestine in response to Ammonium chloride toxicity in the fish Clarias batrachus (Linn.). So, it is suggested that more suitable to culture at water fertilizer, ammonium chloride concentration of < 20 mg/l for optimum growth performance and survival rate than other water conditions.*

Key words : Ammonium chloride, *Clarias batrachus*, chronic, fertilizer, histopathology.

INTRODUCTION :

The Rainfall washes away fertilizers and other agricultural chemicals from widespread area. Natural waters are the ultimate recipients of fertilizer residues used for agricultural purposes which are transferred from land to water. Aquatic organism can survive in very low concentrations of the pollutants. But when these concentrations increased abnormally, they become fatal to the sensitive organisms like fishes (Awasthi *et al.*, 2008).

Nitrogen pollution from agricultural sources is now considered to be a major problem in many regions of the world (Vidal *et al.*, 2000). The aquatic organisms are sensitive to environmental changes. Sub-lethal concentrations of fertilizers may cause ecological imbalance of these organisms after sufficiently long time of exposure probably as a result of cumulative impact of impaired metabolic functions (Abedi *et al.*, 2013).

Ammonia is mainly excreted as the un-ionized form NH_3 unionised ammonia (UIA). The relative proportion of the two forms depends upon pH, temperature and, to a lesser extent, salinity. The NH_3 molecule is non-polar and readily soluble in lipids (Whitfield, M., 1974). It is 300 to 400 times more toxic than NH_4^+ (Thurston *at el.* 1981 and Haywood, G.P., 1983).

Clarias batrachus, which is an integral part of paddy field culture on this subcontinent, is also subjected to severe ammonia toxicity from ammonium fertilizers during the intensive fertilization of the crop fields.

Hence, in this paper efforts have been made to illustrate the histopathological alterations induced by this inorganic fertilizer, ammonium chloride on the liver and gastro-intestinal tract and toxicity impact on fish behavior of air breathing teleost *Clarias batrachus.*

MATERIALS AND METHODS :

The air-breathing teleost *Clarias batrachus* procured live from the local fish market were washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish of average length (15–18cm) and weight (30–34 g) were acclimated for 15 days to laboratory conditions. Commercial diet containing 26.58% crude protein was used through the experiment period with daily ration rate 3% of fish weight in the in morning (10.00 AM). Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done.

Static acute bioassays were performed to determine LC_{50} values of ammonium chloride, the mortality was recorded after 24, 48, 72 and 96 h, and were calculated by the Finney method (1978). The LC_{50} values for these periods were 275 ppm, 240 ppm, 221 ppm and 209 ppm respectively. 1/10th value of the LC_{50} value for 96 hr was taken as the sublethal concentration (Sprague, 1971). Twenty acclimated fish were exposed to a sub-lethal concentration (20.9 ppm) of ammonium chloride for 20 days. Side by side same number of fish as that of experimental one was maintained as the control group. At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. On 15^{th} day and 20^{th}day fish were taken out, sacrificed and the intestine, kidney and liver were excised out and fixed in 10% Neutral Buffered Formalin for 18-24 hours fixed tissue samples were then processed and paraffin embedded tissue blocks were cut into serial sections (5-7 µ thick) by a rotary microtome and all the tissues was prepared using the standard histological methods (Luna, 1968).

RESULT :

BEHAVIOURAL RESPONSE :

The control fish shows a tendency to remain at the bottom of the aquarium with little disturbance. However, mortalities were removed immediately, and behavioural abnormalities were assessed at these regular intervals using a modified behavioural protocol checklist (Klesius *et al*. 2000). Scores were assigned daily to individual fish in the experiment and were based on the following scoring system: 0, no observed changes in behaviour; 1, swimming abnormally, lethargic or unresponsive, changes in skin coloration; 2, hyperactive or excitable, rapid operculation; 3, death. Mean behaviour scores were calculated per replicate treatment.

Just after introduction to test solution fishes showed increased swimming, surfacing and hyperactivity. Restlessness, rapid surfacing, peeling of skin and colour fading were prominent after 24 hrs exposure. After 48hr exposure the fishes showed slightly reduced activity and gradual increase in colour fading. Gill adhesion and a thin film of mucous were noticed on gills, operculum and general body surface at this stage. After 72h exposure increased surfacing and gulping of air was observed. At this stage fishes showed loss of balance and jerky movements during swimming. The school formation, a characteristic of this fish, was found weakened in test animals as compared to controls at this stage. After 96h ulceration on trunk, base of caudal and pectoral fins were prominent in 95% of the animals. A thick film of mucous on whole body and gills was observed in almost all test fishes. Test fishes lost their natural colouration and become almost reddish in colour. Loss of equilibrium before death is a symptom shown all the test fish.

