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Histopathology of Liver and Kidney of *Rasbora daniconius* Exposed to Paper Mill Effluent

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Abstract: The toxicity of paper mill effluent was evaluated histopathologically, using fish, *Rasbora daniconius*. The acute toxicity test was based on 96 h static renewal bioassay which resulted in 96 h 50% value of 9.5% (v/v). Liver and kidney were examined in a 96 h LC_{50} acute test. Histopathological examination reveled, swollen hepatocytes, nuclear hypertrophy, rupture sinusoids, hemorrhages, vacuolation in hepatic cells and broken central vein in liver, kidney showed hypertrophy of hematopoietic tissues, cell necrosis, blocked glomerules with full of blood stain, dilated renal tubules with pyknotic nuclei and tubular necrosis. Overall, data from this research indicates that a paper mill effluent brought histopathological changes in the fish *Rasbora daniconius*.

Key words: Paper mill effluent, liver, kidney, histopathology, *Rasbora daniconius*

INTRODUCTION

In India, first paper mill was started in West Bengal at Serampure. In Maharashtra, first paper mill was started as Deccan Paper Mill at Pune in 1951. Now, there is tremendous expansion in these industries during last 25 years. Controversially, the study industry as it stands now as one of the largest major industries and contributes a lot towards the water pollution.

The pulp and study mill effluent contains a wide variety of inorganic and organic compounds which are partly removed (if at all). These effluents have been found to be containing >200-300 different organic compounds (Fortin *et al*., 1998; Houk, 1992; Perez-Alzola and Santos, 1997; Suntio *et al*., 1988) and approximately 700 organic and inorganic compounds (Cernakova and Golis, 1994). The effluent contains a mixture of substances stemming from wood, the chemicals used (particularly Cl_2, ClO_2 and other chlorine compounds) and compounds arising during the pulping and bleaching processes (Muttray *et al*., 2001).

Due to high chemical diversity of the organic pollutants in study and pulp mill effluent, a high variety of toxic effects on aquatic communities in recipient water courses have been observed (Oanh, 1996; Yen *et al*., 1996). A significant number of these substances have been classified as carcinogenic, mutagenic and clastogenic (Ericson and Larsson, 2000; Houk, 1992) and endocrinic (Zacharewski *et al*., 1995). The pollutants concerned also kill fish or affect their reproductive physiology (Van den Heuvel and Ellis, 2002) or may induce male-biased sex ratios among fish embryos (Larrson and Forlin, 2002).

In the fish, it has been observed that the external organ is affected due to the toxic chemicals causes' loss of equilibrium, increase in opercular movement, irregular movement and finally leads to death. This may be attributed with significant damage to the internal organs. Industrial effluent pollute aquatic ecosystem and find their way in the body of aquatic animals by means of gills, digestive tract and general body surface. As effluent accumulates in the different tissues of body, it is necessary to study in detail the histopathological changes produced by industrial effluent in different organs of fishes thoroughly, investigate them in order to assess the extent of damage.

Histopathological studies have been conducted to establish fundamental relationships between contaminant exposure and various biological responses. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments (Schwaiger *et al*., 1996). The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Bucke *et al*., 1996). Liver (ICES, 1997) and kidney (Bucher and Hofer, 1993) are

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suitable target organs for histological examination to determine the effect of pollution. Fish liver is regarded as a major site of storage, biotransformation and excretion of toxic substances. In fish, the kidney performs an important function related to electrolytes and water balance and maintenance of a stable internal environment. These organs have been proved to be indicators of possible pollution (Hinton and Lauren, 1990).

Very few studies have been carried out in connection with the histopathological effects of pulp and study mill effluent in fish. Khan *et al.* (1992) reported the occurrence of fin necrosis, kidney tumors, anemia change in parastiofauna, low condition factors and organ somatic indices of winter flounder living in the vicinity of a pulp and paper mill. Discharge of untreated pulp and study effluent into receiving waters is known to be toxic to some aquatic organisms.

