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ORIGINAL ARTICLE

IMPACT OF INDUSTRIAL EFFLUENT ON HISTOPATHOLOGICAL CHANGES IN LIVER TISSUE OF *ARIUS MACULATUS*

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ABSTRACT

In the present investigation, *Arius maculatus* were exposed with sub lethal concentration industrial effluents from Cuddalore District. The histopathological changes were observed in the liver tissues of *Arius maculatus* after exposure with industrial effluents (2% and 6%). The present study shows that pycnotic nuclei, irregular hepatocytes and vacualization were observed in effluents trreated fish, *Arius maculatus*

Keywords: Industrial effluents, Histopathalogy,liver, *Arius maculatus*

1.INTRODUCTION

In the present investigation, *Arius maculatus* were exposed with sub lethal concentration industrial effluents from Cuddalore District. The histopathological changes were observed in the liver tissues of *Arius maculatus* after exposure with industrial effluents (2% and 6%). The present study shows that pycnotic nuclei, irregular hepatocytes and vacualization were observed in effluents trreated fish, *Arius maculatus*

Pollution of estuaries is difficult to assess because of the special qualities of this ecosystem; estuarine pollution is different from river pollution as the pollutants remain trapped in the ecosystem for a long period due to tidal isolation; and pollution damage of estuaries is the product of man's as well as nature's activities. Effluent and run off from fields comprising chemicals of versatile nature, exert their toxic effects on fish population by depleting the dissolved oxygen, altering the pH, salinity and changing the carbondioxide content (Sankaran *et al.,* 2011) thereby directly or indirectly affecting the life cycle as well as the metabolic pathways of the fish at the biochemical level (Puvaneswari *et al.,* 2009). Man- made pollution is perhaps the biggest threat to the estuaries, in many instances; estuaries are being used as “sewers and sinks” for untreated waste water..

Contrary to the opinion that the seas are bottomless pits, man is now realizing that they have very specific ocean floors and a limit to the quantum of solute they can hold. Water pollution is now proving to be one of the main causes for public health hazards. Most of the Indian rivers are seriously polluted by industrial effluents carrying toxic chemicals which bring about death or sublethal pathological changes particularly in liver, kidney, respiratory, reproductive and nervous tissues of the aquatic animals (Singh *et al.*, 1997; Purushothaman and Chakrapani, 2007; Pandey *et al.*, 2008).

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In recent years, there has been a global awareness for increased fish production under natural and culture conditions in the vicinity of coastal estuaries which could constitute one of the most valuable and vulnerable natural resources of a nation's economy (FAO, 1986). Indeed estuary forms an ideal buffer zone with productive water for the inhabitation of diverse fauna of lotic and marine origin.The priority list of pollutants compiled by the Environmental Protection Agency of United States contains the eight more widespread heavy metals such as arsenic, chromium, cadmium, copper, lead, mercury, nickel and zinc (Moore and Ramamoorthy, 1984), of which the last six are generally known as "toxic heavy metals" (Dara, 1997), while cobalt, manganese, molybdenum and selenium as "essential heavy metals." According to George (1987) aluminium, arsenic, antimony, cadmium, chromium, copper, iron, manganese, mercury, lead, uranium and zinc are more toxic to the aquatic system

Fish is an important as well as cheap source of animal protein and hence farming of fish has become imperative to meet the growing demand for protein worldwide. In recent years in India too, intensive aquaculture practices are on the increase, and aquaculture programmes at present largely depend on riverine and estuarine seed resources (Meehan, 2002). Wide variable effects of different toxicants on a given species (Dalela, 1977; Savinov *et al.,* 2003) and the variable effects of the given toxicants on different species (Ruangsomboon and Wongret, 2006) warrants, no generalization be made on the toxic effects of disposed industrial wastes surrounding aquatic environment. Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish (Tulasi *et al.,* 1992; Kumaresan and Karuppasamy, 2011b). The fishes which are largely being used for the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits *et al.,* 2004; Lopes *et al.,* 2001).

2.MATERIALS AND METHODS

Arius maculatus was collected from three different stationsat Uppanar estuary and they were immediately transferred to ice box.

From the experimental station of Uppanar estuary, fish were caught either using cast net or hand lines. Cast net are made of nylon. The mesh varies from 10 to 15 mm. Hand lines with mono filament and hooks were used with prawn or mussel as bait for the capture of *Arius maculatus.* The fish were transported to the laboratory and maintained in the same way as the control fish collected from Perumal Lake. The water was renewed once in two days.

