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HISTOPATHOLOGICAL CHANGES IN GILLS AND LIVER OF *CIRRHINUS MRIGALA* FINGERLINGS EXPOSED TO BARIUM CHLORIDE

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ABSTRACT
The Indian Carp *Cirrhinus mrigala* fingerlings were exposed to different concentrations of barium chloride for 96 hours and the LC_{50}value for the exposure period was noted as 400 ppm.The mortality of fingerlings increased with the increase in concentration of barium chloride. The effect of sub lethal concentration in gills and liver of *Cirrhinus mrigala* was investigated. The behavioural changes seen in fingerlings were erratic swimming, loss of equilibrium, bubble formation and swimming towards the water surface. The gills and liver were removed for histological examination. The results showed hyperplasia of secondary lamellar epithelium, fusion of secondary lamellae and clavate lamellae in gills whereas in liver it showed cytoplasmic vacuolation in the hepatocytes and infiltration of R.B.C's in the central vein.

KEY WORDS: *Cirrhinus mrigala*, Barium chloride, LC_{50}, Histology, Gill, Liver.

INTRODUCTION

In our day to day life we often come across many types of pollution such as water, air, thermal and noise pollution etc. Of all the various types of pollution, water is considered important since it can affect not only aquatic organisms but also man. Water pollution is defined as any water body whether fresh or marine that is adversely affected due to the addition of large amount of unwanted materials. Aquatic water bodies are most often polluted with various potentially hazardous substances (Battaglin and Fairchild, 2002). Recently it has brought increasing concerns for potential adverse ecological health effects resulting from the production, use, and disposal of numerous chemicals that offer improvement in industry, agriculture, medical treatment and even household conveniences (Daughton and Ternes, 1999). Heavy metals are one of the main components of industrial effluent which along with other products from industries are discharged into the aquatic environment and it is toxic to aquatic life (Korai *et al.*, 2008).Fish are more susceptible to aquatic pollution caused by toxic substances. The main organs affected are gills, liver, kidney, intestine and gonads. In all these organs there is an architectural and structural alteration, necrosis of epithelium and plasma alterations etc. There can also be tumor formation (Benign or malignant tumor) in gills, liver and kidney of fish (D. Bernet *et al.*, 1999).

Barium chloride is one such industrial effluent. It is one of the water soluble salts of barium. Barium chloride is widely used in the purification of brine solution, in the manufacture of heat treatment salts, pigments, other barium salts and impart bright green colour to fireworks (ATSDR, 2007).This study investigates the toxicity of barium chloride on *Cirrhinus mrigala.* The main objective of this study was to determine the acute pathological effect of barium chloride.

MATERIALS AND METHODS

Live 150 healthy fingerlings of *Cirrhinus mrigala* of mean weight 4.502 grams were collected from Manjri Fish Farm in Hadapsar using net and brought to the laboratory where they were cleansed with dilute $KMnO_4$ to ward off any skin infection. The fingerlings were then kept in glass tanks containing dechlorinated tap water for 15 days for acclimatisation. The water was constantly aerated and the fingerlings were fed regularly with commercially available fish food. Before the start of experiment aquaria water was analysed according to the procedure outlined in Standard Method for Examination of water at public Health laboratory, St. Mary's School, Pune. The physico-chemical characteristics such as, pH, alkalinity, turbidity, hardness were checked and results were recorded. (Table 1).

LC_{50} was determined using four different concentrations of barium chloride (200 ppm, 300ppm, 400ppm, 500ppm).for 96 hours. At the end of 96 hours LC_{50} value was 400 ppm. (Table2). All data obtained in the test were analysed using Finney's method (Finney, 1971).

To understand the impact of low concentration 100 ppm was selected as the sub lethal concentration of Barium chloride.4 litre glass tank containing 10 healthy live fingerling of same size and weight in 3 litre of acclimatized water was kept for 24 hours before the experiment. After 24 hours, 100 ppm of Barium chloride was added to the tank and stirred with glass rod. One glass tank was kept as control that contained acclimatized water and fingerlings but no Barium chloride. No aeration and food were fed to fish during this experimental period. Both control and experimental fish were sacrificed by decapitation after every 24, 48, 72 and 96 hours. After every 24 hours, the test solution was changed and fresh test solution was added. Immediately after sacrificing the fingerlings, the gills and liver was removed, and cut in required size for fixation in Bouin's fixative for 24 hours and processed for routine histological examination using standard histological techniques .Similar procedure was followed for. 24, 48, 72 and 96 hours. Haematoxylin- Eosin stained slides were examined under the microscope and then photographed.

RESULTS

The behavioural changes seen in fingerlings during the main experiment were up and down erratic swimming with bubble formation at the water surface. There was loss of equilibrium and rapid opercular flap movement

Table 1. Values of Physico-Chemical Parameters of Water:

S.R.No	Parameters	Value
1.	Alkalinity	14.6mg/l
2.	pH	7.1
3.	Turbidity	0.82 NTU
4.	Hardness	16mg/l

Table 2. Mortality of fingerlings of *Cirrhinus mrigala* exposed to different concentrations of Barium chloride for LC_{50} for 96 hrs.

