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HISTOPATHOLOGICAL CHANGES IN LIVER OF FRESHWATER MAJOR CARP, *LABEO ROHITA* AFTER ACUTE AND CHRONIC EXPOSURE TO TEXTILE MILL EFFLUENT

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ABSTRACT
In present investigation, fingerlings of *Labeo rohita* were exposed to acute (96h) and chronic (30 days) dose of TME. The histopathological studies revealed conspicuous changes in the liver. The acute dose (18 % of Textile Mill Effluent) in *Labeo rohita* showed an increase in binucleated cells and a light infiltration of lymphocyte after 96h. Dilation of sinusoids with reduced blood cells. There were few distinct hepatocytes. Necrotic cells showed displaced nucleus at the periphery with basophilic cytoplasm. Chronic dose of 1.8 % TME for 30 days in *Labeo rohita* resulted in severe damage to liver, which can be visualized by necrotic areas. Congestion in the sinusoids, infiltration of mononuclear lymphocyte around the vena centralis, dilation in the sinusoids resulted in irregular arrangement of sinusoids. Hepatocytes showed hemorrhage, disconnection among the cells and necrotic areas near central vein. The TME collected from Ichalkaranji was analyzed for physico chemical parameters and heavy metals, which showed high range of pH and high values of alkalinity, hardness, chloride, sulphate, TDS, COD, BOD and absence of oxygen. The heavy, metal contents in TME was in the order of Iron > Lead > Manganese > Chromium > Aluminum > Nickel > Zinc > Copper > Cadmium. The results indicate severity of physico-chemical parameters and heavy metals resulting in massive destruction in normal architecture of Liver which is concentration and time dependent.

INTRODUCTION

Direct discharge of industrial effluents in to rivers and run off from fields in to the ponds, lakes and rivers are causing serious concern about water pollution particularly with respect to inland fisheries. These effluents and their toxic effects on aquatic animals by depleting the dissolved oxygen, altering the pH, salinity, CO_2 content, and thereby directly and indirectly affecting the life cycles as well as the metabolic activities of the aquatic animals at the biological levels. Our present knowledge of industrial effluent is still limited to few (David and Ray, 1960; Stephen, 1987).

The effluent when discharged into receiving water body without adequate treatment can cause irreversible changes like high temperature and increased pH of the received stream. The soluble chemicals and dyes present in waste water will interfere with the green aquatic plants. Colloidal organic matter and soluble inorganic salts will increase turbidity of this water. All these lead to a large increase of COD and BOD values of that water body (Hussain *et al.,* 2004) and make that water unfit to use by aquatic animals and plants. Toxic effects of the chemicals and textile wet processing effluent can be seen on the receiving water bodies e.g. sodium sulphide, free residual chlorine, azo dyes and heavy metal like chromium are all toxic to fish and aquatic animals as well as resistant to bio-degradation (Hussain *et al.,* 2004).

Water pollution induces pathological changes in fish. As an indicator of exposure to contaminants, histology represents a useful tool to assess the degree of pollution, particularly for sub-lethal and chronic effects.

In 1990s, the concept of biomarkers has become increasingly established (Hinton and Laurén, 1990; McCarthy and Shugart, 1990; Huggett *et al.,* 1992; Myers *et al.,* 1993). According to Huggett *et al.* (1992), the most common usage of the term biomarker has been for biochemical, physiological or histological indicators of either exposure to or the effects of xenobiotic chemicals at the sub-organismal or organismal level. During toxicological and pathological studies the variations in the histology are exploited for the evaluation of physiological state of the animal. Histological alterations in marine organisms have been identified as useful biomarkers for environmental contamination (Hinton and Lauren, 1990 a, b, Hinton *et al.,* 1992, Munday and Nowak, 1997).

