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SUGAR MILL EFFLUENT INDUCED HISTOLOGICAL CHANGES IN LIVER OF *CHANNA PUNCTATUS* (BLOCH.)

Suman Prakash	Department of Zoology, Agra College, Agra
Ajay Capoor	Department of Zoology, Agra College, Agra
H.N. Sharma	Department of Zoology, S.L.S., Dr. B.R. Ambedkar University, Agra

ABSTRACT

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 gonads. 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. The fish liver appears as does the liver of other vertebrates, as a key organ it controls many life functions and plays a prominent role in fish physiology, both in anabolism and catabolism including detoxification. The liver has an important function in the metabolism and biochemical transformations of pollutants from the environment, which inevitably reflects on its integrity by creating lesions and other histological alterations of the liver parenchyma or the bile duct. So it is necessary to study the toxic effects on liver.

INTRODUCTION

The liver is a detoxification organ and is essential for both the metabolism and excretion of toxic substances. 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 Lauren, (1990). The liver has the ability to degrade toxic compounds but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds and could subsequently result in structural damage. Similar studies on various fish species exposed to different toxicants showed histological changes in the livers of those specimens as recorded in the present observations. Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structures and functions. Histological analysis does however require the ability to discriminate between toxically induced lesions and normal variations in structure.

The Chhata Sugar Mills (Mathura) have been taken as a case study for this study purpose to justify the pollutional standards. The Chhata Sugar Mill was established in 1975 and the first production was started in 1977-78. It is situated at Agra-Delhi highway (NH-2), 84km from Agra on left side of the road. Initially the capacity of mill was 1250 T.C.D. which was enhanced to 2500 T.C.D. in 1992 and is maintained till now. Mill produces sugar while many waste products are effluxed from this process. Latter include baggase, final molasses, press cake or press mud, dirty water, oil gas, diammonium sulphates, urea, suspended solids etc. Baggase is used as fuel for burning, molasses is used for alcoholic purpose for which there storage are three tanks of 40000 liters capacity each in the mill, press mud is

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used as fertilizer in fields while dirty water is transferred to E.T.P. (Effluent treatment plant) which contains less oxygen with a capacity of 1000 hectoliters/day. In this plant animal faeces, urea and lime are used in purification which separate grease and oil while the residual water is tested for purity, if it is upto the mark for the use then it is transferred to a Nala to Nari Semri but if it is not good for any use then it is stored in the ponds of mill. For the above said purpose, the present study is designed to assess the effect of toxicants on this fish, on the basis of histology of liver.

MATERIALS AND METHODS

EXPERIMENTAL FISH: The air breathing teleost *Channa punctatus* (Bloch.) have been selected for the present investigation (Plate-1). Fishes were collected from Government Fish Farm, Laramada village, Agra and other local fresh water resources. The experiments were done at Research Laboratory of Zoology Department, Agra College, Agra.

MAINTENANCE AND FEEDING OF EXPERIMENTAL FISH: The experimental fishes *Channa punctatus* (Bloch.) were kept in clean large glass aquaria measuring 75 cms X 37.5 cms X 37.5 cms. The water, used for keeping fishes, was stored before one week to remove unfavourable gases. Dechlorinated water was used throughout the experiment. Fishes were kept in aquaria at the temperature ranging from 30^0C to 35^0C. The experimental fishes were acclimatized to the laboratory conditions for one week prior to experiment. The water of aquaria was changed every alternate day. The fishes were fed on readymade fish food. The food was given daily two times and feeding was disrupted 24 hours prior to the ***experiment.***

EXPERIMENTAL CHEMICAL: Sugar mill effluents collected from Chata Mill, Mathura which contains various organic and inorganic effluents was used for the histochemical experimentation.

TISSUE COLLECTION**:** The control and experimental fish; *Channa punctatus* (Bloch.) were killed under light chloroform anesthesia. They were dissected carefully and the liver was taken out for histological examination accordingly.