S.R NO.	Concentration of pollutant ppm	No of fish	Total fish dead	Percent mortality	Log_{10} concentration	Probit
1	200	10	2	20%	2.30	4.48
2	300	10	4	40%	2.47	4.75
3	400	10	5	50%	2.60	5.00
4.	500	10	9	90%	2.69	6.28

Histopathological changes were seen in the gills and liver of Cirrhinus mrigala fingerlings exposed to Barium chloride (Table 3 and 4).

Table 3: Histopathological changes in gills of *Cirrhinus mrigala* fingerlings exposed to 100 ppm of Barium chloride

Histopathological changes	Exposure Time in hours				
	Control	24 hours	48 hours	72 hours	96 hours
Hyperplasia in secondary lamellae	-	+	+	+	+
Hyperplasia in secondary lamellae resulting in fusion of secondary lamellae	-	-	++	++	++
Clavate lamellae seen in secondary lamellae	-	-	-	-	+

Legend: -Absent + Present++ Present in large numbers.

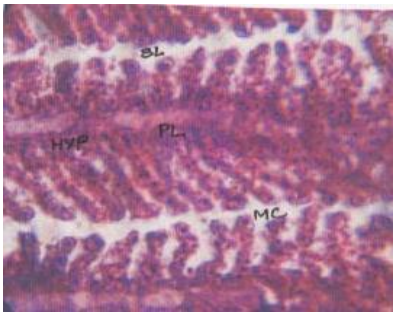
Table 4. Histopathological changes in liver of *Cirrhinus mrigala* fingerlings exposed to 100 ppm of Barium chloride

Histopathological changes	Exposure Time in hours				
	Control	24 hours	48 hours	72 hours	96 hours
Cytoplasmic vacuolation in hepatocytes	-	+	++	+++	++++
Infiltration of R.BC's in the central vein	-	-	+	+	+

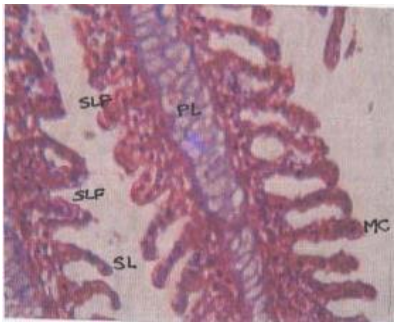
Legend-Absent + Present++ Present in large numbers

Plate1: Sagital section of Gills.

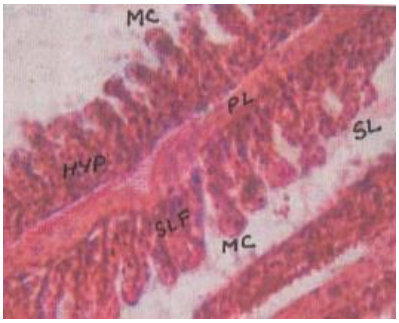
Control gills (10x45X)



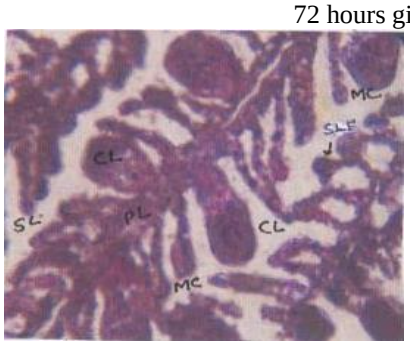
24 hours gills (10x45X)



48 hours gills(10x45X)



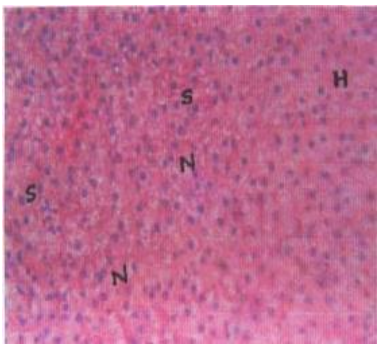
72 hours gills(10x45X)



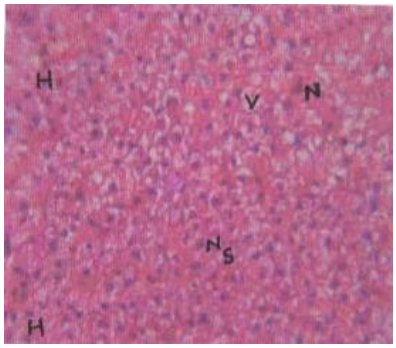
96 hours Gills (10x45)

Legend: *PL: Primary lamella, PCN: Pillar cell nucleus, MC: Marginal SL: Secondary lamella, CL: Clavate lamella cells, HYP: Hyperplasia, SLF: Secondary lamellar fusion.*

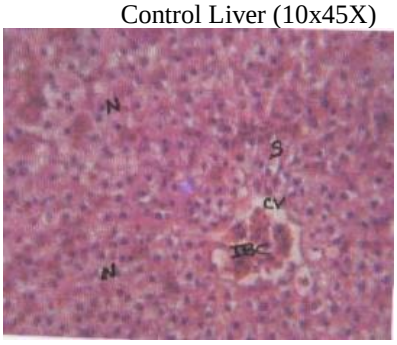
Plate2: T.S. of Liver.



