EVALUATION OF POTENTIAL BIOMARKERS FOR EFFLUENT INDUCED HEPATOTOXICITY

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Abstract

The study was conducted to investigate the histo pathological and biochemical studies of the liver of Mystus tengara. The effluents were collected from non point source of River Chambal and the chemical parameters assessed .The COD, BOD, TSS, TS and TDS were much higher than the standard quality. The toxicity of these effluents on Mystus tengara and the LC 50 values were determined. The liver showed vacuolar degeneration, focal areas of necrosis and fibrosis, and aggregations of inflammatory cells . The experimental fishes revealed gradual decrease in albumin and INR. RBC.Plasma level of glucose was also lower in the exposed fish when compared to the control. However high levels of ASP and ALT were observed. In conclusion, the changes observed indicate that hematological and biochemical parameters can be used as an indicator of toxicity related stress in fish exposed to effluents.

Keywords Effluent, AST, ALT, INR, hematology, Biomarkers

I. INTRODUCTION

Municipal effluents have been shown to contain a cocktail of endocrine disrupting chemicals (EDCs).Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissue, directly from water through respiration and also through their diet. Fish are frequently subjected to pro oxidant effects of different pollutants often present in the aquatic environment.

Discharges of metal effluents into rivers may cause deleterious effects to the health [1]. Fish contaminants can reach man through the food chain [2]. Metals are released to the environment by both natural processes and anthropogenic sources, [3]. Like in man, changes in the blood parameters of fish, which occur because of injuries or infections of some tissues or organs, can be used to determine and confirm the dysfunction or injuries of the organs or tissues. Many studies have demonstrated changes in blood variables such as hemoglobin, haematocrit, ESR, RBCand WBC used as indicators of disease, stress and presence of contaminants. [4], [5].The biochemical hub of the body, the liver, has a variety of transaminases to synthesize and breakdown amino acids and to interconvert energy storage molecules. The concentrations of these enzymes in serum are normally low. However, if liver is damaged the hepatocyte cell membrane becomes more permeable and some of these enzymes leak out into the blood stream. In general, any damage to the liver will cause elevations in transaminases [6]. Biochemical measurements have been referred to as biomarkers and, in the majority of the studies, fish have been used as test organisms. Limited knowledge is available on fish's physiological responses to muncipal effluents as compared to effluents from mechanical pulp production [7]. In toxicological studies of acute exposure, changes in concentrations and enzyme activities often directly reflect cell and organ damage in specific organs. [8]

Previous studies reported that exposure of fish to pollutants (industrial, agricultural and sewage) resulted in several pathological and biochemical alterations in different tissues of the fish. The liver, as the major organ of metabolism comes into close contact with xenobiotics absorbed from the environment and liver lesions are often associated with aquatic pollution. Therefore, the present study aimed to investigate the impact of the environmental conditions of Chambal River on biochemical and histological structures of liver and kidney of *Mystus tengara*

II. MATERIAL AND METHODS

A. Study Area

Chambal River in Nagda is very close to tropic of cancer at 23'27 N and 75'25 and 517 meters above MSL. More than one lakh of residents in and around the Nagda rely on water from Chambal River for public and industrial use. Waste after coming from the factory complex runs in a channel for about 1 km and finally joins with municipal channel which runs for 2 km and finally joins River Chambal near Juna Nagda.

B. Sampling

Sampling: Effluents were collected in sterilized phosphate free cleaned polythene bottles, near Mukteswar temple at Juna Nagda where discharges of industrial complex and domestic waste are drained into this station. The samples after collection were immediately placed in dark boxes and processed for physico chemical analysis like pH, electrical conductivity(EC),total dissolved solids (TDS), total hardness (TH) dissolved oxygen(DO),Biochemical oxygen demand (BOD) and chemical oxygen demand(COD).The procedure for analysis followed 'Standard methods of analysis of water and waste water(9).

C. Experimental design

Living healthy specimens of fishes were collected from local fresh water sources and acclimatized for lab conditions. Some fishes were used for the determination of LC50 value and it was found to be 35 %.(96h).Acclimatized fishes were divided into 3 groups. GroupI control, Group II and Group III were treated with 6.25 and 12.5% doses respectively. The experiment was conducted for 28 days. All experimental groups were maintained under similar laboratory conditions. At the end of the treatment period the control and treated fishes were dissected and liver was processed for histological and blood was processed for biochemical studies.