HISTOPATHOLOGY:

Tissue samples liver, kidney and intestine of *C. batrachus* were treated with sublethal ammonium chloride concentration 20 mg/l at 15 day and 20 day after sacrificed and processed by conventional method, sectioned at 5-7 µm and stained with Haematoxylin and Eosin (Luna 1968).

LIVER:

Liver is the major metabolic center and any damage to this organ would subsequently do, so many physiological disturbances leading to subsequent mortality of fish (Ojolo *et al* 2005; Saxena *et al*. 2008; and Ogamba *et al*. 2016). In histopathological examination, the tissue samples taken from control groups were in normal structure. The liver is composed of hepatic lobule in which the central vein obscure. The parenchyma of the hepatic lobule is formed from hepatocytes which are arranged around the blood sinusoid in cord-like structure known as hepatic cell cord. There are bile ductile in between the cord of hepatic cells which are directed toward the periphery of the lobule to open in the bile duct (Figure:- A1). Liver lesions consisted of hydropic degenerations and cloudy swelling in the hepatocytes with focal aggregation of melanomacrophage cells in between the hepatocytes (Figure:-A2). Focal areas of necrosis, mononuclear inflammatory cells and hyperplasia in the wall of the bile duct were also detected (Figure:-A3). In the liver tissue of the group treated with fertilizers, vacuolar degeneration, necrosis, hyperemia and mononuclear cells filtration in portal regions were observed (Figure:- A2, A3).

KIDNEY:

The fish kidney consists of head and body kidneys. The head kidney is the anterior portion of the kidney and consists of lymphoid tissue. The body kidney is composed of nephron and renal tubules. The nephron is formed of renal corpuscle and Bowman's capsule. The capsular epithelium is continuous with the renal epithelium. The renal tubules begins with :- a) short neck portion lined by low cuboidal epithelium with long cilia, b) proximal convoluted tubule which has divided into segment I lined with acidophilic cuboidal to columnar epithelium with distinct brush border. The epithelial cells of the segment II are columnar and taller than those of segment I. The epithelium becomes lower and more cuboidal in the intermediate segment. The distal convoluted tubules have epithelium with lightly eosinophilia and have no brush border (Figure:- B1) and kidneys displayed glomerulonephritis (Figure:- B2), vacuolar degenerative changes in the tubular epithelium and slight congestion (Figure:- B3).

INTESTINE:

The intestinal wall of *Clarias batrachus* comprised of four distinct layers, viz. mucosa, submucosa, muscularis and serosa. The mucosal layer being thrown into finger like villi, which is made up of simple, long columnar cells and numerous goblet cells (mucous cells) with centrally placed nuclei. Sub-mucosa is thin and projected into mucosal folds constituting the lamina propria. This layer is composed of loose connective tissue with numerous collagen fibres and blood cells. Muscularis consists of inner, thick, circular, and outer, thin, longitudinal muscular layers. Serosa is formed of peritoneal layer and blood capillaries (Figure:- C1).

In the present study, marked histopathological changes in the intestine of *Clarias batrachus* have been observed in intestinal tissue, hydropic degeneration, necrosis and desquamation in epithelium cells at the apex of the villi were determined and mononuclear cell infiltration in the lamina propria was slightly observed (Figure:- C2, C3).

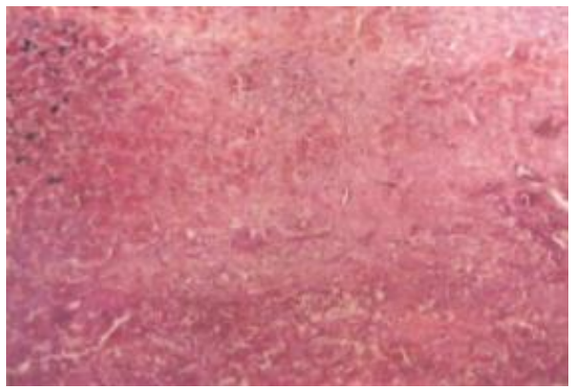

Figure:- A1. Photomicrograph of liver section from control *Clarias batrachus* at day 20 showing the central vein (V) and normal place of hepatocytes . H&E (mag. ×100).

