

Histopathology and Proteotoxicity Studies in the Liver Samples of Freshwater Fish *Channa punctatus* (Bloch), on Exposure to Fungicide, Bordeaux Mixture

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Abstract: Fish are one of the major sources of protein for human beings and the nutritional value of fish depends on their biochemical composition like protein, amino acids, vitamins, mineral contents, etc. The present study was intended to evaluate the impact of pesticide toxicity stress symptoms in fresh water fish *Channa punctatus*. The toxicity of Bordeaux mixture, a fungicide was assessed by exposing the experimental species *Channa punctatus* to sub lethal concentration of Bordeaux mixture under static bioassay condition. From the experiments done, it was observed that at ppm concentration of Bordeaux mixture, 50% mortality occurred. After 96 hrs of exposure, the fish tissue samples were collected from liver. The samples were analyzed for histopathological changes as well as alterations in the protein profile using SDS PAGE technique. Noticeable degenerative histopathological changes were observed in the liver of *Channa punctatus* exposed to Bordeaux mixture. The protein profile also found to be varied much from the normal liver samples.

Keywords: *Channa punctatus*, Bordeaux mixture, histopathological changes, protein profile, bioassay, SDS PAGE

1. Introduction

The use of pesticides has been recognized as part of agricultural practices throughout the world. Unfortunately the indiscriminate uses of these pesticides to improve agricultural production and yields have a negative impact on non target organism especially aquatic lives and environment.

In India, a wide variety of pesticides has been used in agriculture for broad spectrum control of insect pest of crops such as caterpillars, green leaf hoppers, brown plant hoppers, and gall midges, stem borers leaf folder of paddy and white aphids of chilly. (Giri *et al.*, 2002).

Fish as a bio-indicator species play an increasingly important role in monitoring water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fishes indicates heavy pollution of aquatic ecosystem. The fishes respond specific to a single pollutant or a group of contaminants (Svobodova, 1997).

In the present investigation, the impact of fungicide Bordeaux mixture on the histology and protein profile of liver in *Channa punctatus*.

The ability of Bordeaux mixture to adhere to plants in rainy weather makes it an excellent choice for a winter fungicide. The copper ions react with the Sulphur containing ligands in the enzyme of the plant and inhibit the metabolism of the fungus. Bordeaux mixture has been found to be harmful to fish and livestock due to potential build up of copper in the soil.

Liver is the major detoxification centre in the body so the liver is susceptible to a number of toxic disturbances mainly the pesticides. In the present study the SDS PAGE analysis and histopathological studies have been conducted on liver to understand the pathological effects of pesticide Bordeaux mixture.

2. Materials and Methods

Adult specimen of healthy *Channa punctatus* of both the sexes, measuring average length 14.18 ± 1.03 cm, weight 41.6 ± 7.9 gm were collected during the pre monsoon season using cast net from "Chirakadavu pond" at Neduvathoor, Kottarakara (Taluk), Kollam (Dist.)

The collected fishes were transported to the laboratory in aerated containers. Fishes were treated with 2% KMnO₄ solution for 15 minutes to remove external contamination and kept in tanks having capacity of 70 liters filled with 50 liters of well water, and acclimatized for 15 days with continuous aeration and fed with dry shrimps and pieces of earthworms; the water was renewed in every 24 hrs.

Ten fishes were taken both in control and treated tanks. The physicochemical parameters of water in which the fishes were kept were analyzed prior to setting up of the experiment and during the course of experiment. Bioassay experiments have been organized as the standard method for the measurement of toxicity, (APHA, 2005).

Histopathological Alterations of Liver Exposed to Bordeaux mixture.

The experiments were conducted during pre monsoon season. Prior to each toxicity experiment, range finding tests were carried out to determine the range of toxicant to be used for definite toxicity tests. The range finding tests were

conducted in aquarium tanks of 70 liter capacity with 50 liter water and ten fishes of equal size were exposed to each concentration for a period of four days and mortality was noted. After preparing the different concentration of test solutions, (2, 4, 6, 8, 10, 12 ppm) with Bordeaux mixture, the fishes were immediately transferred from the acclimatization tank to the test solution (APHA 1992).