The advantage of histopathology as a biomarker lies in its intermediate location with regard to the level of biological organization (Adams *et al.,* 1989). Histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect the effects of irritants, especially chronic ones, in various tissues and organs (Johnson *et al.,* 1993). The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs (Sindermann, 1979; Bucke *et al.,* 1996). Gills (Malatt, 1985; Poleksic and Mitrovic-Tutundzic, 1994), kidney (Oronsaye, 1989; Bucher and Hoffer, 1993), liver (Hinton and Lauren, 1990; Myers *et al.,* 1993; ICES, 1997) and skin (Vethaak, 1994) are suitable organs for histological examination in order to determine the effect of pollution. Jiraungkoorskul *et al.* (2002) reported histopathological

changes in the liver and gills of Nile tilapia, *Oreochromis niloticus*, exposed to glyphosate herbicide. In the gills, filamentous cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm were observed. In liver, there were vacuolations of hepatocytes and nuclear pyknosis.

Considering the effects of effluents in an ecosystem and their hazardous effects on the aquatic organisms particularly fish species, the present work was undertaken to analyze TME and sediment from Panchganga River near Ichalkaranji for their physicochemical characteristics and heavy metals. In present study acute and chronic toxicity of the TME on histopathology of liver of *Labeo rohita* has been studied.

MATERIALS AND METHODS

Experimental animals: collection and maintenance

The fingerlings of the freshwater fish, *Labeo rohita* measuring 6 to 7cm in length and weighing about 6 to 7g were collected from the Government fish seed rearing centre, Rankala, at Kolhapur, Maharashtra. After collection, they were brought to the laboratory and stocked in rectangular glass aquaria containing aerated water for seven days for acclimatization. During this period, water was half-changed twice in a day and fishes were fed with fixed staple diet (Red Rose TM) once in a day. Feeding was stopped 24h prior to the exposure of fishes to the effluent. The effluent from textile mill, at Ichalkaranji town near Kolhapur, was collected at a fixed point source, when the discharges from all the stages of processing were released in to the Panchganga River together. Static renewal bioassay (acute tests) was conducted by using textile mill effluent (TME) to determine LC_{50} and LC_{50} values for 96h exposure. Chronic toxicity (30 days) experiments were also conducted using $1/10^{th}$ and $1/20^{th}$ of LC_{50} concentration of this effluent.

Experimental sets: The physico-chemical parameters of TME and test solution, like Temperature, Solids -Total Dissolved (Filterable) solids and Total Settle able solids: pH, Acidity, Alkalinity, Dissolved Oxygen, Biochemical Oxygen Demand (BOD), Free $C0_2$, Chloride and Sulphate, were studied according to APHA (1998) After acute (96h) and chronic (30 days) exposure, the live fishes (five from each group) were sacrificed and tissues (Liver) was quickly excised.

Histopathological studies

During toxicological and pathological studies, the variations in the histology are used for the evaluation of physiological state of the animal. Therefore during present study, liver was dissected out and cut into pieces and fixed in Bouin's fixative. The tissues were processed for wax sectioning.The sections were cut at 5.0-6.0μ and stained with hematoxylin and eosin. The observations were made under Olympus microscope and wide and narrow field eyepieces.

RESULTS

Physico-chemical characteristics of TME

The analysis of TME showed wide range of pH 2 to 12, which was highly fluctuating during the study period. High alkalinity was also recorded (393.00 ± 28.78mg/L) with total hardness of 330.00 ± 60.00 mg/L. Samples analyzed for the dissolved oxygen content indicates absence of dissolved oxygen. Residual chloride content was 1.08 ± 0.11 mg/L. Sulphate content in the effluent was 292.00 ± 175.00 mg/L, which was insignificantly, varies with sampling time. Total dissolved solids were 1761 ± 780.00 mg/L. Effluent analyzed for free CO_2 showed 5.85 ± 0.53 mg/L and DO was absent. The COD content was 650.0 ± 168 mg/L. While BOD for 3 days at 27ºC was recorded as 306.0 ± 175.00 mg/L (Table 1).