Manifestations of toxicity in fish includes fin necrosis, increase of parasites, changes in physiology, detoxifying enzyme activities, hematology, osmo regulation and reproduction (Andersson *et al*., 1987, 1988; Myllyvirta and Vuorinen, 1989; Lindesjoo and Thulin, 1990; Lindstrom-Seppa and Oikari, 1990).

Lesions have also observed the gill and liver of effluent exposed fish (Lehtinen *et al*., 1984). Therefore, the objective of study is to assess histopathological alterations in liver and kidney of fish, *Rasbora daniconius* exposed to lethal concentration of the paper mill effluent.

MATERIALS AND METHODS

Experimental fish: The *Rasbora daniconius were* collected from Godavari river at Kaigaon Toka (latitude 19°37.463 and longitude 75°01.409) 45 km away from Aurangabad (MS) India. The fishes were kept in glass aquaria, acclimatized for the period of four weeks. During period of acclimatization, the fishes were fed after every 24 h with pieces of live earthworms. Healthy fishes with active movements were only considered for the experimentation.

Paper mill effluent: The paper mill effluent was collected directly from the Kaigaon study mill at releasing point 45 km away from Aurangabad (MS) India. The physicochemical parameters of effluent and tap water were analyzed according to APHA *et al.* (2000) recorded in Table 1. The percentage concentration of test solution obtained by using formula (FAO, 1984):

$$\text{Volume percent} = \frac{\text{Volume of effluent}}{V_E + V_{DW}} \times 100$$

Table 1: Physico-chemical parameters

Parameters	Tap water	Paper mill effluent
Temperature (°C)	26±2°C	28±2°C
pH	7.2	6.4
Dissolved oxygen (DO)	6.9	0.98
Alkalinity	27	100
Total hardness	116	510
Biological Oxygen Demand (BOD)	10	276
Chemical Oxygen Demand (COD)	25	1183
Nickel (Ni)	-	0.416
Zinc (Zn)	-	0.02

*Except temperature and pH, values express in mg L^{-1}

V_E = Volume of effluent
V_{DW} = Volume of dilution water

Acute toxicity test: The LC_{50} value for 96 h was determined by static renewal bioassay following probit analysis (Finney, 2009) due to its advantage over other bioassay techniques. This method has advantage of replacing the toxicant solution a fresh every 24 h so that metabolic waste (ammonia) which itself highly toxic can be removed.

Histological biomarkers: The fish, *Rasbora daniconius* (length 8-8.5 cm and weight 4-4.5 g) were exposed to lethal concentration (96 h LC_{50}) of paper mill effluent for 96 h. At the end of exposure period, the fishes survived were sacrificed, dissected carefully to isolate liver, kidney and fixed in bounis fluid.

After 24 h, they were processed following the standard technique. Tissues were embedded in paraffin wax and serial section of 4-6 μM thickness were cut, deparaffinised, stained in haematoxylin and counterstained with eosin. The sections were examined under light microscopy, using Takashima and Hibiya (1995) as a reference and photographed using a digital camera.

RESULTS

Physicochemical parameters: The values of physico-chemical parameters were observed and recorded in Table 1. The values of physicochemical parameters of study mill more or less effluent fall within acceptable limits, except values of Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and hardness. The raised values of physicochemical parameters may imply an increased toxicity (Pathan *et al*., 2009).

Acute toxicity test: The 96 h LC_{50} is the basic value in the acute toxicity test. For *Rasbora daniconius*, the 96 h LC_{50} value was 9.5% concentration (Pathan *et al*., 2009).

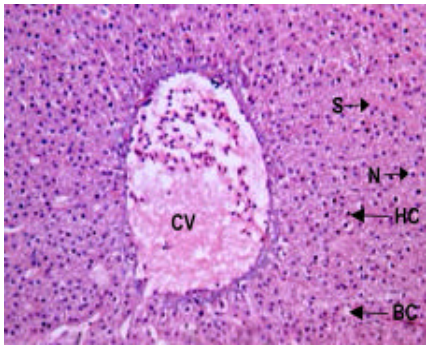

Fig. 1: Transverse section of the liver of control fish, *Rasbora daniconius* showing Central Vein (CV), Sinusoid (S), Nucleus (N), Hepatic Cell (HC), Bile Canaliculi (BC) (400x)

