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Research Article

SODIUM CYANIDE INDUCED HISTOPATHOLOGICAL CHANGES IN KIDNEY OF FRESH WATER FISH *CYPRINUS CARPIO* UNDER SUBLETHAL EXPOSURE

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ABSTRACT

Sodium cyanide which has a wide range of applications in mining industries also has harmful effects on various organisms. The cyanide utilized by mining industries is let off into streams seriously compromises with the survival of the fish population. In the present study an attempt was made to analyze the toxicity of sodium cyanide to the kidney of freshwater fish *Cyprinus carpio* by histopathological aids. The results obtained suggested that sodium cyanide at a concentration of 0.2mg/L ($1/5^{th}$ of LC_{50}) can impact the fish kidney in a catastrophic manner and cause the variation in histoarchitechture of kidney. Based on the outcome of the present study, it is therefore suggested that appropriate measures be taken for detoxification of sodium cyanide before it is discharged in to streams, as it can compromise the survival of aquatic habitat consequently resulting in the disturbances of aquatic ecosystem.

Keywords: Freshwater fish, Nephrotoxicity, histopathology, sodium cyanide.

1. INTRODUCTION

Cyanide is extremely destructive chemical which can kill both target and nontarget organisms when expelled in the environment (Dube and Hosetti, 2011). Industries dealing with metal plating and finishing, mining and extraction of metals such as gold and silver, production of synthetic fibres and the processing of coal generate large quantities of cyanide-containing wastes (Rocha-e-Silva *et al.,* 2010). Consequently release of cyanide containing wastes into the environment has generated considerable interest. Cyanide is found to be highly toxic to the aquatic organisms, primarily due to the formation of complexes with metal ions that are present as enzyme cofactors. The discharge of toxic pollutants into waterways may result in acute or chronic toxicity in fish (Ballantyne, 1987; Leblanc, 1997). Cyanides are used widely and extensively in the manufacture of synthetic fabrics, plastics, in electroplating baths and metal mining operations, as pesticidal agents and intermediates in agricultural chemical production, in predator control devices, and constitute a hazard to aquatic ecosystems in certain waste-receiving waters and to livestock (EPA 1980; Towill *et al.,* 1978). The toxicity of cyanide is a consequence of its high potency as a respiratory poison in all aerobic forms of life (Yen *et al.,* 1995). Acute doses of cyanide are usually fatal, due to the marked susceptibility of the nerve cells of the respiratory centre to hypoxia (Greer and Jo, 1995).

Kumar and Pant (1984) have stated that histopathological studies are useful to evaluate the pollution potential of various toxicants, which do not cause animal mortality over a given period, but are capable of producing considerable original damage. Kidney serves as a major route of excretion of metabolites of xenobiotics, and receives the largest proportion of postbranchial blood, and therefore, it is more likely to undergo histopathological alterations under toxic stress (Ortiz *et al.,* 2003). Although a number of studies have investigated the effect of

cyanide exposure on liver (Rutkowski *et al.*, 1986; Ma and Pritsos, 1997; Buzaleh *et al.*, 1990), little is known about the effect of direct cyanide exposure upon the structural conformation of biomolecular components in kidneys of fish. Thus in the present study, an attempt is made to understand the potential threat of sodium cyanide to kidney of freshwater fish *Cyprinus carpio* by histopathological aids.

2. MATERIALS AND METHODS

2.1 Procurement and maintenance of fish

Healthy *Cyprinus carpio* (Length 6±1cm and weight 5±2 g) were procured from the State Fisheries Department, Dharwad, India and were acclimatized to laboratory conditions for 15 d at 24 °C. Further they were held in dechlorinated tap water in large cement tanks which was previously washed with potassium permanganate to free the walls from any microbial growth. Fish were fed regularly and 12–16 h of photoperiod daily during acclimation. Water was renewed daily, whose physico-chemical characteristics were analyzed following the methods mentioned in APHA (2005) and found as follows, temperature, 25 ± 2 °C; pH, 7.6± 0.2; dissolved oxygen, 7.7 ± 0.8 mg/L; total hardness, 30.4 ± 3.1 mg as $CaCO_3$/L; salinity, nil; specific gravity, 1.003; conductivity less than 14 µS/cm; calcium, 17.86 ± 0.92 mg/L; phosphate, 0.4 ± 0.004 µg/L and magnesium, 0.8 ± 0.3 mg/L.