Heavy metal detected in TME were in order of Fe > Pb > Mn > Cr and Al > Ni > Zn > Cu which acts as pollutants under chronic exposure (Table 1).

Histopathology: control

In control group, liver showed polyhedral symmetry of hepatocytes arranged around the central vein. Arrangement of hepatocytes was in muralium duplex fashion. Hepatocytes showed centrally placed nucleus with eosinophilic cytoplasm. Few vacuolated hepatocytes were observed which indicates the presence of glycogen. Blood cells were evenly distributed all over the sinusoids Plate I.

Acute

The results of the light microscopic studies showed that the morphological changes were evident in the liver of exposed animals as compared to control. In the fish exposed to LC_0 concentration of 11 %, the cells of liver, showed occurrence of a light hypertrophy, dilation of sinusoids, and vacuolization of cell cytoplasm was observed after 96h of exposure. Textile mill effluent caused a disorganization and dilation of vena centralis, a frequent woven of cell cords, and an increase in the infiltration of lymphocytes. A light vacuolization and dilation of sinusoids was still apparent.

With the 18% textile mill effluent, an increase in the cells containing binucleated cells and a light infiltration of lymphocyte were observed after 96h. Dilation of sinusoids was observed with reduced blood cells. There were few distinct

Table 1: Physico-chemical characteristics of textile mill effluent from Ichalkaranji. (All values are expressed in mg/L, except pH)

Sr. No.	Parameters	mg/L
1	pH	2.00 to 12.00
2	Alkalinity	393.00 ± 28.78
3	Acidity	10.30 ± 2.19
4	Total Hardness	330.00 ± 60.00
5	Chloride	500.00 ± 147.00
6	Residual Chlorine	1.08 ± 0.11
7	Sulphate	292.00 ± 175.00
8	Free CO_2	5.85 ± 0.53
9	Total Dissolved Solids	1761.00 ± 380.00
10	Dissolved Oxygen	Nil
11	Chemical Oxygen demand	650.00 ± 168.00
12	B.O.D. 3 Days at 27^0 C	306.00 ± 75.00
13	Copper	0.0054 ± 0.0007
14	Nickel	0.0088 ± 0.0008
15	Cadmium	0.0029 ± 0.0005
16	Zinc	0.0074 ± 0.0006
17	Lead	0.74 ± 0.09
18	Chromium	0.038 ± 0.005
19	Aluminum	0.038 ± 0.005
20	Iron	1.88 ± 0.16
21	Manganese	0.17 ± 0.08