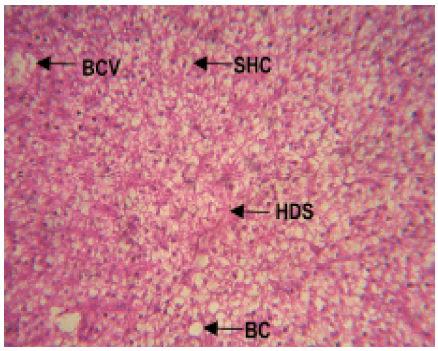

Fig. 2: After exposure lethal concentration at 9.5% (LC50 of 96 h) of paper mill effluent gill showing Broken Central Vein (BCV), Swollen Hepatocyte (SHC), Hemorrhage with Destruction of Sinusoid (HDS), Bile Canaliculi (BC) (400x)

Histological biomarkers

Liver: The liver of control *Rasbora daniconius* was composed of hepatocytes (parenchymal cells) arranged in typical tubular architecture, sinusoids and blood vessels filled with numerous blood cells. The hepatocytes were morphologically polygonal and had conspicuous nuclei with densely stained nucleoli (Fig. 1). In contrast, the liver of fish treated with lethal concentration for 96 h at 9.5% (LC_{50}) exhibited marked pathological changes as mildly swollen hepatocytes in which the nucleus retained nearly normal shape. The sinusoids were found to rupture with hemorrhages at several places, necrosis of hepatic cells and broken central vein was observed. The inter-cellular space became wide due to damaged connective tissue (Fig. 2).

Kidney: The kidney of the species examined had a pattern similar as found in other vertebrates. They were composed of nephrons consisting mainly of a Malpighian corpuscle, of a tubule and of a collecting duct system to which the filtrate was led. The Malpighian corpuscle had a capillary network that forms the Glomerulus (Fig. 3). In contrast, the kidney of treated fish with lethal concentration for 96 h at 9.5% (LC_{50}) exhibited marked pathological changes such as hypertrophy of hematopoietic tissues with cell necrosis. Glomerulus was found completely blocked with full of blood stain. Renal tubules were found dilated with pyknotic nuclei. Tubular necrosis and atrophy of dilated nature was also noticed (Fig. 4).

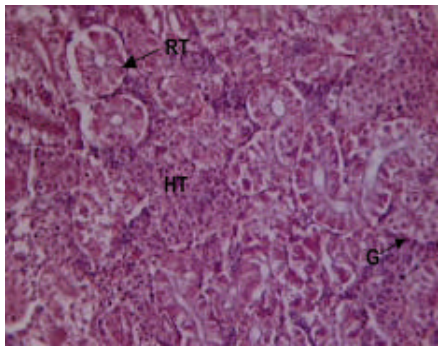

Fig. 3: Transverse section of the kidney of control fish, *Rasbora daniconius* showing Glomerulus (G), Renal Tubule (RT), Hematopoietic Tissue (HT) (400x)

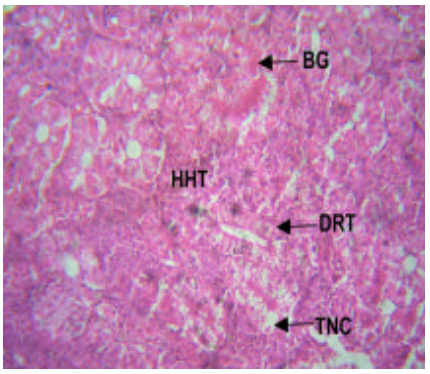

Fig. 4: After exposure lethal concentration at 9.5% (LC50 of 96 h) of paper mill effluent kidney showing Blocking of Glomerulus (BG), Hypertrophy of Hematopoietic Tissue (HHT), Dilated Lumen of Tubule (DRT), Tubular Necrosis (TNC) (400x)

