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Histological and Protein Profile Alterations of Liver of Freshwater Fish *Channa punctatus* (Bloch), on Exposure to Fungicide, Fytran

Deepasree .M .I[1], M. S. Rajendran Nair[2]

P.G Department of Zoology and Research centre, Mahatma Gandhi College, Thiruvananthapuram, India

Abstract: ***The present study was to evaluate the impact of pesticide toxicity stress symptoms in fresh water fish Channa punctatus. The effect of Fytran (Copper oxy chloride),a fungicide was assessed by exposing the experimental species Channa punctatus to sub lethal concentration of the toxicant for 24, 48, 72 and 96 hrs under static bioassay condition. From the experiments done, it was observed that at 11.82 ppm concentration of Fytran, 50% mortality occurred. The results obtained from the calculation of LC50 showed that Fytran is toxic to the experimental species Channa punctatus. The impact of 96 hrs exposure to lethal concentration of Fytran in fresh water food fish Channa punctatus was observed. 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 liver of Fytran treated fish samples. The protein profile also found to be varied much from the normal liver samples.***

Keywords: *Channa punctatus*, Fytran, LC50, histopathological changes, protein profile, bioassay, SDS PAGE

1. Introduction

Pesticide usage is a critical concern because it has an adverse effect on the delicate ecosystem. The transfer of pesticides to the aquatic ecosystem therefore creates a need to fully understand their effect in the resident biota. In many areas of the world, the sensitive aquatic ecosystems are at a risk because of a non point source runoff of pesticides from agricultural and urban sources to aquatic ecosystems affecting aquatic biota (Austin, 1999).

Pesticide pollution severely affects aquatic organisms and organisms at higher trophic levels including human beings through food chain. For instance, consumption of fish contaminated by pesticides cause severe health problems in human beings (Svensson *et al*., 1994; Kumar *et al*., 2007).

Copper Oxy Chloride (Fytran) is the common name for basic cupric chloride having the chemical formula 3Cu (OH) $2.CuCl_2$, it is a protective fungicide in many crops. This fungicide is toxic to fish and aquatic invertebrates and contaminates water through runoff from catchment area (Indira Pala *et al*., 2008). This product showed slow disintegration nature and have potential for runoff for several months after application (Nikam *et al*., 2011). *Channa punctatus* was one of the most abundant species in our fresh water rivers, paddy fields and other water channels. But now it is very rare to get the species in our fresh water bodies. The major reason for this alarming condition is the pesticide pollution, house hold waste materials and chemical pollutants from factories.

Liver is the principal organ which carries out the detoxification. It performs a prominent role in the metabolism of carbohydrates, proteins and lipids, it also involved in the storage of glycogen, and synthesis of amino acids. The secretion of bile is a vital function of liver. The liver is susceptible to a number of toxic and metabolic disturbances either through the pollutants or parasitic invasion.

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% $KMnO_4$ 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).

3. Histopathology

Fishes were not fed during the experiment period. Twenty fishes were taken in both control and treated groups. The test solution was prepared from Fytran. After the preparation of stock solution, the different concentrations of the test solution were prepared by serially diluting the stock solution. Prior to each toxicity experiment, range finding tests were carried out to determine the range of toxicant to be used for definite toxicity tests. Ten healthy individuals of *Channa punctatus* were introduced into the tank with utmost care. Control tanks were also setup during the experiment

and also maintained at the same conditions as that of the experimental but tap water instead of test solution. After preparing the different concentration of test solutions, (2, 4, 6, 8, 10, 12 ppm) with Copper Oxy Chloride (Fytran), 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 and Lloyd 1982; Sprague, 1969a). This was done to prevent the depletion of toxic materials by adsorption, absorption, or ventilation when exposed to air. Observations continued for a period of 96hrs. A fish was considered dead when its respiratory activity ceases and it did not respond to the external stimuli. Care was taken to remove the dead fish from the test containers because this might deplete the level of dissolved oxygen and affect the other fishes. Median lethal concentration (LC_{50}) was calculated by following a computerized program namely SPSS/Windows (SPSS.10.0.LNK) for probit analysis.

