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# Histopathological Studies on Nitrite Toxicity to Freshwater Fish, Cirrhinus Mrigala

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***Abstract:*** *Nitrite is an intermediate component of nitrogenous compound and is a naturally occurring inorganic salt found in industrial effluent as a by product of corrosion inhibitors, production of explosives and used extensively in agriculture for crop fertilization. When used at low doses nitrite is toxic. The effect of nitrite on histopathological parameters were evaluated exposing Indian freshwater fish, Cirrhinus mrigala to a sublethal concentration of nitrite (28.31) ppm for different periods from 7$^{th}$ to 35$^{th}$ days. Exposure of fish to nitrite showed changes. During 7$^{th}$ day exposure, in sublethal treatment, the tubular epithelial cells of the kidney exhibited pyknotic and karyohectic nuclei and distrupted luminal surface was observed. Further the kidney showed tubular necrosis, hyaline droplets degeneration, and renal tubular separation as the exposure period extended (14$^{th}$ and 21$^{st}$ days). At the end of 20$^{th}$ day, kidney showed marked contraction of glomeruli, dissolution of lymphoidal tissues and its replacement of blood forming sinuses, leading to formation of cellular debris, tubular necrosis and renal tubular separation. At the end of exposure period (35$^{th}$ day), kidney appeared spongy and friable; the glomeruli were conspicuously shrunken and collapsed, pyknotic nucleus in tubules, coagulative necrosis, nuclear pycnosis, renal tubular separation and hyaline droplets degeneration.In control fish, the histology showed normal disposition of renal tubules, glomeruli and interspersed lymphoid tissue. In acute treatment (24h) the glomeruli of kidney contracted markedly resulting in a wide gap between the capsular walls, extensive degeneration of lymphoid tissue and nephric tubules were noticed*
***Key words****: Cirrhinus mrigala, fish kidney, nitrite*

## I. Introduction

Nitrite an intermediate oxidation product of nitrogenous compound more toxic to fish in freshwater (Jenson, 2003). In freshwater nitrite is actively taken up across gill in competition with chloride (Eddy and Williams, 1987). Nitrite can reach high concentration in recirculating aquaculture systems in which high densities of fish are kept and bacteria transform ammonia, the main nitrogenous waste product of fishes to nitrite (Palachek and Tomasso, 1984). In many aquaculture farms nitrite is used as preservatives. Nitrite concentrations are high in streams receives effluents from wastewater treatment plants and imbalances among species of nitrifying bacteria. In both cases, nitrite levels becomes toxic even at low concentration and are toxic for many fish species (Huertas et al., 2002). Acute toxicity of nitrite has been investigated in number of fish species (Handy and Poxton, 1993; Das et al., 2004), but the effects of acute nitrite exposure on Indian major carps has not been investigated in detail. Sublethal effects, such as reproductive and endocrine end points are gaining prominence in chronic ecotoxicity testing regimes (Hutchinson et al., 2003). Kidney is the predominant organ involved in the regulation of extracellular volume and control of electrolyte and acid-base balance and major sites of foundation of hormones that influence systemic metabolic functions (Hook, 1990).

In fish, as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment. The kidney excretes nitrogen containing waste product from the metabolism such as ammonia, urea and creatinine. In teleost, the kidney, together with the gills and intestine, are responsible for excretion and maintenance of homeostasis of the body fluids (Hinton et al., 1992; Evans, 1993) and besides producing urine, act as an excretory route for the metabolites of a variety of xenobiotics to which the fish may exposed. Since a large volume of blood flows through the kidney, lesions found in this organ can be useful as a signs of environmental pollution (Hinton and Lauren, 1990).

The effects of pollutants on fish kidneys have been studied in some species and the severity of damage seen depends on the sensitivity of the species to the substances released into the environment (Hinton et al., 1992; Vinodhini and Narayanan, 2009). Panday et al. (1997) observed that morphological changes in kidney damage. Hypertrophy and Vacuolation of the epithelial cells lining, proximal convoluted tubules, shrinkages in the glomeruli resulting in dilation of Bowman's space, necrotic changes in Liza paresis exposed to lead. Abdel-Tawwab et al. (2007) observed kidney damage in Nile tilapia. Oreochromis niloticus (L) exposed to copper, damage kidney such as disintegration of glomerulus, shrinkage of Bowman's capsule, vacuolation formed around the capsule, enlargement of tubular lumen and distal and proximal tubules were observed in Channa punctatus exposed to tannery effluents Mohanta et al. (2010). Following exposure of fish to toxic agents such as

pesticides, tissue alterations have been found at the level of the tubular epithelium and glomerulus (Teh et al., 1997). Shrinkage of glomerular tuft, dialation of the lumina of the kidney tubules and necrosis of tubules has also been reported in fish exposed to different pollutants (Das et al., 2004; Venkateswara Rao, 2006). Toxicant impairs the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. The extent of severity of tissue damage is a consequence of the concentration of toxicant and is time dependent (Tilak et al., 2001).