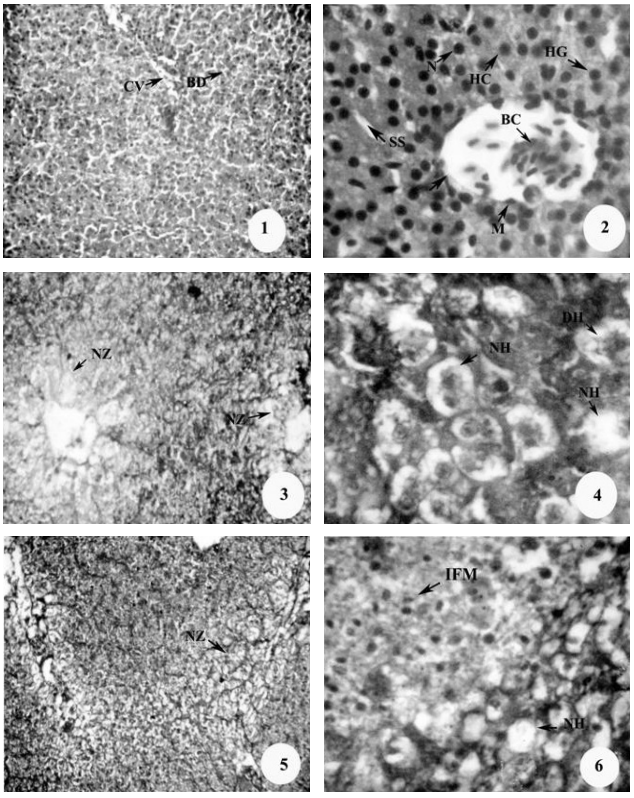

Plate 1: Microphotographs of *Labeo rohita* liver after acute exposure to textile mill effluent are presented ; (1) Gross morphology of liver (X 10); (2) Control liver showing hepatic cords and central vein (X 400); (3) Gross morphology of liver exposed to LC_0 TME (X 10); (4) Liver showing hepatic cords and central vein exposed to LC_0 TME (X 400); (5) Gross morphology of liver exposed to LC_{50} TME (X 10); (6) Liver showing hepatic cords and central vein exposed to LC_{50} TME (X 400)

HC – Hepatocytes **NZ** – Necrotic zone **CV**- Central vein **NH**– Necrotic hepatocytes **BD**- Bile duct **DH** – Degenerating hepatocytes **BC** – Blood cells **IFM** – Infiltration of macrophage **SS**- Sinusoidal space **HG –** Hepatocytes with glycogen vacuole **N** – Nucleus **M** – Macrophage

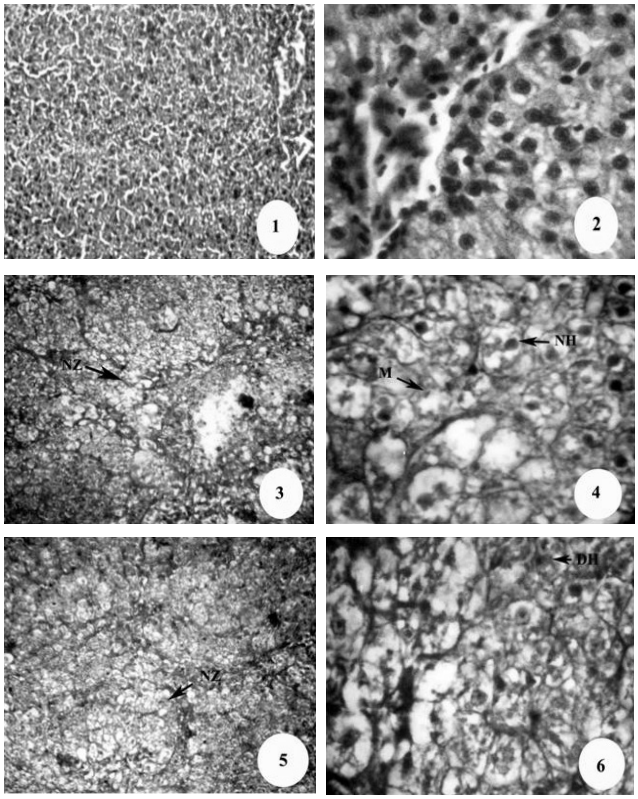

Plate 2: Microphotographs of *Labeo rohita* liver after chronic exposure to textile mill effluent are presented; (1) Gross morphology of liver (X 10); (2) Control liver showing hepatic cords and central vein (X 400); (3) Gross morphology of liver exposed to 1/10^{th} of LC_{50} TME (X 10); (4) Liver showing hepatic cords and central vein exposed to 1/ 10^{th} of LC_{50} TME (X 400); (5) Gross morphology of liver exposed to 1/ 20^{th} of LC_{50} TME (X 10); (6) Liver showing hepatic cords and central vein exposed to 1/20^{th} of LC_{50} TME (X 400)

HC – Hepatocytes **NZ** – Necrotic zone **CV**- Central vein **NH**– Necrotic hepatocytes **BD**- Bile duct **DH** – Degenerating hepatocytes **BC** – Blood cells **IFM** – Infiltration of macrophage **SS**- Sinusoidal space **HG –** Hepatocytes with glycogen vacuole **N** – Nucleus **M** – Macrophage

hepatocytes. Necrotic cells showed displaced nucleus at the periphery with basophilic cytoplasm. Few binucleated necrotic calls were also observed near the central vein.

