

Elucidation of Nitrite Toxicity on Hemoglobin Content and Histopathological Parameter in Freshwater Fish “*Cirrhinus mrigala*”

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Abstract: Nitrite is an intermediate and important product in bacterial nitrification and denitrification processes in the nitrogen cycle. The concentration of nitrite in natural water is typically low in the μM range. Elevated concentrations of nitrite can be found in water receiving nitrogenous effluents, in various hypoxic environments or in effluents from industries producing metals, dyes and celluloid. Intensive rearing methods are commonly used today. These methods mostly rely on recirculating water systems that remove waste ammonia from water, which leads to the hazard of possible incomplete oxidation of ammonia accompanied by accumulation of nitrite in the system. Freshwater fish *Cirrhinus mrigala* ecologically and economically important fish exposed to nitrite in the aquatic environment. This study evaluates the changes on hemoglobin content and histopathological lesions on kidney tissues in *Cirrhinus mrigala* at sublethal concentration. This research explains about the changes in the hemoglobin content of fish *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days. There is an increase and decrease in hemoglobin content throughout the study period. In histopathological parameter the kidney cells appeared spongy and friable; the glomeruli were conspicuously shrunken and collapsed, pyknotic nucleus in tubules, coagulative necrosis, nuclear pyknosis, renal tubular separation and hyaline droplets degeneration.

KEYWORDS: *Cirrhinus mrigala*, nitrite, Hemoglobin content, kidney cells, Sublethal studies, Histopathology.

I. INTRODUCTION

Pollution from toxic chemicals affects human health and environment. Contamination of natural waters from anthropogenic sources of nitrogen is a widespread problem. In the aquatic environment, the most common ionic forms of inorganic nitrogen are nitrite (NO_2^-). These ions present naturally in aquatic ecosystems as a result of atmospheric deposition, surface and groundwater runoff, dissolution of nitrogen-rich geological deposits. Nitrogen is essential for all living things as it is a component of protein. Nitrogen exists in the environment in many forms and it moves through the nitrogen cycle. However, excessive concentrations of nitrite-nitrogen in drinking water can be hazardous to health, in human and animals especially in fish *Cirrhinus mrigala*. Nitrite an intermediate oxidation product of ammonia and nitrate is more toxic to fish in freshwater compared with sea water. In fresh water nitrite is actively taken up across the gill in competition with chloride. (Jensen, 2003).

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. In many aquaculture farms nitrite is used as preservatives (Palachek and Tomasso, 1984). Nitrite is present in natural water only in smaller quantities. It has been found to be toxic to fish, as NO_2^- combines with haemoglobin, and forms methaemoglobin, causing the brown coloration of blood. The presence of chloride ions and calcium inhibits nitrite toxicity (Jensen 1990, 2003). From the blood plasma, nitrite diffuses into red blood cells, where it oxidizes iron in hemoglobin (Hb) to the +3 oxidation state. The main toxic action of nitrite on aquatic animals are due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen, causing hypoxia and ultimate death. In fish, 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 and besides producing urine, kidney act as an excretory route for the metabolites of a variety of xenobiotics. (Evans, 1993). In water it receives excess nitrogenous waste, an imbalance between bacterial nitrification and denitrification, eventually leading to nitrite accumulation (Offem and Ayotunde, 2008).

The effects of pollutants on fish kidneys have been studied and the severity of damage seen depends on the sensitivity of the species to the substances released into the environment (Vinodhini and Narayanan, 2009). Pandey 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 paretis* exposed to lead. Abdel – Tawwab et al. (2007) observed kidney damage in Nile tilapia, *Oreochromis niloticus* (L) exposed to copper. Kidney damage 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, dilatation 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).

The nitrite continuously released into aquatic environment from natural process, from water industrial process and from waste. Nitrogen constitutes a core group like (Nitrite, Nitrate, and Ammonia) of aquatic pollutants. The crux of the problems lies in fact that these elements not only accumulate in water and sediment but also concentrate in the tissues causing alterations at various functional levels of the organisms (Vutukuru et al., 2005). In fish nitrite are absorbed by the digestive tract and gill and can accumulate in kidney internal tissue. Although 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. Hence it is useful to have an insight into histological analysis. The extent of severity of tissue damage is a consequence of the concentration of toxicant and is time dependent. Also, the severity of damage depends on the toxic potential of particular compounds or salts accumulated in the tissues (Jayantha Rao 1985). Microscopic examination of target tissues is an important end point in the evaluation of toxic potential and risk assessment of chemicals in the environment. Hence the present study was aimed to investigate the nitrite toxicity at sublethal concentration on hemoglobin content and histopathological lesions in kidney tissues of *Cirrhinus mrigala*.