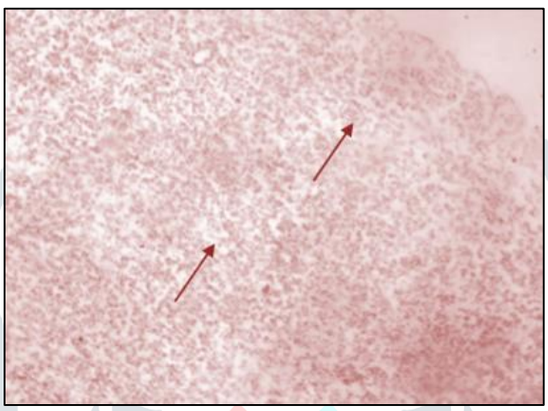

Figure:- A2. Histologic section of liver of *Clarias batrachus* treated with 20 mg/L ammonium chloride at 15 days showing multifocal areas of hepatocyte degeneration. (H&E) mag ×100.

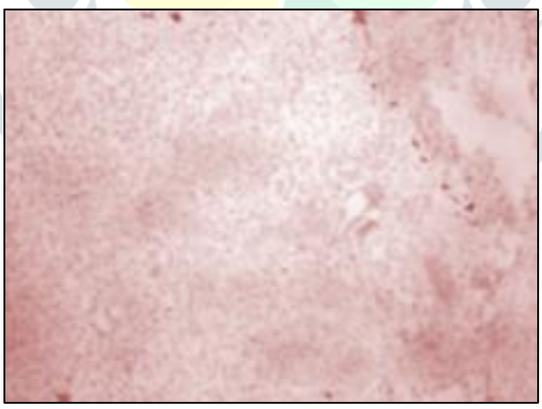

Figure:- A3. Photomicrograph of liver of *Clarias batrachus* treated with 20mg/l ammonium chloride at day 20 showing centrilobular vacuolation of hepatocytes. H&E (mag ×100).

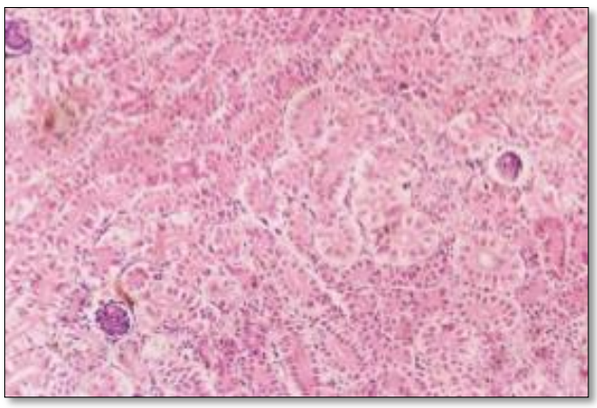

Figure:- B1. Photomicrograph of kidney of *Clarias batrachus* from control group showing normal. H&E, X 250.

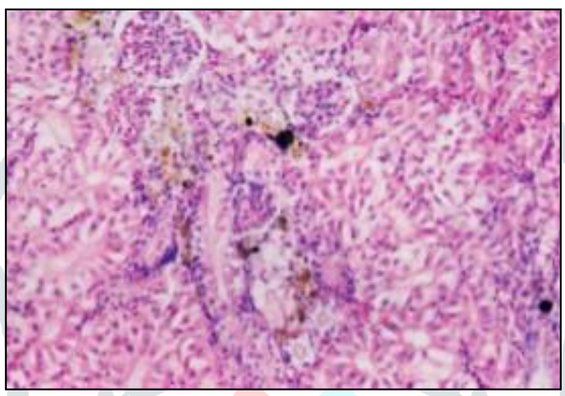

Figure:- B2. Photomicrograph of kidney of *Clarias batrachus* treated with 20 mg/l ammonium chloride at day 15. Degeneration of renal tubular epithelium (a), vacuolation and necrosis of renal tubules (b) along with necrosis of melanomacrophage center (arrow). H&E, X 10.

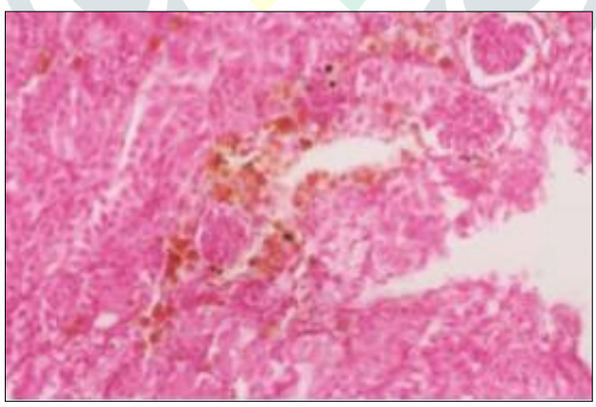

Figure:-B3. Photomicrograph of kidney of *Clarias batrachus* treated with 20mg/l ammonium chloride at day 20 Infiltration of melanomacrophage center between the renal tubules. H&E, Mag X 20.