Procurement of fish

Live specimens of *Arius maculatus* with an average length of 8.5 ± 0.50 cm and weight of 15.0 ± 0.5 g were collected from Uppanar brackish water by operating cast net. The fish were acclimatised in the aquaria of 120 litres capacity containing well aerated sea water (salinity 28 ‰; pH 7.69; oxygen content 4.32 mg/l) and water temperature (32.6°C) for a period of one week prior to experiment. During acclimatization, the fish were fed withchopped prawn and clams. Food was withheld one day before the' commencement of the experiment. The water was changed along with waste feed and faecal matter every 24 hours. Fish collected from Perumal lake (Plate 1A) were used as control and Uppanar brackishwater area was selected as experimental site (Plate 1B).

Acute Toxicity Test

The raw and partially treated effluent was collected from the discharging point of industries surrounding the Uppanar eaturry for acute toxicity test. In the acute toxicity bioassay, mortality could be observed within a short period. LC_{50} was calculated by the following method of Finney (1978) to observe mortality and behavioural response of the test fish, *Arius maculatus* on exposure to effluents of different concentrations.

Static acute toxicity was employed to evaluate the adverse effects of industrial effluents surrounding the Uppanar estuary on the fish, *Arius maculatus* under standardised laboratory conditions.

Food was withheld one day before the toxicity test with a view to avoid the possible change in the toxicity of the pollutants after addition of the effluent into the test tank with 100 litres of sea water having 10 fishes. Mortality was recorded after 24, 48, 72 and 96 hr and five replicates were maintained simultaneously for the purpose. Fishes showing respiratory and lack of response to tactile stimuli were considered, nearing dead and removed immediately. Percentage mortality was calculated and the values were subjected to Probit analysis (Reddy *et al.*, 1992). Confidential limits (upper and lower) of the Regression coefficient with Chi - square test were calculated.

Design of sublethal toxic study

Sublethal studies are helpful to assess the response of the test organism to stress caused by the effluents. Based on acute toxicity test two sublethal concentrations (2% and 6%) on *Arius maculatus* were derived and used as the experimental concentrations. Sublethal of safe level concentration were derived from 96 hr LC_{50} value.

In the present study 2% (1/50) and 6% (1/30) dilution (in *Arius maculatus*) of the 96 hr LC_{50} were selected as sublethal concentrations. The experimental fish were exposed in each concentration for a period of 7, 15 and 30 days. A control batch corresponding to each test group was maintained simultaneously.

Light Microscopy

At an interval of 7, 15 and 30 days, a fish from each concentration (2% and 6%) of the effluent was sacrificed and the tissues of gill, liver, kidney, muscle and intestine were excised out and subjected to standard histological technique of Culling (1957).

Each tissue was fixed separately in aqueous Bouin's fluid for histological examinations. After fixation, the tissue were dehydrated in ascending series of alcohol, cleared in methyl salicylate and embedded in paraffin wax. Serial sections were taken at 5 to 8 µ thickness and stained with Ehlrich's haematoxylin with aqueous eosin as the counterstain.

3.RESULTS

The light microscopy revealed a mass of parenchymal cells (hepatocytes) characterized by hexagonal or polygonal contour with distinct nucleus and dense cytoplasm and it was more prominent in the control sample (Plate 35-A). The plasma membrane appeared continuous with collagenous matrix. Some of the cells were characterized with 2 nuclei and vary greatly in morphology. The intensity of cytoplasm was however, usually much lesser than that of nucleus reflecting the lower concentration of nucleic acid. The cytoplasm also revealed lipid droplets and appeared as unstained vacuoles.

Contrastingly the liver cells exposed to 2% concentration of effluent for 7, 15 and 30 days (Plate 36 - A, C and E) and 6% concentration exposed to the same periods (Plate 36- B, D and F) disclosed remarkable variation in the structural features. Between the two concentrations, 6% influenced more hepatopathy than 2%; similarly 15 days of exposure showed more variation in the histological organization. In both the concentrations, dead and dying cells were a common finding in the parenchymatic tissues which arose as a result of normal cellular turn over. 15 days exposure to 6% concentration of effluent showed phenomenal damage to the hepatocytes causing a void in the organ (Plate 36-D).