II. METHODS

Hemoglobin content of blood was estimated by Cyanmethemoglobin method (Drabkin, 1946).

Haemoglobin + $K_3Fe(CN)_6$ -- Methaemoglobin

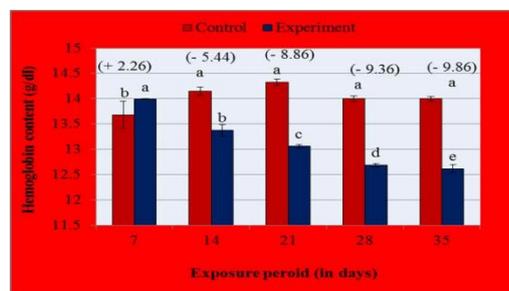
Methaemoglobin + KCN -----Cyanmethaemoglobin

Haemoglobin can undergo several reactions; it binds with oxygen and carbon monoxide to form oxyhaemoglobin and carboxyhaemoglobin, respectively. Oxidation of the ferrous ion to the ferric form results in the formation of methaemoglobin and it binds with cyanide ions to form cyanmethaemoglobin.

Histopathological studies for kidney cells by Humason (1979).

III. RESULTS

Fig 1. Represents the changes of hemoglobin content in fish *Cirrhinus mrigala*



Hemoglobin content of *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days. Error bars indicate the standard error of the mean. Bars bearing same letter are significantly different according to DMRT ($P > 0.05$). The numericals in the parenthesis indicates percent change.

Fig 1. represent the data on changes in the hemoglobin content of fish *Cirrhinus mrigala* exposed to sublethal concentration of nitrite for 35 days. On day 7, the hemoglobin content in the nitrite exposed fish was found to be

slightly increased showing a minimum percent increase of 2.26. However, after 7th day, a decrease in hemoglobin level was observed in the rest of the study period showing a minimum percent decrease of 5.44 at the end of 14th day and a maximum percent decrease of 9.86 at the end of 35th day. There were significant ($P < 0.05$) variation among the treatments ($F_1, 40 = 232.02$; $P < 0.05$), periods ($F_4, 40 = 14.54$; $P < 0.05$) and their interactions ($F_4, 40 = 35.08$; $P < 0.05$).