## II. Materials And Methods

Histopathological profiles of kidney were studied by the methods Pearse (1968), Roberts (1978) and Humason (1979). The changes in physico-chemical characteristics, such as temperature, PH, Dissolved oxygen, alkalinity, hardness, salinity, calcium and magnesium of experimental water were recorded throughout the experimental period. Freshwater fish Cirrhinus mrigala, weighing 5.0-6.0 gm and measuring 7-8 cm were collected from Tamilnadu fisheries development corporation limited, Aliyar fish farm, Aliyar, Tamilnadu, India. Fish of same age and size which hatched from the same lot of eggs were collected, the age of fish being 2 to 3 months old. They were safely brought to the laboratory in well *packed* polythene bags containing aerated water and stocked in a large cement tanks (36' x18'x19'). Fish were acclimatized for about 20 days before the commencement of the experiment. During acclimatization period, fish were fed with ad libitum, with rice bran and ground nut oil cake in the form of dough once in daily. Water replaced every 24h after feeding in order to maintain a healthy environment for the fish. This ensures sufficient oxygen supply for the fish and the environment is devoid of any accumulated metabolic waste. The feeding was withheld for 24h before the commencement of the experiment and to keep the specimens in the same metabolic state. The fish were introduced into glass aquarium (26'x18'x18.5') cm which was washed thoroughly and maintained in the laboratory. Separate circular plastic tubs of 50 litres of water capacity were taken and different concentrations of nitrite were added. 10 healthy fishes were introduced into each tub. A control tub (no toxicant) with 50 litres of water and 10 fishes were also maintained. Three replicates were maintained for each concentration groups. The mortality/ survival of fish in control and nitrite treated tubs was recorded after 24h and the concentration at which 50% mortality of fish occurred was taken as the median lethal concentration (Lc50) for 24h. Sublethal values were found to be 28.31 ppm. For histopathological studies fish were treated with nitrite and various steps were followed for this study. Fixation, washing, dehydration, clearing, infiltration, embedding, sectioning, staining.

**FIXATION**

The kidney were dissected out from the control and nitrite treated fish were cut into bits of 1 to 2 cm in diameter. These tissues were immediately put in Bouin's fluid for 24h to avoid post-mortem changes and shrinkage during further process like dehydration, embedding and sectioning.

**WASHING**

After fixation the tissues were taken and the excessive fixative was removed by transferring the tissues to 50% alcohol (to prevent interference with subsequent process).

**DEHYDRATION**

In dehydration process the tissues were put into alcohol series like 30%, 50%, 70%, 90% and absolute alcohol and duration of 30 min. was given in each alcohol series. To ensure complete removal of water from the tissues a minimum of two or three changes were given in absolute alcohol at an interval of 30 minutes.

**CLEARING**

During clearing alcohol was replaced from the tissues by using a clearing agent xylene. The tissues were kept in xylene for about 30 min to 1h until they become transparent.

**INFILTRATION**

During infiltration the tissues were kept in a paraffin embedding bath (metal cups filled with paraffin at 58-60$^0$C). Then the tissues were transferred directly from xylene to molten paraffin. A minimum of three changes were given in paraffin wax with 30 minutes duration in each.

**EMBEDDING**

For embedding, L blocks were filled with molten paraffin wax. Then the tissues were placed with proper orientation. After embedding process, the blocks were kept in water overnight to ensure complete solidification. Finally, blocks were removed for sectioning.

**SECTIONING**

Tissues were cut with 7µ thickness using a rotator microtome. Then the sections were spread on a glass slide using egg albumin as an adhesive. After complete spreading, the sections were placed in an oven overnight at $37^0$C and the sections were taken for staining.

**STAINING**

During staining paraffin wax from the sections was removed by dewaxing using xylene. Then the sections were hydrated by immersing in descending grades of alcohol (absolute alcohol, 90%, 70%, 50%, 30%) for about 1-2 min. in each. Then, the sections were stained in Heidenhain's iron haematoxylin stain for 2 to 5 min, washed in tap water until the sections become bluish black in colour and then stained in 1% Eosin.

For destaining, 1% iron alum was used. The sections were dehydrated through ascending grades of alcohol (30%, 50%, 70%, 90%, absolute alcohol) for about 5 sections in each. Subsequently, the sections were cleared in xylene and mounted permanently with cover glass using DPX mountant. The histological results of gills of fish from control and nitrite treated were given as photomicrographs in appropriate places in the text.