DISCUSSION

Histopathology provides a rapid method to detect the effect of irritants in various organs Johnson *et al.* (1993). The liver is the primary organ for metabolism,

detoxification of xenobiotics and excretion of harmful substances. The liver has the ability to degrade toxic compounds but its regulating mechanism can be overwhelmed by elevated concentrations of these compounds which could subsequently result in structural damage (Brusle and Gonzalez, 1996). The histo-pathological lesions observed in the liver were mildly swelling of hepatocytes, rupture of sinusoid with hemorrhages at several places and broken central vein. The cellular degeneration in the liver might be due to oxygen deficiency as a result of gill degeneration (Mohamed, 2001). The earlier results correlate with the findings of Nirmala *et al.* (1988) and Das and Mukherjee (2000).

Rana (2007) observed disintegration of hepatic cells, separation of the hepatic cells from the blood vessel and proliferation in the pancreatic tissues. Broken hepatic cells and clear atrophy was also seen in the nucleus of hepatic cells in urea exposed fish, *Ababas testudineus.* Narayan and Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei, loss of glycogen in liver tissue of *Heteropneustes fossilis* while subjecting them to acute thiodon toxicity. Nassr-Allah and Abdel-Hameid (2007) observed inflammation, central necrosis and cell degeneration in liver tissue of *Oreochromis aureus* juveniles while subjecting them to phenol. Figueiredo-Fernandes *et al.* (2007) reported that the hepatic parenchyma of fish exposed to copper showed cytopalsmic vacuolation and hepatocellular necrosis.

Mazher and Boja (2007) observed hepatic lesion with necrosis, vacuolation, damaged blood vessels and accumulation of cytoplasmic granules in the liver of *Tilapia mossambica* due to the toxicity of copper sulphate, lead nitrate and zinc sulphate. Peebua *et al.* (2008) studied alteration in the liver of Nile tilapia *oreochromis niloticus* exposed to 38.19 (acute), 35 μg L^{-1} subchronic of alachlor for 24, 48, 71 and 96 h and 90 days, respectively, found hydropic swelling of hepatocytes, vacuolation and lipid vacuoles were observed in hepatocytes in the 2nd and 3rd month of sub chronic exposure.

Fatma (2008) studied bioaccumulation of selected metals in water and liver, gills, intestine, testis, heart and muscle of *Oreochromis niloticus* and *Late niloticus* from Lake Nasser-Egypt and reported several histopathological alterations, including vacuolar degeneration with focal areas of necrosis in liver.

The kidney of fish receives the largest proportion of postbranchial blood and therefore renal lesions might be expected to be good indictors of environmental pollution (Ortiz *et al.*, 2003).

In this study, hypertrophy of hematopoietic tissue with cell necrosis, blocking of Glomerulus, dilated renal tubules with pyknotic nuclei and tubular necrosis were observed in kidney tissues of fish. The earlier results correlate with the findings of Das *et al.* (1996). Mourad (1995) observed tubular destruction and epithelial oedema, detachment of the epithelial cells form the underling basement membrane, pycnosis of nuclei in effluent of the Egyptian copper work treated fish, *Tilapia zillii.* Veiga *et al.* (2002) studied the histopathological alteration in the kidney of *Prochilodus lineatus* exposed to trichlorofan.

The kidney tissue collected after 24 h exposure showed enlargement of intercapsular space with gomerular atrophy, hypertrophy of the kidney tubule cells with small granules on its cytoplasm and little nuclear alteration. Blood overflowing from capillaries with pyknotic nuclei and vacuoles in the cytoplasm was noticed.

After 48 h, the kidney tissue showed glomerular expansion, impossibly to visualize the intercapsular space as well as cytoplasm limit of many cells. The parietal capsular epithelium and the base membrane present loss of cell content, the tubular cells appeared swollen vacuolated and within thin and thick cytoplasmatic granulation. Some of the cell nuclei kept relatively regular form with a condensed chromatin at its central region while others showed themselves relatively small and pyknotic. Peebua *et al.* (2008) observed hydropic swelling of tubular cells, lipid vacuole accumulation in many tubules and nuclear pyknosis of *Oreochromis niloticus* exposed to alachlor.

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