The term necrosis or cell death was a common feature observed when it was exposed 2% and 6% of effluent for 15 days (Plate 37-C and D). In the case of 30 days of exposure at 6% concentration the liver was characterized by complete destruction of cytoplasmic and nuclear material and vacuolization of the hepatocytes and blood sinusoids. Consequently nuclear changes were the most characteristic feature of necrosis. It was reflected by the first sign of pyknosis, characterized by the condensation of chromatin to small densely staining mass. Later the nuclear material became fragmented; a process called karyorrhexis and ultimately broke down with loss of the nucleus altogether (Karyolysis) (Plate 36-F).

Furthermore, due to the intoxication of the effluent, denaturation of cytoplasmic protein resulted and made the cytoplasm amorphous. When the intoxication of the effluent was more, as it was evident in the field exposure of the fish (Plate 36-B). In the effluent habitat for a period of 30 days revealed the dissolution of cell membrane. Consequently the cellular outline became increasingly difficult to distinguish leading to cytolysis and progressive death of the constituent cells, the hepatocytes.

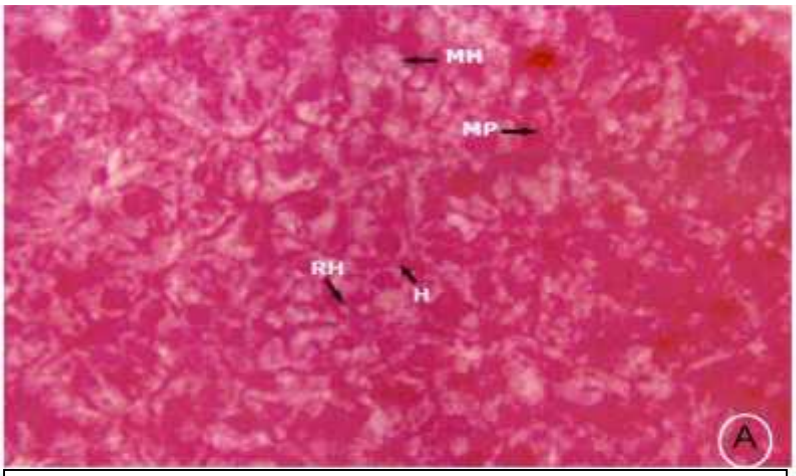
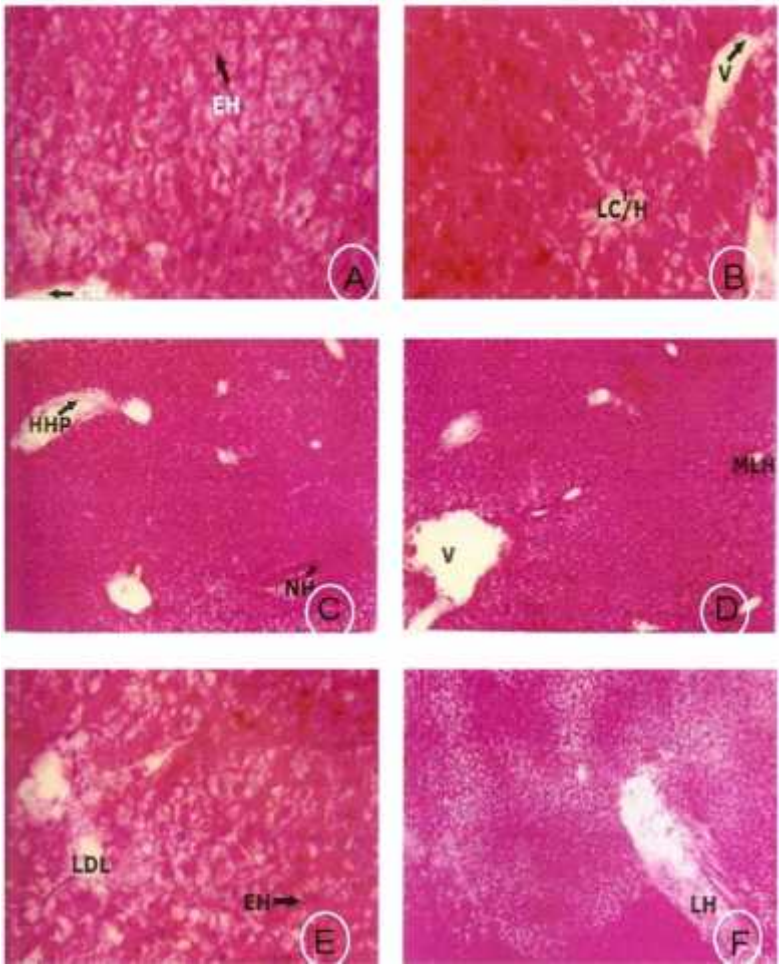


Fig. 1 shows histology of liver tissues in control fish

Light micrographs on the histopathology of the liver of *Arius maculatus* exposed to 2% (A, C & E) and 6% (B, D & F) sublethal concentrations of the effluent for an exposure period of 7 days (A&B); 15 days (C&D) and 30 days (E&F) (360X).