During the experiment period, the test solution was renewed in every 24hrs with fresh solution of same concentration, (Alabaster, 1982; Sprague, 1969a). This was done to prevent the depletion of toxic materials by adsorption, absorption, or ventilation (Alabaster, 1969) when exposed to air. Observations continued for a period of 96hrs.

The fish died after pesticide treatment was collected. The methods for processing of tissues for histological studies were adopted from Roberts, (1978), and fixed in Bouin's fixative. The fixed tissues were cleared in chloroform. The tissues were dehydrated and embedded in paraffin wax at 60°C. Sections cut at 5µm thickness were stained with Haematoxylin & Eosin (H&E) and photomicrographs were taken.

SDS PAGE

The clinical value of the protein analysis by electrophoresis depends upon the change in the environment of experimental species and which results in the alteration of the protein fractions due to stress conditions. In the present investigation, tissue samples from the *Channa punctatus* exposed to Bordeaux mixture was take for the SDS-PAGE analysis.

3. Results

Histopathological alterations of liver

In the normal histological section, liver is covered with a serous membrane and connective tissue. Liver is composed of hepatic cells and lattice fibers, which support the hepatic cells. Hepatic cells are roundish polygonal, containing clear spherical nucleus.

Bordeaux mixture (13.06 ppm) exposures showed 50% of the death of the species and have induced discrete pathological changes in the liver tissue of the fish *Channa punctatus*. The changes include, degenerated hepatic tissue, blood cells among hepatocytes, appearance of blood streaks among hepatocytes, formation of vacuoles along with atrophy, necrosis and disappearance of hepatocytic cell wall and disposition of hepatic cords. The degenerative changes were intensified in lethal exposures.

The histology of the liver of *Channa punctatus* exposed to 13.06 ppm of Bordeaux mixture, showed shrinkage of hepatocytes with increased sinusoidal blood spaces. The hepatocytic hemorrhage was observed after 96 hrs of exposure. In this acute pesticidal exposure liver cells became irregularly arranged with ruptured hepatic cell membrane

SDS PAGE

Table 1: Log Molecular Weight of Standards used in SDS-PAGE

	Standard (Da) (y)	Rf (x)	Rf standard Log mol.Wt
β-galactosidase	118000	0.050	-
Phosphorylase-b	97000	0.083	4.988
BSA	66000	0.166	4.820
Amylase	51000	0.216	4.707
Recombinant DNA	25000	0.366	4.397
Lacta globulin	18000	0.483	4.262
Lysozyme	14400	0.616	4.16

Table 2: Rf value of protein fractions in the normal and Bordeaux mixture treated liver samples

Rf value Normal liver	Rf value Treated liver	M.W (Da)
0.06	0.06	105000
0.15	0.15	75000
0.18	0.18	60000
-	0.23	47000
0.36	0.36	25000

In the present study, the toxic effects of the pesticide Bordeaux mixture (13.06 ppm), on the electrophoretic protein fractions of liver tissues of *Channa punctatus* was observed and is compared with respect to the protein fractions of control liver sample.

The molecular weight of all protein fractions of the Bordeaux mixture treated liver sample obtained by SDS PAGE was calculated as 105000Da, 75000Da, 60000Da, 47000Da, 25000Da. In the normal liver sample, the protein fraction with molecular weight 47000Da was absent.

The Rf value of protein subunits present in the treated liver sample was 0.06, 0.15, 0.18, 0.23 and 0.36. The Rf value of the protein fraction 0.23 with molecular weight, 47000 Dalton was observed in Bordeaux mixture treated liver sample, but the same protein band was absent in the control liver sample of *Channa punctatus*. The control fish liver sample showed 4 polypeptide bands, where as in the Bordeaux mixture treated liver sample, showed five polypeptide bands

4. Discussion

Liver is the major detoxification centre in the body so the liver is susceptible to a number of toxic disturbances mainly the pesticides. In the present study the histopathological studies have been conducted on liver to understand the pathological effects of pesticide Bordeaux mixture.

The most remarkable histopathological changes observed in the liver were, loss of polygonal shape of hepatocytes, degeneration of hepatocytes, necrosis and loss of cell boundaries of hepatocytes. Similar findings have been reported by Mathur, (1962, 1965, and 1967) in *Ophiocephalus punctatus*, *Barbus stigma*, and *Heteropneustes fossilis* due to DDT, dieldrin and lindane toxicity. Konar (1970), Chakrabarti *et al.*, (1986); Amminikutty and Rege (1977); Anees (1978); Dubale and Shah (1981) also reported hypertrophy of hepatic cells, loss of characteristic polygonal shape of liver cells, degeneration,

shrinkage, cell rupture, necrosis and hepatic cell vacuolization.