Microphotographs of *Labeo rohita* liver after chronic exposure are presented in Plate II.

Chronic

After chronic dose of 1.8% textile mill effluent for 30 days, liver showed clear congestion, infiltration of mononuclear lymphocyte around the vena centralis. Sinusoids were dilated and appeared irregular. Sinusoids showed hemorrhage and disconnection among the cells. Necrotic areas were observed near central vein. Necrotic cells showed varied intensity of vacuoles. Hepatocytes showed swollen nuclei, in pyknotic condition.

Chronic dose of 1.8% textile mill effluent for 30 days resulted in severe damage to liver, which can be visualized by necrotic areas. Congestion in the sinusoids was observed as a result of chronic toxicity. Infiltration of mononuclear lymphocyte around the vena centralis was noticed. Dilation in the sinusoids resulted in irregular arrangement of sinusoids. Hepatocytes showed hemorrhage and disconnection among the cells. Necrotic areas were observed near central vein**.** Necrotic areas were greatly increased as compared to chronic 1.8 % textile mill effluent treated fish. Necrotic cells showed varied intensity of vacuoles. Hepatocytes showed swollen nuclei, in pyknotic condition**.**

In general, there were remarkable histopathological alterations in the liver after acute and chronic exposure to TME as compared to control. These alterations were more marked at higher concentration than lower ones in both the exposures. Microphotographic alterations in histological architecture of liver of, *Labeo rohita* after acute and chronic exposure to TME are presented in Plate I (Fig. 1 to 6) and II (Fig. 1 to 6).

DISCUSSION

The freshwater forms very important media for the production of protein-rich fishes, prawns and crabs. But the fresh water media are ecologically deteriorating due to discharge of industrial effluents (Thingran, 1974).The disposal of industrial effluents in the aquatic environment is toxic to fishes. Mortality

of fishes has been recorded in rivers receiving various pollutants. Abnormal physico-chemical characteristics of industrial effluents are responsible for mortality of fishes (Mishra *et al.,* 1988; Pawar, 1988).

Some water quality parameters are more likely to be involved with fish losses, such as dissolved oxygen, temperature, and ammonia. Others, such as pH, alkalinity, hardness and clarity affect the fish, but usually are not directly toxic. Each water quality parameter interacts with and influences other parameters, sometimes in complex ways (NRAC, 1993).

The non availability of oxygen created by high BOD, fluctuating pH of the industrial effluent may affect the normal metabolism of fish in order to overcome the toxic stress exerted by the pollutants in the textile effluent.

Fish are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may, however, be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and kidney (Dutta, 1996). A histological investigation may therefore prove to be a cost-effective tool to determine the health of fish populations, hence reflecting the health of an entire aquatic ecosystem in the bio-monitoring process. In this study, the gill, histology of the fresh water fish, *Labeo rohita* was analyzed.

As a principle metabolic organ (detoxification organ), the liver plays a major role in the uptake, accumulation (Couch, 1975, Gluth *et al.,* 1985), biotransformation (Braunbeck, 1998), and excretion (Köhler, 1990) of xenobiotics. Exposure to toxicants may cause histological changes in the liver, which in turn could be used as a biomarker to indicate prior exposure (Hinton and Laurén, 1990). The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations of harmful substances, and this could subsequently result in structural damage. Similar studies on various fish species, exposed to various toxicants, showed histopathological changes in the liver of those specimens (Van Dyk, 2003).

Vacuolations of hepatocytes (fatty change) is a common response associated with exposure of fish to a variety of different agents (Meyers and Hendricks, 1985). This histological change could signify the following biochemical lesions: (1) Inhibition of protein synthesis; (2) energy depletion; (3) desegregation of microtubules; (4) shifts in substance utilization.