- **Histological Studies:** For liver histology, liver tissue sample was fixed in Bouin's solution for 24 hours and transferred to 70% ethanol. The samples were kept in ethanol before dehydration and embedding in paraffin and sectioning (6 um). Tissue samples were stained with hematoxylin-eosin Histological structure of the liver was evaluated under a light microscope and photographed using digital camera (Sony, 14.1MP, Japan) attached to the microscope.

- **Hematological studies**: After the completion of the experiment blood was collected from caudal vessels with a disposable syringe using containing ethylenediamine tetra acetic acid -potassium (EDTA-K2) as an anticoagulant. Blood hematocrit (packed red blood cell volume) was determined immediately with Remi centrifuge. The percentage of hematocrit was determined by the use of a micro capillary reader. Erythrocytes (RBC) were counted immediately after blood collection in hemocytometer (Improved Neubauer. Weber scientific Ltd.) according to [10].Hemoglobin concentration was measured by the cyanmethaemoglobin method [11] using a commercially available kit (Span, India). Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and Mean cell volume (MCV) were calculated using the formulae mentioned by [12].

- **Biochemical studies**: Serum ALT and AST activity was assayed following the modified International Federation for Clinical Chemistry(IFCC) method laid down in monoenzyme kits (Siemens Diagnostics,Ltd, India). Total plasma glucose concentration was determined using commercial kits (Siemens Diagnostics, Ltd, India).

- **Coagulation Test** The blood in the tube was maintained at room temperature for coagulation. (INR) The International Normalized Ratio (INR) was measured on the basis of blood clotting time.
  Statistical analysis: Student's' test was applied to observe the significance of difference between control and experimental groups [13].

III. RESULTS

A. Water analysis

A summary of physico chemical parameters obtained in Chambal River are shown in Table.1. Results clearly indicate that the physico chemical parameters showed high levels of pH, temperature, low DO, alkalinity, hardness and high turbidity and exceed the limits WHO standards. This must have been as a result of the nature of effluents discharged from the industries.

Table 1. Water Parametrs of Textile Industrial Effluent

S.No.	Parameter	Values
1	pH	8.540 ± 0.38
2	Temperature	26.31 ± 1.3°C
3	D.O	3.32 ± 1.04 mg/L
4	Alkalinity	37.8 ± 1.75 mg/L
5	Hardness	144.56 ± 11.75 mg/L
6	Turbidity	11.305 ± 0.07 mg/L

B. Histological Studies

Sections of control group (I) show the normal histological structure but Group II treated with 6.25% effluent show mild swelling and degeneration of hepatocytes. In GroupIII the micro sections of liver show degeneration of hepatocytes, hypertrophy of kupfercells and proliferation of bile ducts was observed.

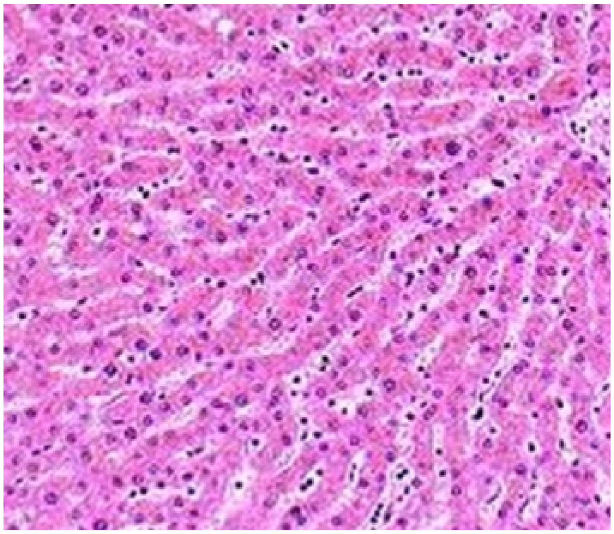

Fig. 1. Microphotograph of liver of control fish. (Group I, 300x)