## III. Result

Ph.m. 15 presents the normal structure of kidney of Cirrhinus mrigala. In control fish, the histology showed normal disposition of renal tubules, glomeruli and interspersed lympoidal tissue. In acute treatment (24h), the glomeruli of kidney contracted markedly resulting in a wide gap between them and capusular walls extensive degeneration of lymphoid tissue and nephric tubules were noticed (Ph. m. 16). In sublethal trearment, the tubular epithelial cells of the kidney exhibited pycknotic and karyohectic nuclei and a distrupted luminal surface at the end $7^{th}$ day (Ph. m. 17). Further the kidney showed tubular necrosis, hyaline droplets degeneration, and renal tubular separation as the exposure period extended ($14^{th}$ and $21^{st}$ day) (Ph. m. 18, 19). At the end of $28^{th}$ day, kidney showed marked contraction of glomeruli, dissolution of lymphoidal tissue and its replacement by blood forming sinuses, leading to formation of cellular debris, tubular necrosis and renal tubular separation (Ph. m. 20). At the end of the exposure period ($35^{th}$ day), kidney appeared spongy and friable; the glomeruli were conspicuously shrunken and collapsed, pyknotic nucleus in tubules, coagulative necrosis, nuclear pycnosis, renal tubular separation and hyaline droplets degeneration (Ph. m. 21).

## IV. Discussion

The teleost kidney is one of the first organs to be affected by contaminants of water (Thophon et al., 2003) in the present study control fish showed normal structure of renal tubules and haemopoetic tissue. In the present study during acute and sublethal treatment of nitrite the kidney of fish C. mrigala shows glomerular expansions, swelling of tubules, dilation of capillaries in the glomerulus and hyaline droplet degeneration. The toxic action of nitrite may be direct on the cellular components or occur by inducing stress responses.

Exposure to metals frequently causes alterations in the tubules and glomerulus in perch Lates calarifer exposed to cadmium (Thophon et al., 2003). Handy and Penrice (1993) noticed swollen Bowmen's capsule cells and melanomacrophages in the kidney of trout Salmo trutta and Tilapia Oreochromis mossambicus exposed to mercuric chloride. First Mullet Valamugil georgii exposed to nitrite, showed tubule regeneration and dilation of capillaries in glomerulus and tubules in rainbow trout Oncorhynchus mykiss (Bucher and Hofer,1993). Crandall and Goodnight (1963) observed pathological symptoms like dilation of renal tubules and reduction of lymphoid tissue in the kidney of Lebestes reticulates in response to prolonged (3 months) exposure to lead. Establier et al. (1978) observed renal epithelial necrosis, sloughing of the epithelium, and accumulation of necrotic debris with in lumen of the renal tubules in Mugil auratus exposed to organic and inorganic mercury. Accounting to severe pathological changes noticed in the kidney may be due to renal excretion of under toxified fenvalerate (Tilak et al., 2001)

A toxicologic insult to the kidney could affect any or all of these functions, the cellular response to a toxic insult may vary from an imperceptible biochemical aberration to cell death with resulting necrosis; functionally, toxicity may be reflected as a minor alteration in transport capability or as rank renal failure (Hook 1980). Depending on the magnitude of the insult, these changes may be reversible, permanent or lethal. Kidney is under the influence of the sympathetic nervous system and changes in neural activity can markedly influence renal function; renal nerve activity might directly influence proximal tubular function as well; so any change in systemic homeostasis that would alter sympathetic nerve activity could also influence the kidney (Hook, 1980). Renal excretion is one of the ways of eliminating the undertoxified toxicant molecule resulting in severe pathological changes in haemopoietic tissues resulting severe necrosis, cloudy smelling of renal tubules, disintegration of interstitial tissue, pycnotic nuclei etc. (Tilak et al., 2001).

A single dose of nephrotoxin agent may produce profound acute changes in renal function; similarly, chronic administration of a low dose of a nephrotoxin chemical may bring about significant changes in structure

of the kidney (Hook, 1980). Even sublethal concentrations of a toxicant, which lack the capacity to dramatically increase the rate of mortality in exposed populations, may still cause their ecological death after a sufficiently long time exposure, probably as a result of the cumulative impact of impaired metabolic functions which may explain the enhanced nephral damage of fish from sublethal toxicity chronic exposure. It can be stated that in chronic conditions of exposure some sort of repair mechanism takes place in fish kidney (Ahmad and srivastva, 1995). In general fish seems to acclimatize with sublethal nitrite exposure in chronic conditions.