The present results are in agreement with the observations of Mohamed, (2001); Ptashynski, et al., (2002) and Fanta, et al., (2003). They have studied the effects of different pollutants on fish liver and also observed degeneration of hepatocytes and necrosis in the liver of *Oreochromis niloticus* and *Clarias gariepinus* exposed to lead. Exposure of *Oncorhynchus mykiss* to copper sulphate was found to induce degeneration of hepatocytes, sinusoidal dilation and congestion in the blood vessels of the liver (Atamanalp, et al., 2008).

The electrophoretic techniques are promising tools for identifying protein profile in response to stress and lethal level of pesticides. In the present investigation, the fishes were exposed to 13.06 ppm of Bordeaux mixture for 96hrs. The Bordeaux mixture contain metal ion, copper. Metal binding proteins such as ferritin, ceruloplasmin, and metallothioneins (MTs) have special functions in the detoxification of toxic metals, and also play a role in the metabolism and homeostasis of essential metals (Kelly et al., 1998). Metallothioneins are low molecular weight proteins rich in cysteine residues that can bind various metals, including mercury, silver, copper, cadmium, lead, zinc, and cobalt, with varying affinities (Hamer, 1986).

The molecular weight of all protein fractions of the Bordeaux mixture treated liver sample obtained by Gel Electrophoresis was calculated as 105000Da, 75000Da, 60000Da, 47000Da, 25000Da. In the normal liver sample, the protein fraction with molecular weight 47000Da was absent. Formation of the new protein fraction may be attributed to the cellular damages caused by this pesticide. Tissue damage would result in "leakage" of intracellular enzymes from the plasma membrane into the blood. The studies conducted by Bouck and Ball, (1966) showed that the changes in protein fraction could occur as a result of an increase in the rate of cellular degeneration or due to the leakage of proteins across the affected cell membranes.

5. Conclusion

The present study entorched into the impact of Bordeaux mixture on the liver histopathology and protein profile of fresh water fish *Channa punctatus*.

Bordeaux mixture exposures have produced the following pathological changes in the liver tissue of the fish *Channa punctatus*. The changes include, degenerated hepatic tissues, blood cells among hepatocytes, appearance of blood streaks in hepatic tissue, formation of vacuoles along with atrophy, necrosis and disappearance of cell wall of hepatocytes and disposition of hepatic cords. The degenerative changes were intensified in lethal exposures.

The toxic effects of the pesticide Bordeaux mixture (13.06 ppm), was studied by electrophoresis, it was observed that in treated *Channa punctatus* there was a new protein fraction 0.23 with molecular weight, 47000 Dalton but the same protein band was absent in the control liver sample of *Channa punctatus*. It can be concluded that, Bordeaux

mixture is highly toxic to fish *Channa punctatus*, even at short term exposure producing hepatic histology and protein profile also supports the above findings.

6. Acknowledgement

The authors are grateful to the Head of the department, Zoology and Principal of the college for giving all lab facilities to complete the work. Equally thankful to other researchers for their help and encouragement.