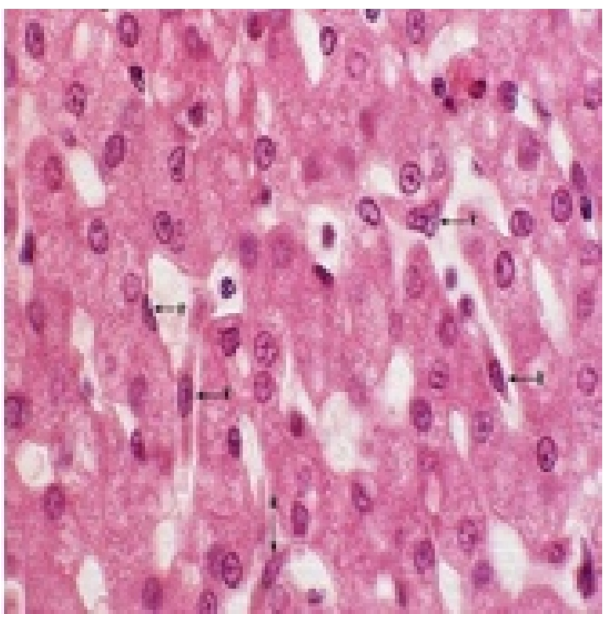

Fig. 2. Microphotograph of liver of Group II fish, showing swelling and degeneration of hepatocytes (400 xs)

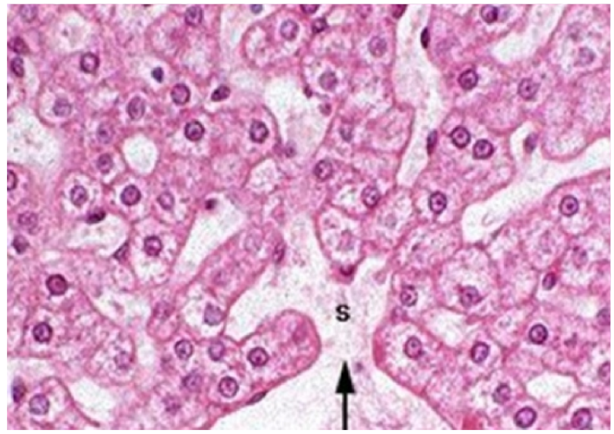

Fig. 3. Microphotograph of liver of Group III showing degeneration and proliferation of bile ducts (400x)

B. Hematological studies

Table 2. Toxicity of industrial Effluent on Some blood parameters of Mystus tengara.

Parameter	Group I	Group II	Group III
Plasma Glucose (mg/L)	234.5 ± 6.1	166.3 ± 2.3	158.1 ± 3.4 ***
Haematocrit (%)	31.1 ± 0.94	24.8 ± 0.25	19.2 ± 0.18**
Hemoglobin (g/100ml)	13.3 ± 0.8	9.71 ± 0.21	8.11 ± 0.42**
RBC(106 mm-3)	1.82 ± 0.12	2.22 ± 0.89	2.23 ± 0.23**
ESR (mm/h)	21.80 ± 1.00	18.63 ± 1.1	19.1 ± 0.9
MCHC (%)	29.11 ± 2.11	25.1 ± 1.0	23.3 ± 2.11**
MCH (g)	78.1 ± 9.11	72.1 ± 3.8	59.8 ± 6.51**
MCV (ug)	178.3 ± 12.2	154.3 ± 4.3	148.4 ± 5.3

The glucose values of fish held in both 6.25% and 12.5% concentrations were significantly decreased in both experimental groups. (P <001). The hematological indices of MCHC, MCH and MCV were similarly decreased in both experimental fish.The hemoglobin and haematocrit values in Group II decreased significantly (P < 0.01) compared to those of the control. The hemoglobin value also decreased significantly (P < 0.05) in both the exposed fish. But there was a increase in red blood cell and decrease in erythrocyte sedimentation rate values of fish exposed to both 6.25% and 12.5% of effluent (P >0.05) compared to the control fish.(Tab.2)

C. Biochemical studies

Table 3. Toxicity of industrial effluent on some biochemical parameters in Mystus tengara.

Parameter	GroupI	GroupII	GroupIII
1.ALT(ul/L)	38.2 ± 0.2	54.1 ± 2.1	187.3 ± 6.2**
2.AST(ul/L)	26.1 ± 0.9	44.2 ± 2.1	69.2 ± 2.8**
3.Albumin (g/dL)	4.8 ± 0.32	2.9 ± 0.2	1.23 ± 0.2**
4.INR (Sce)	13.1 ± 0.6	34.2 ± 1.1	64.7 ± 3.2***

Alanine transaminase (ALT): The serum ALT levels of fish in control group was found to be 38.01IU/L.But in the fish treated with 6.25% effluent showed elevated levels of enzyme when compared to control. (54.21 IU/L, P> 0.01).A further increase in ALT level was observed in Group III (187.3IU/L P> 0.01) when compared to Group II.