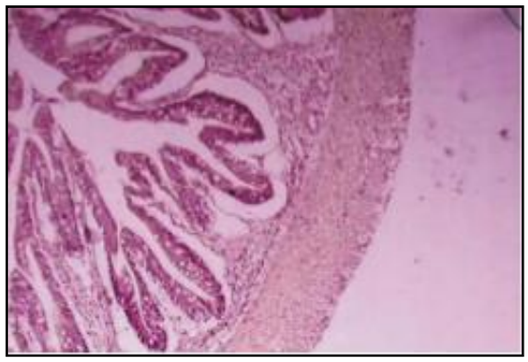

Figure:- C1. Photomicrograph of Intestine tissue of *Clarias batrachus* in control groupshowing normal appearance of circular muscles, longitudinal muscles, serosa and villi. H&E 125X.

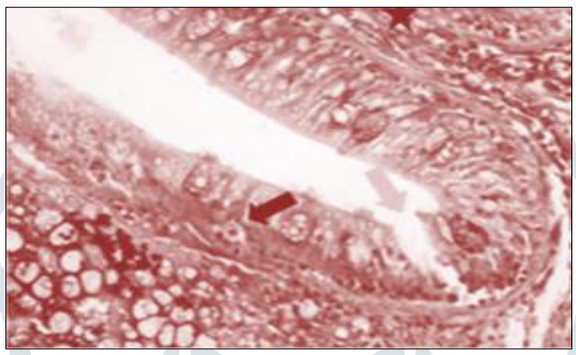

Figure:- C2. Photomicrograph of Intestine tissue of *Clarias batrachus* exposed to 20 mg/L ammonium chloride at 15 days. Desquamation (orange arrow), mononucleear cell infiltration (MHI) in connective tissue.H.E. 40X

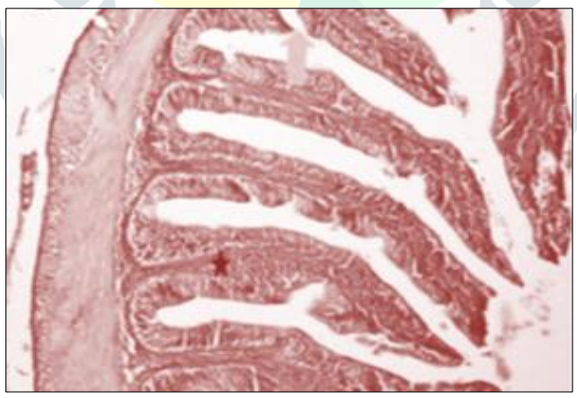

Figure:- C3. Photomicrograph of Intestine tissue of *Clarias batrachus* exposed to 20 mg/L ammonium chloride at 20 days. Desquamation (orange arrow) and mononuclear cell infiltration (MHI) (arrow). H-E. 20X

DISCUSSION :

In the present study, certain deformities and unusual swimming patterns were found in fish exposed to 209 mg/L and above concentrations. The results of the present study also indicate that the fish exposed to this fertilizer recover quickly when they were moved to freshwater. It is concluded that the fertilizers may have toxic potentials in the shallow water and therefore it should be carefully used in the areas closed to waterside. The responses recorded for the fish in this study are similar to those reported by other authors under various stress conditions

(Paul and Banerjee, 1996; Rani *et al.*, 1997; Palanivelu *et al.*, 2005; Ufodike and Onusiriuka, 2008; Lata *et al*., 2008). Behavioural responses of fish to most toxicants are the most sensitive indicators of potential toxic effects (EIFAC, 1983). Acute toxic effect mercuric chloride was observed on zebrafish by Vutukuru SS, Basani K. (2013). The toxic effects of surfactant, dodecyl dimethyl benzyl ammonium chloride (1227) on larval locomotors of zebrafish was observed by Yanan, W. *et al.* (2015). It is, therefore, conclude that the toxicity of the fertilizer ammonium chloride depend upon a number of physical, chemical and biological factors. Each of which may be used as a tool for fertilizer toxicity to fish.

Histopathological studies on fish are a noteworthy and promising field to understand the structural organization that occurs in the organs due to pollutants in the environment. These structural changes vary with the body parts, nature of the pollutant, medium and duration of exposure. Water quality characteristics also influence histopathological manifestations of toxic effects (Galat *et al.*, 1985). The structural changes in the organs at microscopic cellular and organ level leads to alterations of the function systems (Bhatkar, N.V., 2011).