The fish died after pesticide treatment was collected. The methods for processing of tissues for histological studies were adopted from Roberts, (1978). The tissues from liver was dissected out, pooled 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 Haematoxilin & Eosin (H&E) and photomicrographs were taken.

SDS PAGE

Laemmli's system which is a continuous system and is by the most widely used electrophoretic system for proteins. In this system the acrylamide gel consists of two parts. The separating gel which has pore size in the dimension to permit the sieving of macromolecules to be analyzed is located at the bottom. On the top of the separating gel is the stacking gel, which has large pores and thus exerts no marked sieving effects. The denatured sample containing the tracking dye is layered on the stacking gel. During electrophoresis the polypeptides are concentrated as a sharp band in the stacking gel. The polypeptide is then separated according to their size and charge in the homogenous electrophoretic medium that is in the resolving gel and resolution of polypeptide is superior in this system.

In the present investigation, tissue samples from the Fytran exposed *Channa punctatus* was take for the SDS-PAGE analysis.

4. Results

Histopathological alterations of liver:

The observed LC50 value for 96 hrs of pesticide exposure was 11.82 ppm. The fishes were treated with LC_{50} dose of Fytran (11.82 ppm) for 24hr, 48hr, 72hr and 98 hrs of exposure. After the pesticide exposure period, histopathological observations of liver showed that Fytran induced discrete pathological changes in the liver tissues of *Channa punctatus*. Most degenerative changes of hepatocytes were observed at 98hrs of exposure of Fytran viz., the cytoplasmic degeneration, necrosis, vacuole formation, rupture of blood vessels, disappearance of the wall of hepatocytes and degeneration of hepatocytes.

Liver is a bi-lobed organ, with a homogenous mass of hepatic cells with granular cytoplasm. The liver of untreated fish shows parenchymatous appearance and mainly consists of polygonal shaped hepatocytes with a central nucleus. Sinusoids are irregularly distributed between the polygonal hepatocytes.

The fish exposed to lethal concentrations of Fytran showed moderate to severe changes. The degree of histopathological lesions showed an increase as the period of exposure in Fytran was increasing. The intensive hepatic vacuolization (steatosis) was raised from 72 hrs exposures of Fytran and more pronounced on 96 hrs of treatment. The major alterations observed were degeneration of hepatocytes with dilation of sinusoid lumen. Cellular breakdown and cytoplasmic vacuolization were distinguished in combination with pyknosis.

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	4.158

Table 2: Rf value of protein fractions in the normal and Fytran 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 Fytran (11.82 ppm), on the electrophoresis protein fractions of liver tissues of *Channa punctatus* was observed and is compared with respect to the protein fractions of control liver sample.

The Rf value of protein subunit 0.23 with molecular weight, 47000 Dalton was observed in Fytran treated liver sample, but the same protein band was absent in the control liver sample of *Channa punctatus*. The control fish liver sample was observed to have 4 protein bands, where as in the Fytran treated liver sample, the polypeptide bands were found to be 5.

From the electrophoresis studies, it is clearly visible that, *Channa punctatus* exposed to Fytran, showed profound effect on the liver protein pattern. A new electrophoresis protein band was appeared after the treatment of pesticide.

5. Discussion

Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structure and functions. Some of these environmental factors include bio toxins, parasites, infectious germs, pollutants, pesticides, hydrocarbons, PCBs (polychlorinated biphenyls) and heavy metals (Brusle and Anadon, 1996).

The normal architecture of hepatocytes in the liver of *Channa punctatus* was markedly disrupted by the exposure of Fytran (11.82 ppm). Sinusoids were distended and central veins appeared to be severely damaged due to excessive swelling and degeneration of hepatocytes.