2.2 Preparation of stock

Sodium cyanide of 95% purity was procured from Loba Chemie Pvt. Ltd., Mumbai, India. Stock solution was prepared by dissolving sodium cyanide in double distilled water in a standard volumetric flask. Water was renewed every day over test periods. Henceforth, the replacement of the water medium was followed by the addition of the desired dose of the test compound. The fish were exposed in batches of 10 to a fixed concentration of sodium cyanide with 20 L of water in three replicates for each concentration. One fifth (0.2 mg/L) of the LC_{50} (1mg/L) was selected as sub lethal concentration for studies and the duration of exposure were 10 and 20 days. Further, the fish were allowed to undergo a recovery period of 14 days. This study was conducted under OECD Guideline for static-renewal test conditions (OECD, 1992). At the end of 10 and 20 days and that of post recovery of 14 days, fish were sacrificed and were sampled for histopathological studies.

2.4 Histopathology

For the histopathological examination, the method was followed as described by Humason (1972) the liver was dissected. The sample was isolated immediately and was fixed in Bouin's fluid for 24 to 48 h. The tissue was processed in a series of graded alcohol and embedded in paraffin which was being filtered thrice earlier. The organs in paraffin were sectioned into 5 µm thick ribbons by using semi-automated microtome (LeicaRM 2255) and sections were stained primarily with haematoxylin and counter stained with eosin (H & E) for light microscopic examination (Lille, R.D., 1969). The sections were observed under 200X and 400X magnifications respectively. The microscopic view was photographed by using an Olympus phase contrast microscope (Olympus BX51, Tokyo, Japan) with attached photography machinery (ProgResC3, Jenoptic-Germany). The photographed images were further observed for differences and the findings were recorded.

3. RESULTS AND DISCUSSION

The results from the present study indicated variations histopathological aspects in kidney of fish exposed to 0.2mg/L (1/5th of LC_{50}) of NaCN. However, no changes were observed in control group of fish.

The kidney of *Cyprinus carpio* comprises of functional units, the nephrones. Each of which consists of a renal corpuscle and a renal tubule. The renal corpuscle of nephrone consists of glomerulus and Bowman's capsule. A tubular neck follows the Bowman's capsule. Other regions of the renal tubule are proximal distal and collecting tubules. The interstices of the tubules are enriched with hematopoietic tissue, which contain round to polygonal cells possessing hyperchromatic nuclei (**Fig. 1**). Histopathological analysis of section of control showed no changes, the collecting tubules appeared to be normal in shape, no changes were noticed in glomerulus and bowmans capsule, the lumen of distal tubules were also normal **(Fig-1, A).** On the other hand, the fish exposed to 0.2mg/L of sodium cyanide showed certain degree of variation. The findings like, cytoplasmic vacuolation, shrinkage in lumen size of collecting tubules, and glomerular degeneration, were noticed **(Fig-1, B)** in fish exposed to 0.2mg/L of sodium cyanide for duration of 10 days. In the kidney of fish exposed to same concentration but for a duration of 20 days, the changes were severe, which included, portal inflammation, increase in lumen size, degeneration of glomerulus, increased cytoplasmic vacuolation, blood coagulation and necrosis were witnessed (**Fig1,**

C). Nevertheless, the fish under recovery group showed slight improvements in histoarchitechture, these included, slight regeneration of distal tubules, reduced lumen size, minor blood coagulation, less instances of cytoplasmic vacuolation **(Fig-1, D)** which suggested the possible tendency of restoration by the experimental fish during the recovery period, yet complete recovery was however not possible, thus indicating the need of furthermore duration of post exposure period for complete recovery.