HISTOLOGICAL STUDY**:** All the tissues were fixed in the Bouin's solution. After washing and dehydration, the tissues were embedded in paraffin wax. The sections were cut at 5 micron and stained with haemotoxylin and eosin (Humason, 1979). Sections were examined under trinocular research microscope and photomicrographs were taken.

RESULTS AND DISCUSSION

The fish liver appears as does the liver of other vertebrates, as a key organ it controls many life functions and plays a prominent role in fish physiology, both in anabolism and catabolism including detoxification; Brusle and Gonzalezi (1996). The liver has an important function in the metabolism and biochemical transformations of pollutants from the environment, which inevitably reflects on its integrity by creating lesions and other histological alterations of the liver parenchyma or the bile duct. The liver is a detoxification organ and is essential for both the metabolism and excretion of toxic substances. 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 Lauren, (1990). The liver has the ability to degrade toxic compounds but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds and could subsequently result in structural damage. Similar studies

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on various fish species exposed to different toxicants showed histological changes in the livers of those specimens as recorded in the present observations.

Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structures and functions. Histological analysis does however require the ability to discriminate between toxically induced lesions and normal variations in structure. This is not always a simple task, even in mammalian pathology, which has a much more extensive database if compared to that of fish pathology; Hinton and Lauren, (1990). The present study also demonstrates that the liver of control fish exhibits a normal architecture and there were no pathological abnormalities. The hepatocytes present a homogenous cytoplasm and a large central or subcentral spherical nucleus. The histology showed that intoxicant caused some alterations of the liver parenchyma, like vacuolization and necrosis. The liver histological changes observed were more evident in fish exposed for longer time. These alterations are often associated with a degenerative-necrotic condition; Myers *et al*. (1987). Several studies had shown a variety of changes in the liver of *O. niloticus*, resulting from exposure to different toxic chemicals; Visoottiviseth *et al*. (1999) and Figueiredo-Fernandes *et al*. (2006a,b). Moreover, it was also reported by several studies that chronic intoxicant accumulation in the liver of fish causes hepatocyte lysis, cirrhosis and ultimately death.

A series of experiments by Sastry and Gupta (1978a) have shown that significant pathological changes were produced by 0.3 mg/L mercuric chloride in the liver of snake head fish *Channa punctatus* over a period of 30 days. Histological changes included hepatocellular granulation and vacuolation of the cytoplasm, hypertrophy of the nucleus, necrosis, fatty infiltration, proliferation of connective tissue, glycogen depletion and cirrhosis. Dixon and Leduc (1981) studied the chronic cyanide poisoning of rainbow trout and its effects on the growth, respiration and liver histopathology. Juvenile rainbow trout *Salmo gairdneri* (Richardson) exposed to concentrations of 0.01, 0.02, or 0.03 mg/L hydrogen cyanide for 18 days showed widespread degenerative necrosis of hepatocytes at all concentrations tested. Hepatic damage has to be severe and diffused to result in the liver failure as the hepatic reserve is large. Liver toxicity does, however, result in altered fat metabolism and digestion, thereby resulting in generalized stress of the fish; Klaassen, (1996). Recent evidence indicates that increases in oxidative stress and associated biochemical alterations could be a toxic mechanism in fish exposed to pulp mill effluent; Oakes and Van Der Kraak, (2003). Also, high amounts of ions especially nitrogen and potassium) in wastewater have drastically changed the water chemistry in water bodies, and affected species composition, the food chain structure and fish health. Despite high fish population densities, histology of liver indicates unfavorable environmental conditions for individual fish under stress of effluents; Tkatcheva *et al*., (2002).