4.DISCUSSION

Histological structures of liver of the estuarine fish *A. maculatus* are almost similar to those described for a number of freshwater teleosts namely, *Siganus rivulatus* (Abdel-Azin *et al.,* 2006), *Carassius auratus* (Olojo *et al.,* 2005), *Oreochromis mossambicus* (Naidu *et al.,* 1983), *Salmo salar* and *S. gairdeneri* (Roberts, 1989), *M. cephalus* (El-Bakary *et al.,* 2010).

On exposure to the effluent the liver of *A. maculatus* exhibited several pathological changes including hyperplasia, degeneration of blood vessels, vacuolization, hypertrophy, pycknotic nuclei, necrosis splitting lesion, hepatocytes, congestion of hepatic tissue and accumulating of blood vessels (Langiano and Martinoz, 2008).

The present study closely agrees with a similar report by Kothari and Suneetha (1990) in the liver of catfish exposed to zinc sulphate. The histopathological alterations observed, were necrosis, degenerative changes and infiltration of blood cells into the hepatocytes. The vacuolation, necrosis and appearance of some typical globular bodies in *Punticus conchonicus* by Atamanap *et al.* (2008), Koehler (2004) due to zinc toxicity have been reported. Disintegration and necrosis in *Cyprinus carpio* (Wong *et al.,* 1977) and reduction in the size of fish liver (Singh and Sivalingam, 1982;Bunton *et al.,* 1991) have been reported.

Earlier workers have revealed similar destructive changes in the liver as seen in *A. maculatus.* Zhou *et al.* (1999) revealed that the ultrastructural changes in the liver were characterized by severe enlargement of hepatocytes. The effect of mercury on *Channa punctatus* showed vacuolization of hepatocytes, focal necrosis and rupture of cell membrane, damage of connective tissue and enlargement of intercellular spaces in the liver. Necrosis was observed in the hepatic cells during the present work. Similar results were also seen in *O. niloticus* (Vicentini *et al.,* 2005).

Liver is the most important organ in vertebrate body as it metabolites the digested food materials and detoxifies the toxicants carried by the portal blood circulation (Leeson and Leeson, 1981). Liver also acts as a sensitive index to toxic substances (Cough, 1975). Many reports indicate that it is the organ with highest concentration of toxicants and with the greatest damages of impairment (Isairasu and Haniffa, 1990). Therefore, it becomes necessary to study the effects of industrial effluent on liver as it provides a sensitive index to the virulence of the toxic substance employed.

The liver has a high concentration of xenobiotic metabolizing enzyme, some of which activate the toxicants to induce leisions locally (Lu, 1985). Toxicants induced changes in the liver tissue of fishes (Coach, 1975). The important abnormality noticed in the liver of *Tilapia mossambica* after the exposure of sub-lethel concentration of arsenic chloride are the necrosis of hepatocytes, loss of adhesiveness between the cells, enlargement nucleus, vacuolization, karyolysis and swelling of liver cards. The similar results were also made by Bonoard Hasan (1990). Jagadeesan (1994). Ramalingam and Reddy, (1981) have reported the shrinkage of nucleus and intra cellular vacuolization in colisc labia when treated with lindane. Jagadeesan (1994) has also observed the necrosis and shrinkage of nucleus in mercury treated *Labeo rohita.*

The necrosis of lepato cytes, cytoplasmolysis, swelling of liver cord and nuclear enlargement are the important histopathological abhormalities observed in the present investigation. The same results were also reported by Nagarthamma and Ramamoorthy (1982); Jayanti Rao *et al.,* (1983); Gupta and Rajbanshi (1979). The histopatholgical abnormalities observed by several workers in different fishes exposed to various metals have deleterious effects which leads to the dysfunction of the liver. Since liver is one of the major sites of metabolic activities, the toxic effects of cadmium are more pronounced and cause malfunction resulting in the altered metabolisom of liver in *Tilapic mossambica.*

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