The present investigation revealed the toxicity of sodium cyanide on kidney of freshwater fish *Cyprinus carpio* under a sub lethal concentration of 0.2mg/L for 10 and 20 days, with a post exposure period of 14 days further. The toxicity of sodium cyanide on the present experimental model was proved to be catastrophic. As most of the cyanide is absorbed and detoxified inside the body by various mechanisms, thus the detoxification process results in nutritional scarcity (Prashanth & Neelagund 2007). Literature on cyanide complexes in lethal and sublethal concentrations have been well established on fishes (Dube & Hosetti, 2010). Authors around the world have concluded that in prolonged conditions of exposure or recovery, some sort of repair mechanism exists which acts in fish kidney (Ahmad and Srivastava, 1985). This was observed in the present study. The current study witnessed harmful changes during the exposure period of 10 and 20 days but however, the changes during recovery period of 14 days was in such a way that the fish seems to acclimatize when the condition of cyanide free medium persisted. Thus the study can therefore be termed as a pioneer work on the histopathological changes in kidney of *Cyprinus carpio* since no record was available regarding histopathological changes in kidney of a fish due to sodium cyanide exposure earlier.

Histopathology provides a rapid method to detect effects of irritants in various organs (Johnson *et al.,* 1993). Kidney is a vital organ of body and proper kidney function is a must to maintain the homeostasis. Along with the function of removing wastes from blood, it is also responsible for selective reabsorbtion, which helps in maintaining volume and pH of blood and body fluids and that of erythropoieses (Iqbal *et al.,* 2004). The kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.,* 2003). Histological alterations in the kidney at the level of glomerulus and tubular epithelium in fish after exposure to toxic agents have been reported by many workers. Das and Mukherjee, (2000) reported dilation of renal tubules and necrotic changes characterized by karyorrhexix and karyolysis.

In one report, *Cyprinus carpio* when exposed to dimethioate, the changes noticed were, cytoplasmic vacuolation, shrinkage in lumen size of collecting tubules, and glomerular degeneration (Ram Nayan Singh, 2012). This matches with the results of the present study. One more report showing necrosis, cellular atrophy, granular cytoplasm and vacuolization in kidney tissues of *Ctenopharyngodon idella* after exposure to fenvalerate (Tilak *et al.,* 2001) shows similar kind of findings as that of the present investigation. Degeneration in the epithelial cells of renal tubules, degeneration of glomerulus, cytoplasmic vacuoles in the epithelial cells with atrophy and hypertrophied cells and narrowing of the tubular lumen are some of the findings which are in agreement with the study reported by Cengiz, 2006, this was witnessed after fish were exposed to deltamethrin. Velmurugan *et al.,* (2007) reported hypertrophied epithelial cells of renal tubules, contraction of the glomerulus and expansion of space inside the Bowman's capsule in the kidney of *Cirrhinus mrigala* exposed to monocrotophos, the findings show relevancy to the current study wherein similar kind of changes are noticed. Gill *et al.,* (1989) reported various histopathological changes such as degeneration of tubular epithelium, nuclear deterioration like karyorrhexis and karyolysis, and collapsing glomeruli in the kidney of *Puntius conchonius* following exposure to cadmium. They also found progressive increase in severity of degenerative changes with increasing duration of exposure. This can be compared to the present investigation wherein two different durations of exposure showed variation of symptoms with 10 days possessing minimum and 20 days showing maximum damage. However, it is to be noted that during the period of 14 day recovery, the fish were able to overcome the stress and showed positive signs of development by strengthening the histoarchitechture of fish kidney.

Thus histopathological observation indicated that sodium cyanide had the similar kind of harmful effects which were observed for various other toxicants including pesticides and heavy metals. The symptoms observed in the present study matched with that of previous studies wherein the freshwater fishes were exposed to different concentrations of differenct to sublethal concentrations of sodium cyanide caused destructive effect in kidney of freshwater fish *Cyprinus carpio* which could be seen in earlier studies. The kidney histopathological alterations, such as those observed in these

studies and findings from previous studies, could result in severe physiological problems, ultimately leading to the death of fish.