Anthony et al. (1986) studied the effects of sublethal concentrations of diazinon on degeneration of the liver, they noticed lesions in the liver of *Clarias* and enlargement of mucous cells. Histological changes in the liver of *Tilapia mossambica* after exposure to the organophosphate monocrotophos were reported by Desai *et al*. (2004) while Armstrong and Millman (1974) observed acute toxicity of sevin in histopathology of *Macoma nasuta* at the initial stage of intoxication, necrosis and vacuolization of hepatocytes were recorded, while fatty degeneration was observed later on. Sakr *et al*. (2001) studied the effect of the organophosphorous insecticide; hostathion on the liver of the catfish; *Clarias gariepinus*, their results showed that this insecticide produced histological changes in the liver

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represented by liver cord disarray, cytoplasmic vacuolization of the hepatocytes, damage of blood sinusoids, blood vessel congestion and inflammatory leucocytic infiltrations.

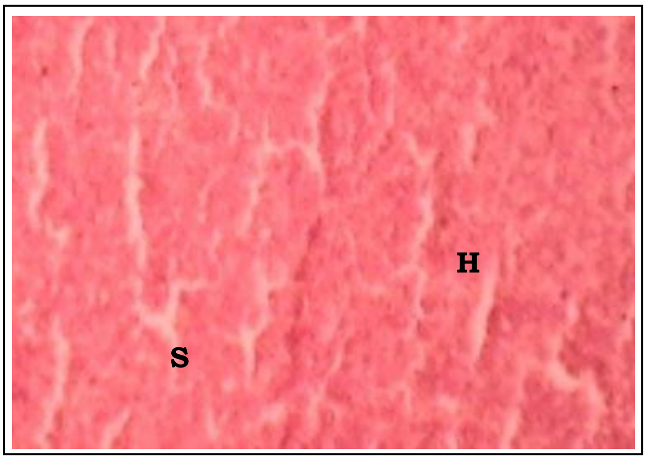

Plate-1a: Control

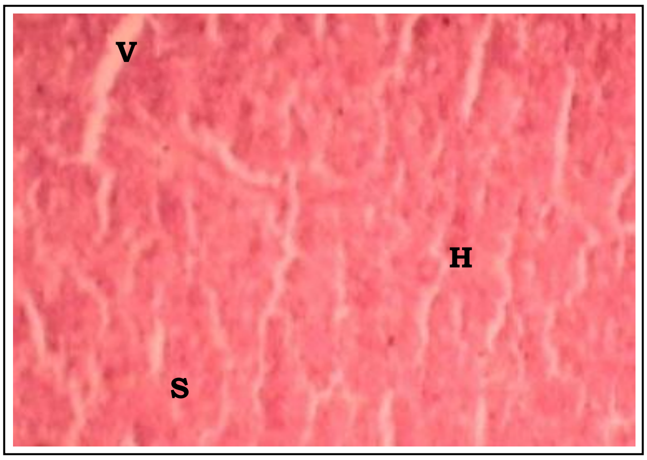

Plate-1b: 24 hrs

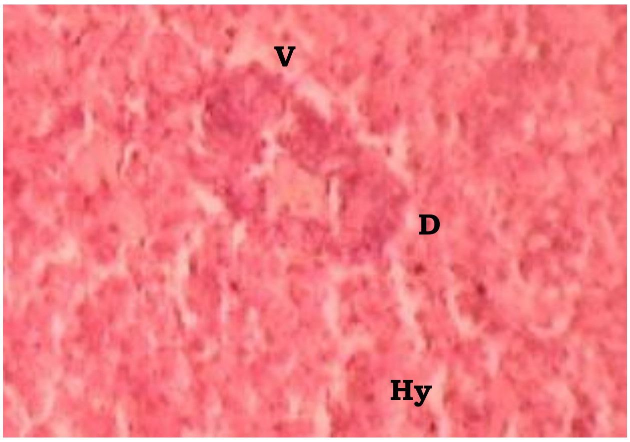

Plate-1c: 48 hrs

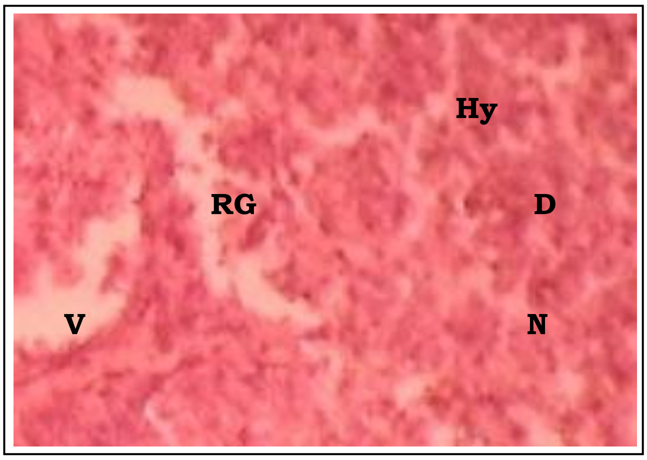

Plate-1d: 72 hrs

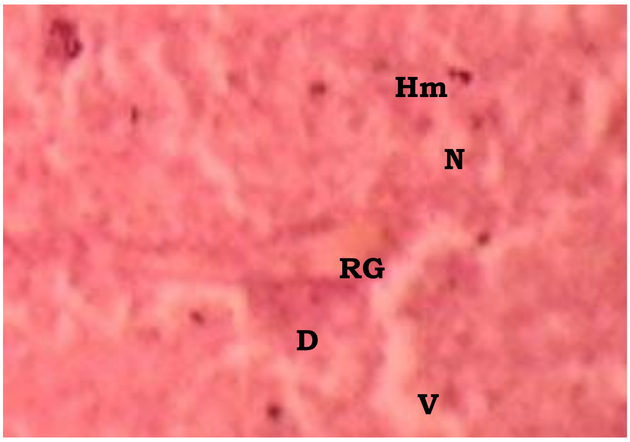

Plate-1e: 96 hrs

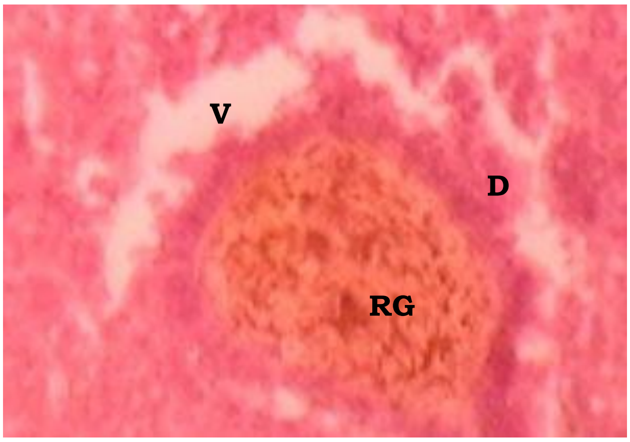

Plate-1f: 1 week

S-Sinusoidal space; H- Hepatocyte; V-Vacuolization; D- Degeneration; Hy-Hypertrophy;

RG-Reduced glycogen; N- Necrosis; Hm- Haemorrhage

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They also reported the marked reduction in glycogen content in the hepatocytes of *Clarias gariepinus*. These results are in agreement with those of Reddy *et al.* (1991) who reported that fenvalerate altered glycogen metabolism in liver and muscles of *Cyprinus carpio*. Singh and Srivastava (1981) mentioned that carbohydrates decreased as a result of exposure to a sublethal concentration of a mixture of aldrin and bromophenol blue, these insecticides induced marked diminution in the glycogen content of the liver and muscles of this fish, these results agree with those of Tripathi and Verma (2004) who recorded the exposure fish *Clarias batrachus* to fenvalerate. Reddy *et al* (1991) also found that total, structural and soluble proteins were decreased whereas the free amino acids and the activities of protease, aspartate aminotransferase and alanine aminotransferase significantly increased in fenvalerate exposed fish; *Cyprinus carpio*, the reason of reduction in protein content in liver of fenvalerate-exposed fish might be due to either arrested metabolism in the liver or to use it to build up new cells or enzymes to reduce the stress. In conclusion, the present study proved that sugar mill effluents affected the structure and histochemical contents of the liver of *Channa punctatus (Bloch.)* and this effect was time-dependent.

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