The damage as more severe and progressive after 15 and 20 days exposure. Histological changes in the liver of fishes have been extensively reported. The results of the present observations in *Clarias batrachus* exposed to ammonium chloride were in agreement with those of the earlier workers especially in the vacuolization and necrosis in hepatic tissue. In the present study, cloudy swelling and hydropic degenerations on the liver were observed where liver being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in the liver. Ammonium chloride release ammonia which can be carried by the hepatic portal vein to the liver as a nutrient and enter liver metabolic pathways (Kucuk, 1999). Ammonia exposure causes liver glycogen vacuolation due to disruption of energy production (Thurston *et al*., 1978). The most frequently encountered types of degenerative changes are those of hydropic degeneration, cloudy swelling, vacuolization and focal necrosis on fish exposed to different kinds of contaminants (Hinton and Lauren, 1990). Wajsbrot *et al.* (1993) observed clear signs of liver pathology in gilthead sea bream (*Sparus auratus*) after 20 days of exposure to 13 mg l_1 TA-N (0.7 mg l_1 NH3–N). Hematopoietic tissues had occurred necrosis and vacuolar degeneration on proximal tubules of the kidney (Çapkın, E., *et. al.*, 2009). An another study, *Oncorhynchus mykiss* applied to 0.1 mg/L NH_3 for 2h, filament and lamella epithelium have superficially folded, the same concentration after 24 hours, telangiectasia in the filament on the 2 or 3 lamellae has been observed (Kirk, R. S., Lewis, J. W.,1993). In Ontario (Canada), in the *Oncorhynchus mykiss* farm in April-May as result of come to toxic levels of ammonia in water was indicated that death of 4000 fish within 48 hours, in pathological examination of fish was reported that telangiectasia in gill lamellae and kidney congestion (Speare, D. and Backman, S., 1988). *Osman et al.*, (2009) recorded congestion and hemorrhage in the hepatic sinusoids with dilation of hepatic vessels, vacuolization and degeneration of hepatic cells with fatty changes with atrophy of pancreatic acini; in liver of the *Oreochromis neloticus* exposed to the polluted water containing heavy metal salts.

In our study, kidney tissues displayed glomerulonephritis and hyperemia after being exposed to different concentrations of sublethal ammonium chloride concentrations where the kidney is a one of the major organs of the toxic effects. Thurston *et al.* (1978) observed hydropic degeneration in the kidney of trout after exposure to 0.34 mg /1 NH_3–N. Intracellular vacuolation, necrosis and shrinkage of nuclei were also apparent in the present study in ammonium chloride treated *Clarias batrachus*. Smith and Piper (1975) and Thurston *et al.* (1984), reported that degeneration of renal tubule epithelia, hyaline droplet degeneration and in some instances, partially

occluded tubule lumens invariably result in impaired glomerular blood flow and filtrations, and eventually may induce renal failure. Hyaline droplets in kidney tubule epithelium suggest re absorption of excessive amounts of proteins from glomerular filtrate (Robert and Rosemarie, 1983).

The intestine is the most important organs in digestion and absorption of nutrients from food, and therefore, monitoring of these organs is considered necessary (Takashima, F. *et al.*1982). Histological analysis of the digestive system is considered a good indicator of the nutritional status and toxicant ingestion of fish (Caballero *et al.* 2003). In the present study, marked histopathological changes in the intestine of *Clarias batrachus* have been observed in intestinal tissue, hydropic degeneration, necrosis and desquamation in epithelium cells at the apex of the villi, mononuclear cell infiltration in the lamina propria was slightly observed (Fig. C1.). All the pathological alterations showed a relationship with prevalence increasing with increasing ammonium chloride concentration and exposure time. Desquamation mononuclear cell infiltration (MHI) in connective tissue was observed in treated fish at 20 days. Similar observations made by earlier workers relating to histopathological changes in intestine in response to various toxicants are being enumerated here. The proliferation, necrosis of serora and mucosa and rupture of villi have been reported by Konar (1970) and in *Labeo rohita*; Wong *et al*. (1977) in *Cyprinus carpio* and *Ctenopharyngodon idellus*; Sastri and Gupta (1978) in *Channa punctatus*; Kumar and Pant (1984) in *Barbus conchonius*; against exposure to heptachlor, zinc and copper salt mercuric chloride, dimecron, aldicarb and furadan, respectively.

CONCLUSION:

It could be concluded that *C. batruchus* with average weight 30.0± 4.0 g, were more suitable to culture at water fertilizer, ammonium chloride concentration of < 20 mg/l for optimum growth performance and survival rate than other water conditions. Therefore, it can be recommended to be carried out under the similar experimental conditions.

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