The liver of fish *Channa punctatus,* when exposed to Fytran caused cloudy swelling of the hepatocytes and even the connective tissue of liver was damaged. Rupture of blood vessels, breakage of the wall of hepatocytes and total deterioration of hepatocytes were also observed after 96 hrs of Fytran treatment. Radhaiah and Jayantha Rao, (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture of blood vessels and appearance of blood cells among hepatocytes in the liver of *Tilapia mossambica* exposed to Fenvalerate. The damage of the hepatocytes may be due to lack of sufficient blood supply. Anita Susan and Tilak, (2003) observed the toxic lethal concentration of Fenvalerate induced atrophy and appearance of blood streaks among hepatocytes in liver of *Crirrhinus mrigala*. Similar observations on the liver of *Cirrhinus mrigala* were reported, when exposed to Chlorpyrifos, Tilak *et al*., (2005).

Fishes 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. In the present study, the clinical value of protein analysis by electrophoresis depends upon the alteration of the protein fractions due to stress conditions.

In the present investigation, the lethal toxic effect of the pesticide Fytran (11.82ppm/96 hrs) on the electrophoretic protein fractions of liver tissues of experimental fish *Channa punctatus* was studied and the protein bands were compared with those of control fish. The results showed that there was an increase in the number of protein bands in the treated group of fish, in comparison with controls. The number of protein fractions in the liver of test fishes was found to be 5 where as in the control fishes the number of protein bands were 4. The The protein subunit with molecular weight, 47000 Dalton was observed in Fytran treated liver sample, but the same protein band was absent in the control liver protein sample of *Channa punctatus*.

El-Bermawy, (2000) have reported that, the protein pattern in electrophoresis revealed a profound difference between control and treated samples due to the production or activation of a new sequence of DNA responsible for synthesizing new types of protein. It is inferred that this protein fraction would be a stress protein produced to overcome the toxic effect of Fytran. Manna and Mukherjee, (1986) have made similar findings in *Tilapia* on exposure to radiation, Malathion and Mercuric chloride.

The present observations are in agreement with the studies of Muthukumaravel *et al.,* (2007) on *Oreochromis mossambicus* exposed to sub lethal concentration of cadmium for a period of 10 days. Similar observations were reported by Kumar *et al.*, (2009) on *Channa panctatus* exposed to Malathion, an organophosphate pesticide, showed profound effect on the protein pattern. Some new electrophoretic protein bands appeared and some others disappeared after the treatment.

Riji John and Jayabalan, (2007) observed that protein pattern of the gill varied at different sampling periods characterised by disappearance of certain protein fractions and occurrence of additional protein fractions in *Cyprinus carpio* exposed to endosulfan and they concluded that the severity of variations seen in the protein pattern in the gill was dependent on both duration and concentrations of endosulfan exposure. Tripathi and Shukla, (1990) performed SDS-PAGE of the cytoplasmic protein fractions of the liver and the skeletal muscle of C*larias batrachus* exposed to endosulfan and Methyl parathion for 1 to 28 days and observed the appearance of new protein bands at different time intervals after the exposure of the pesticide. They emphasised that, these changes in the protein band pattern is in response to the exposure of pesticides and may be attributed to the changes in the synthesis or degradation of various proteins.

These observations are in agreement with the studies of Dhar and Chatterjee, (1984) in *Channa punctatus* on treatment with pesticides, the electrophoretic patterns of serum proteins has resulted in depletion of several protein fractions and appearance of some new fractions. The electrophoretically analyzed total serum protein components of *Tilapia nilotica* treated with Edifenphos and Glyphosate showed a decrease in the protein fractions with time bound exposure of pesticide, when compared with control (El-Gendy *et al.,* 1998).