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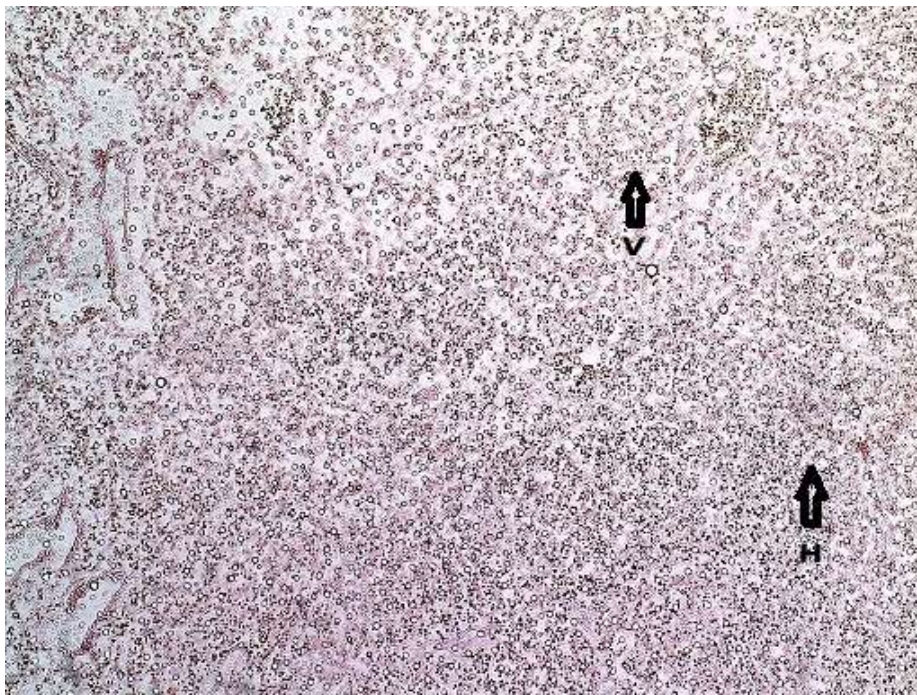


Figure 1: Normal Structure of Liver H- Hepatocytes, BC-Bile Canaliculi (100X)

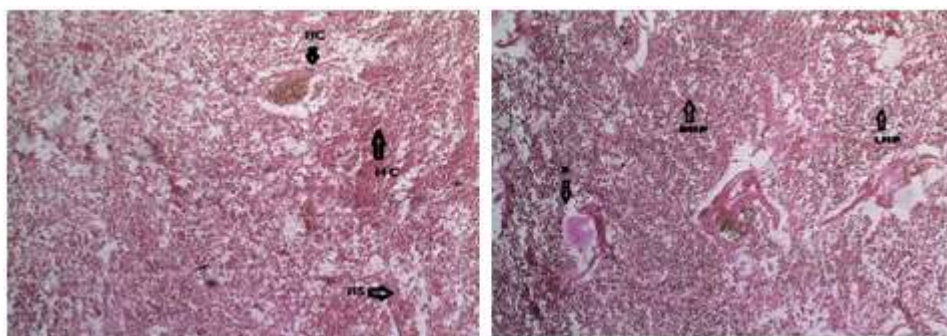


Figure 2, 3: Liver treated with Bordeaux mixture: HE-Haemorrhage, V-Vacuolation, S-Sinusoids, S-Sinusoids, HE-Haemorrhage, V-Vacuolation, LHP-Loosely arranged Hepatocytes (100X)

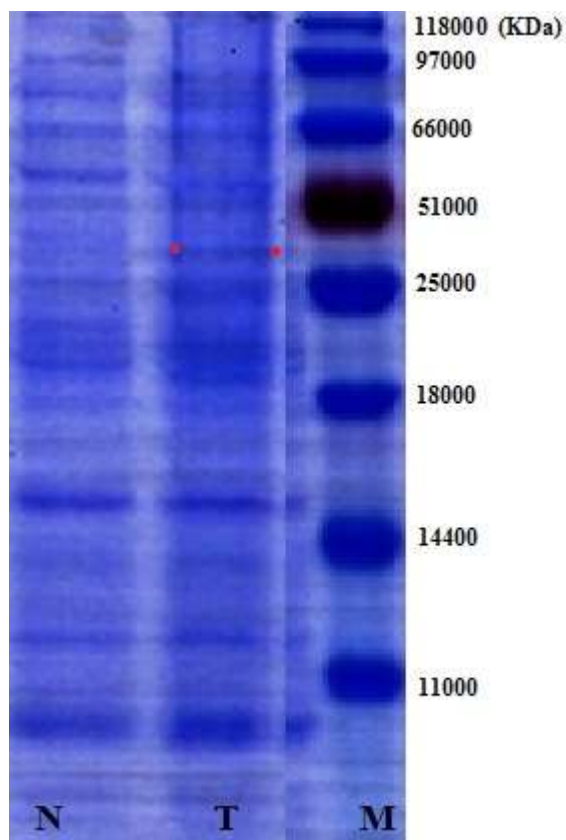


Figure 4: Protein profile of the normal (N) and Bordeaux mixture treated (T) Liver samples of *Channa punctatus* in comparison with marker (M)