Fig 2. Represents the changes of kidney cells in fish *Cirrhinus mrigala*

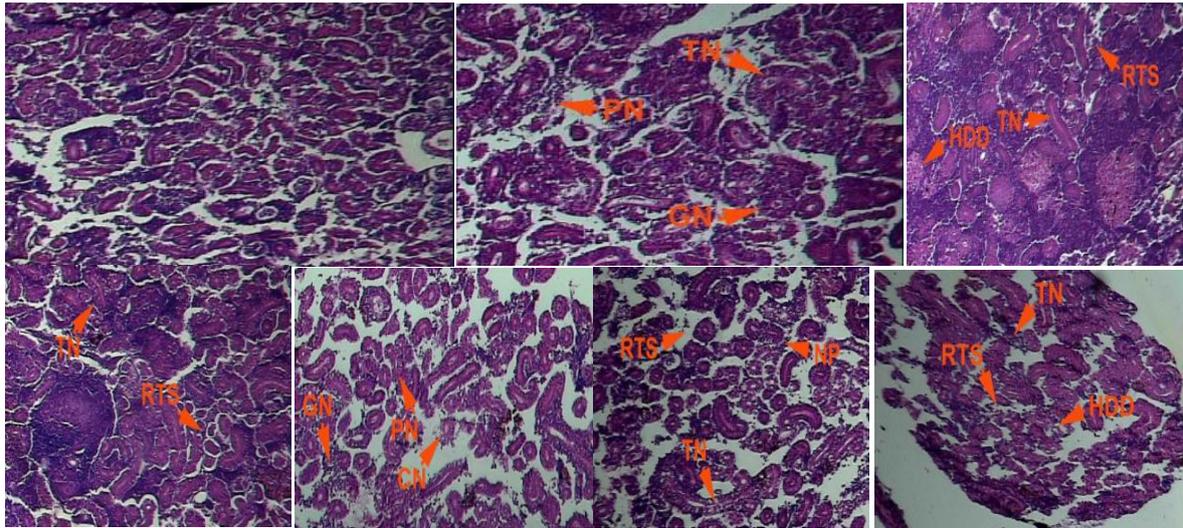


Fig 2. In sublethal treatment, the tubular epithelial cells of the kidney exhibited pyknotic and karyorhectic nuclei and a disrupted luminal surface at the end of 7th day. Further the kidney showed tubular necrosis, hyaline droplets degeneration, and renal tubular separation as the exposure period extended (14 and 21st day). At the end of 28th 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. At the end of the exposure period (35th 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.

IV. DISCUSSION

Aquatic ecosystems are equipped with a variety of physico-chemical and biological mechanism to eliminate or reduce adverse effects of toxic substances. A great deal of research has been conducted to understand the effects of toxicants on the physiology and survival of animals especially in fish. High levels of nutrients discharged from various anthropogenic sources, including agricultural fertilizer, livestock waste, urban runoff, and sewage leaks. Among the major nutrients, more attention has been made to nitrite likely because of their perceived higher toxicity. Nitrite compounds exert environmental stress on fish and affect oxygen consumption, ammonia excretion, and haemolymph (Farah et al., 2004).

Nitrite induced shortage of O₂ results in high stress levels leading to hyperventilation, elevated heart rate, and increased blood pressure in fish. Nitrite reacts with oxygenated Hb to form metHb and nitrate (NO₃), and it reacts with deoxygenated Hb to form metHb and NO (Camargo and Alonso, 2006). The resulting MetHb cannot bind and transport oxygen; hence specimens with high levels of methemoglobinemia suffer functional anaemia owing to a reduction of the oxygen-carrying capacity of the blood. This, in turn, results in tissue hypoxia (Huertas et al., 2002).

Exposure of nitrite concentration leads to several physiological changes like disruption of ion regulatory, cardiovascular, and excretory processes, lead to death of fish (Jensen, 2003). High levels of nitrite in water is a potential factor triggering stress and cause high mortality in aquatic organisms. Water pollution and industrial waste usually elevate the nitrite concentration due to ammonia oxidation. This nitrification process depends on the bacterial activity and oxygen level (Das et al., 2004).

The primary cause of nitrite toxicity may vary among fish species (Doblender and Lackner, 1997). This is not only due to a sole mechanism but to a combination of several toxic mechanisms, such as a reduction in the functional activities, Hb concentration in blood and the subsequent functional anemia, damage of lysosomal and microsomal membranes in the liver, the formation of DNA-damaging nitrous derivatives and changes in plasma homeostasis, such as extracellular hyperkalemia. The physiological disturbance like depression, suppression of hemopoietic activity of the kidney in addition to the increased removal of dysfunctional RBC from blood.

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. 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.

V. IMPLICATION

The significant increase in hemoglobin value during initial period of nitrite toxicity perhaps attributed to the erythropoiesis reactivation mechanism induced by the spleen and liver to compensate the cerebral hypoxia induced by nitrite. Significant decrease during acute and sublethal nitrite treatment may be due to a decrease in nonspecific immunity of the fish due to stress which may cause internal hemorrhage resulting in a decrease in the number of red blood cells. Histological lesions of kidney of fish, *Cirrhinus mrigala* may be nonspecific responses of fish to nitrite toxicity. The magnitude of the above alterations showed a great relationship with degree and duration of stress.

VI. CONCLUSION

Thus it is concluded that the Hematology, histopathology parameters are most sensitive parameters in monitoring the toxicity of nitrite especially at sublethal concentrations in fish *Cirrhinus mrigala*. The stress may be reduced due to denitrification process by bacteria in water such that the nitrite can be converted into nitrate non toxic to fish and it also reduces the ammonia toxicity in water.

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