6. Conclusion

The present study encompasses the Fytran induced histopathological changes in the liver *Channa punctatus* and the change in protein pattern of the liver tissues of Fytran exposed *Channa punctatus*. In the Fytran exposed liver, degenerative changes of hepatocytes were observed viz., the cytoplasmic degeneration, necrosis, vacuole formation, rupture of blood vessels, disappearance of the wall of hepatocytes and degeneration of hepatocytes**.**

In the present study, the toxic effects of the pesticide Fytran, 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 Rf value of protein subunit 0.23 with molecular weight, 47000 Dalton was observed in Fytran treated liver sample, but the same protein band was absent in the control liver sample of *Channa punctatus*. It is inferred that the new protein fraction would be a stress protein produced to overcome the toxic effect of Fytran. It can be concluded that

Fytran is a pesticide which is badly reducing the population rate of *Channa punctatus* as it badly affect its reproductive system.

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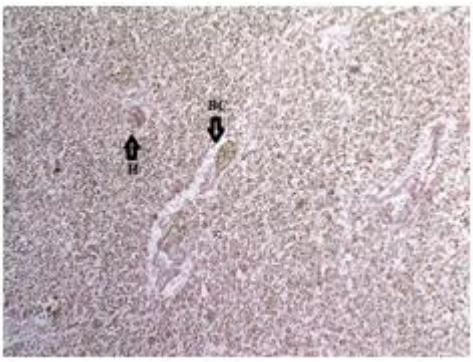

Figure 1: Normal Structure of Liver
H- Hepatocytes, BC-Bile Canaliculi (100X)

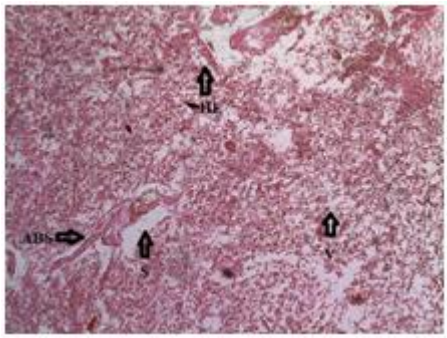

Figure 2: Liver treated with Fytran
HE-Haemorrhage, V-Vaculation, S-Sinusoids, ABS-Appearance of Blood Streaks among hepatocytes (100X)

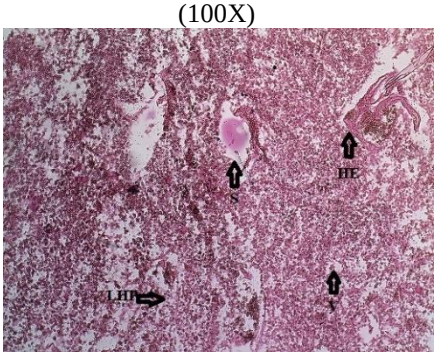

Figure 3: Liver treated with Fytran
S-Sinusoids, HE-Haemorrhage, V-Vaculation, LHP-Loosely arranged Hepatocytes (100X)

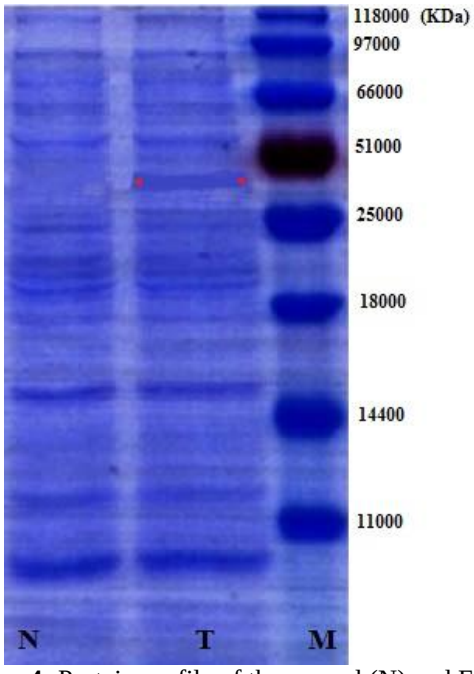

Figure 4: Protein profile of the normal (N) and Fytran treated (T)
Liver samples of *Channa punctatus* in comparison with marker (M)