Asparate transaminase (AST): Similar to ALT the AST level in serum of control group showed normal activity and it was found to be 26.1U/L. The AST level was significantly increased in Group II (44.2IU/L, P> 0.05) and Group III (69.21IU/L P 0> 001) when compared to control fish

- Albumin: It was 4.8g/dL in control. But in contrast to the AST and ALT in both experimental Groups, the albumin content was significantly decreased and it was found to be 2.9g/dL in Group II (P> 0.01) and 1.2g/dL and Group III (P> 0.01).

- Coagulation test: The INR was normal in Group I control fish (13.1sec). However it was enhanced in group II (34.2 sec .P> 0.01) and Group III (64.7 sec. P> 0.001).

IV. DISCUSSION

The toxic effects of industrial effluent on the survival and biochemical profiles i.e. serum ALT activity of fish exposed to lethal concentrations were critically examined and the corresponding results are compared and discussed. The 96 h LC50 value for effluent was found to be 37.5%. The mortality ranged from 20% to 53.5% and increased with a corresponding increase in the effluent concentration and also duration of the exposure demonstrating both time and concentration dependent responses.

Blood parameters are influenced by a variety of environmental stressors. Hematological indices such as erythrocyte count, hematocrit, and hemoglobin concentration has been used as markers for evaluating fish health [14]. The hypothesis has been that these indices could provide information comparable to that given by human blood variables. In the present investigation, a significant decrease was observed in all blood indices except in RBC count. Fish erythrocytes, however, are more responsive to environmental stresses, and often vary in morphology and effectiveness of oxygen transport. The increase in RBC count may be due to respiratory stresses, starvation, bleeding, exposure to heavy metals, and environmental changes in temperature and dissolved oxygen which may increase rate of erythropoesis as in other findings. [15], [16], [17].

In the present investigation serum ALT levels of fish exposed to 6.25% of effluent showed an activity of 54.11IU/L (Table.3), which was significant when compared with the control. Similar trends were also observed in serum AST of fish exposed to 12.5% of effluent. Though the liver plays an important role in metabolic processes and detoxification of many xenobiotics, acute exposures to metals present in industrial effluent like may lead these metals to accumulate in the liver and cause pathological alterations [18]. Moreover, cell injury of certain organs like liver leads to the release of tissue specific enzymes into the bloodstream [19]. Significant increase in transaminases (AST and ALT) activity in fish exposed to effluent in experimental fish could be due to possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of enzymes by the liver. Although its precise biochemical functions in the fish are not fully understood, administration of effluent increased serum AST and ALT activities of reflecting a situation of tissue damage. The increased activity of ALT and AST may be due to leakage of hepatic cells which was also evidenced by our histopathologial studies [20]. Research indicates that ALT and AST can be used as biomarkers of cellular damage in blood plasma, protein degradation and liver damage [5, 21]. The major findings of this study are that heavy metals present in industrial effluent are more toxic and caused liver damage. The experimental fish showed significant increase in serum ALT and AST levels suggesting serious hepatic damage [22, 23] .The present study provided new insights on the hepatotoxicity. In contrast, though an increase in the ALT activity was observed in the fish exposed to 6.25% of the effluent but the difference is not much significant (P>0.05) and it is similar to that of the control fish. Significant increase (P<0.01) in the activity of serum AST and ALT in fish exposed to 12.5% of effluent indicates hepatic damage and distress to the fish. The liver is also responsible for the production of coagulation factors. The International Normalized Ratio (INR) measures the speed of a particular pathway of coagulation, comparing to normal. In the present investigation the INR was increased gradually in both GroupII and III. It clearly indicates that due to the damage of liver in experimental fish the synthesis of vitamin K- dependent coagulation factors have been impaired hence speed of clotting time was delayed.

V. CONCLUSIONS

The work presented here only threatened the chemical quality of the effluent. The present research work is an informative biomarker of exposure, but the role of environmental, physiological, and toxicological factors on transaminase response must be understood in order to properly interpret the biomarker response. The present data in our paper show decrease of hematological indices and increase of level of AST and ALT enzyme in several samples. Our results display that it is needed much more investigation on other fish populations, to resolve the question, about pollution impact of Chambal.

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