These lesions are common biomarkers of metal exposure in mammals but have not been commonly reported in fish. Metal containing cytoplasmic inclusions are usually found in lysosomes and may result from metallothionein degradation. Leland (1983) reported an increase in the number of lysosomes and the presence of electron dense deposits in hepatocytes from trout exposed to both copper and zinc (Hinton and Laurén, 1990).

In the nucleus of the liver cells, electron dense heterochromatin was not present. The degree of the damage in the liver cells was indicated by swollen mitochondria with electron transparent matrix and by dilation and vacuolation of the endoplasmic reticulum system. Epithelial cells decreased in electron density, the endoplasmic reticulum was vesiculated and mitochondria were swollen. Leucocytes increased in number, and empty vacuoles and vacuoles filled with dense granules appeared in them during toxicosis. Copper sulphate or paraquat increased serum transaminase enzyme activities (glutamic acid-oxalacetic acid transaminase, glutamic acid-pyruvic acid transaminase) in all the three fish species. These damages can cause serious disturbances in energy uptake and secretion processes of fish.

The accumulation of glycogen inclusions in hepatocytes that displaces the nucleus to the periphery of the cell is pathognomic of type IV glycogen storage disease (GSD IV, Anderson's disease, amylopectinosis) in humans (Ishak and Sharp, 1979; Sherlock and Dooley, 1997). GSD IV is caused by an enzyme deficiency that results in the synthesis of an abnormal glycogen molecule having decreased branch points and increased chain length. Biochemically the unbranched glycogen, similar to amylopectin, becomes less soluble and glycogenolysis is reduced (Goodman and Ishak, 1999). The abnormal glycogen structure is due to a deficiency of the branching enzyme amylo-1, 4, 1, 6 transglucosidase (Ishak and Sharp, 1979; Sherlock and Dooley, 1997). While GSD may be an inherited metabolic condition, it may also be a part of a toxic process (Goodman and Ishak, 1999). With the exception of Cu and Zn, the metals Pb, Hg, Cd, Cr, Mn, Mo, Ni and Co caused hepatic glycogenolysis (Gill and Pant, 1981; Goodman and Ishak, 1999). In present study, the occurrence of glycogen inclusions in the liver suggest that Cu may be eliciting biochemical stress in exposed fish and that elevated concentrations of Cu in sediments downstream from the abandoned mines is resulting in metabolic disorder in which food is being converted first into glucose then glycogen and stored in the liver but, the glycogen is not being converted back normally into glucose for distribution to the tissues. Decreased energy flow explains why reduced growth and increased mortality is common among individuals with GSD (Ishak and Sharp, 1979; Sherlock and Dooley, 1997).

However, its susceptibility to a number of toxic and the consequential metabolic disturbances cannot be overemphasized (Roberts, 1980; Olojo *et al.,* 2005). The high proportion of fibrotic tissue within the lobules and peribilliary connective tissue of the treated specimens indicate hepatic cirrhosis. It is thus believed that the most dramatic cirrhosis found in fish is the peribilliary cirrhosis of the hepato-renal syndrome associated with dietary toxicity (Anderson *et al.,* 1976). The most frequent, degeneration of hepatocytes observed. There was hepatocytes enlargement with large vacuoles and sinusoid conjection, pyknosis and karyolysis observed in cases of severe intoxication with pollutants (Jiraungkoorskul *et al.,* 2003).

The shrinkage of the hepatic cells can result in cirrhosis - the contracting of the blood vessels thereby greatly impeding the portal flow through the liver. The functions of the liver such as the conversion of glucose to glycogen for storage, regulation of lipids and deamination of amino acids are impaired. The blockage of the sinusoids makes the blood flow from the

hepatic artery and veins into the central vein rather difficult. The sinusoids widened to make up the right volume of blood in the central vein. The function of the canaliculi that forms the bile duct is hampered and as such, bile secreted from gall bladder cannot adequately get into them.

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