4. CONCLUSION

Based on the observations made and results obtained from the present study it is inferred that sodium cyanide is a toxic component to the freshwater fish *Cyprinus carpio* at a concentration of 0.2mg/L and is able to cause catastrophic effects in kidney which can be witnessed histopathologically. It can be also stated that histopathology is a useful biomarker for environmental contamination assessment.

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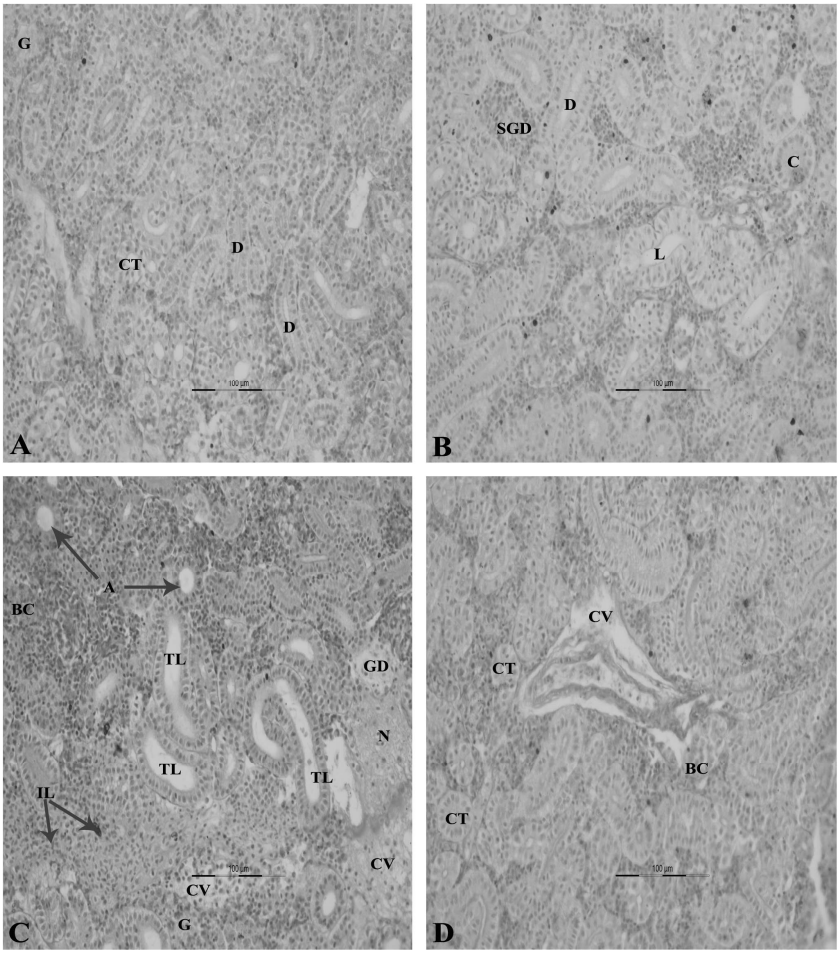

Fig. 1: Showing section of fish kidney exposed to 0.2mg/L NaCN
Kidney of *Cyprinus carpio* showing; A). Control: intact glomerulus (G), Distal tubule (D), collecting tubule; B). 10 day exposure to 0.2mg/L NaCN; slight glomerulus degeneration (SGD), changes in lumen size (L), slightly damaged collecting tubule (C), C). 20 day exposure to 0.2mg/L NaCN; Atrophy (A) change in size of tubular lumen (TL), Necrosis (N), Cytoplasmic vacuolation (CV), Blood congestion (BC), Degenerated glomerulus (GD), Infiltration of lymphocytes (IL) D). Blood congestion (BC), slightly damaged collecting tubule (CT) and Cytoplasmic vacuolation (CV).

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