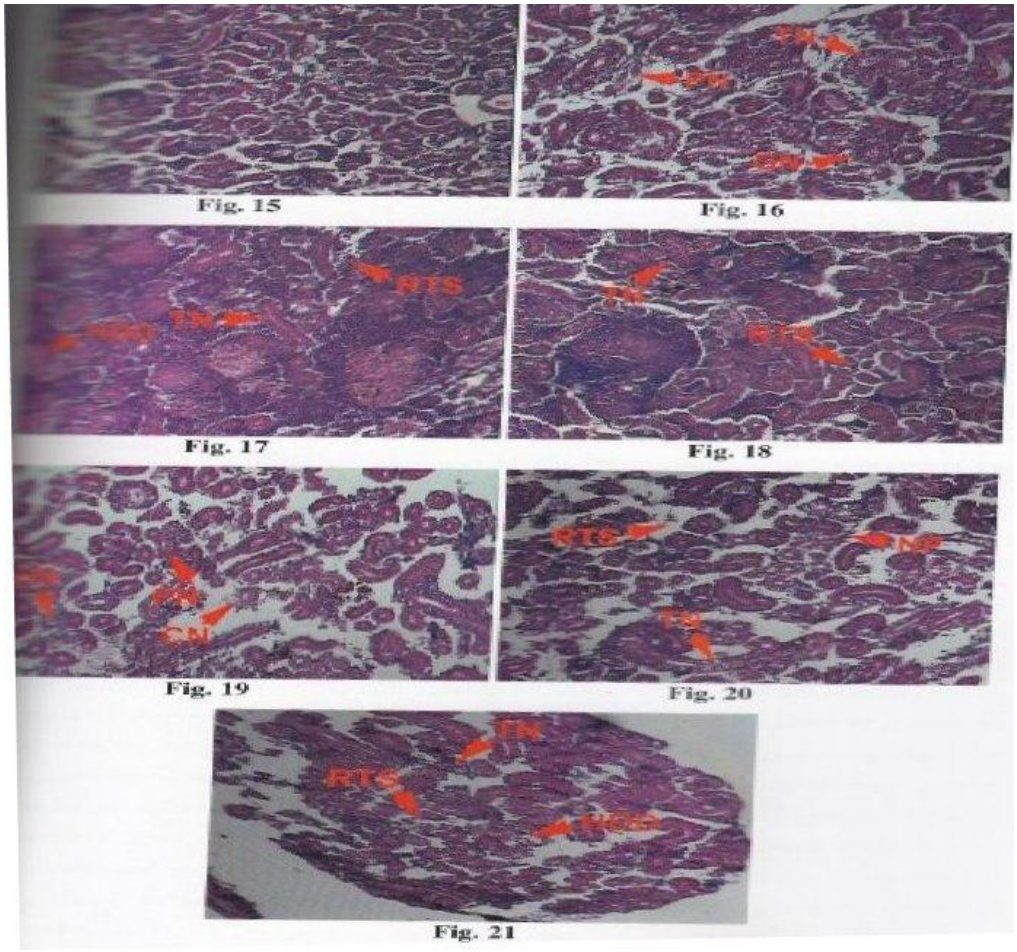


Fig 15-21. (15) Kidney structure of control fish. H&E, x 100,
(16) Kidney tissue of C.mrigala exposed to 28.31 ppm of nitrite 24h (acute): TN: tubular necrosis: PN: pyknotic nucleus in tubules; GN: globular necrosis, H&E, x100.
(17) Kidney tissue of C.mrigala exposed to 2.831 ppm of nitrite for 7 days (sub lethal): TN: tubular necrosis: HDD: hyaline droplets degeneration; RTS: renal tubular separation, H&E, x100.
(18) Kidney tissue of C.mrigala exposed to 2.831 ppm of nitrite for 14 days (sub lethal): TN: tubular necrosis: RTS: renal tubular separation, H&E, x100.
(19) Kidney tissue of C.mrigala exposed to 2.831 ppm of nitrite for 21 days (sub lethal; GN: globular necrosis, PN: pyknotic nucleus in tubules; CN: coagulative necrosis.H&E, x100.
(20) Kidney tissue of C.mrigala exposed to 2.831 ppm of nitrite for 28 days (sub lethal; RTS: renal tubular separation, TN: tubular necrosis: NP: nuclear pycnosis.H&E, x100.
(21) Kidney tissue of C.mrigala exposed to 2.831 ppm of nitrite for 35 days (sub lethal; TN: tubular necrosis; RTS: renal tubular separation; HDD: hyaline droplets degeneration; H&E, x100.

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