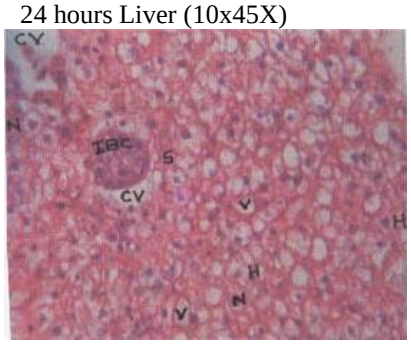
Control Liver (10x45X)



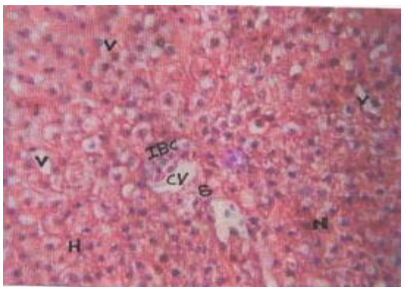
24 hours Liver (10x45X)



48 hours Liver (10x45X)



72 hours Liver (10x45X)



96 hours Liver (10x45X)

Legend: *N: Nucleus, H: Hepatocytes, S: Sinusoids, CV: Central Vein, V: Vacuoles, IBC: Infiltration of blood cells.*

DISCUSSION

In today's so called modern world urbanisation and industrialisation have raised the man's economy by various ways. But at the same time chemical pollutants released from industries have been a major cause of water pollution (EI-Demerdash and Elegamy, 1996). Aquatic organisms such as fish are most commonly affected by water pollution. The effect of Barium chloride was seen on fingerlings of *Cirrhinus mrigala* exposed to 100 ppm of Barium chloride. During the period of exposure various behavioural changes such as swimming towards water surface, bubble formation at the surface, erratic swimming and loss of equilibrium were observed.

GILLS

Histopathological effects of barium chloride were seen in the gills of *Cirrhinus mrigala* fingerlings exposed to barium chloride. Gills remain in close contact with external environment and are particularly sensitive to changes in the quality of water. They naturally become primary target of contaminants (Marina Camarago M.M and Martinez,2007). When *Cirrhinus mrigala* fingerlings were exposed to 100 ppm of Barium chloride for 24 hours, the treated gills showed proliferation of secondary lamellar epithelium indicating the start of hyperplasia of epithelial cells (Plate1). Hyperplasia leads to impairment of respiratory function by affecting diffusion of gases. Similar hyperplasia of epithelial cells was reported by Ashley (1970) when he studied the action of iron salts on gold fish. Jon .J Mallatt (1985) exposed fish to

other toxicants. As the duration of the exposure increased to 96 hours more hyperplasia of epithelial cells was seen resulting in fusion of secondary lamellae. As a result of fusion of secondary lamellae there is decrease in the total respiratory area of gills resulting in low oxygen uptake. Similar lamellar fusion was reported by Banerjee and Chandra (2005) when he had exposed air breathing "murrel" to zinc chloride. Clavate lamella was also seen in many of secondary lamellae. In clavate lamella there is excessive swelling of artery involving breaking of pillar cell system leading to loss of blood channels. Similar clavate lamella was reported by Mazon *et.al*, (2002) when he had exposed *Pronchilodus scrofa* to lead and copper. Hanan (2007) when exposed Nile Tilapia to cadmium also found similar clavate lamella.

LIVER

Histopathological changes were also seen in the liver of *Cirrhinus mrigala* fingerlings. Liver is a detoxifying organ. All the substances which are absorbed by the gastrointestinal tract pass through the liver. In the process of detoxifying chemical pollutants hepatocytes may get damaged. When fingerlings of *Cirrhinus mrigala* were exposed to 100ppm of barium chloride for 24 hours, few vacuoles could be seen in the hepatocytes (Plate2). Similar findings were also reported by Sorenson *et.al.,* (1984) when he treated fish from Belews Lake to Selenium. As the duration of the exposure increased to 96 hours, more hepatocytes showed cytoplasmic vacuoles along with the infiltration of R.B.C's in the central vein of liver. Similar infiltration of R.B.C's in the central veins of liver was reported by Ravindrababu *et.al.,(*2007) when they exposed fish to ammonia. Tripathi M. Mishra, *et.al.,(*2011) when they exposed *Catla catla* to 1.2% lindane also found similar infiltration of R.B.C's.

CONCLUSION

It can thus be concluded that industrial effluents contribute a lot to water pollution by affecting the aquatic flora and fauna and if its release is not controlled it can result in the destruction of aquatic ecosystem. It has therefore become a need to protect the integrity of our environment in the 21st century.

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