

Cyathrin induced Histopathological and Histochemical changes in the liver of the catfish, *Clarias batrachus*

Vijay Shankar Pandey
Ex-Research Scholar, JaiPrakash University, Chapra (Bihar)
Email ID: shankarvijay982@gmail.com

ABSTRACT

The investigation was aimed to assess effect of the pyrethroid – Insecticide used in paddy fields flows in nearby water resources on the histology and histochemistry to 1/10LC₅₀ for 4 and 7 days under ecological conditions of PH, temperature, acidity and hardness of water.

The results showed that the histopathological changes induced in the liver were often represented by cytoplasmic vacuolization of the liver cell (Hepatocytes), blood vessel congestion, inflammatory, leucocytic infiltration, necrosis and lipid infiltration. The histochemical level reveals reduction in glycogen content and total protein content of the hepatocytes with increasing activity of enzymes related to improving filtration mechanism in body.

INTRODUCTION

Pesticides have been widely used in the paddy fields to control insects, pests and disease vectors. They ultimately find their way into aquatic resources and have been found to be highly toxic not only to fish but also to organisms which contribute substantially to the food chain. Such pesticides are also have affinity for residing in animal tissue specially composed by lipid containing cells (Deichmann & MacDonald 1975). Cyathrin (RS) alpha cyano-3 phenoxybenzyl (RS)-2-(4 chlorophenyl)-3 methyl butyrate is a pyrethroid insecticide widely used for insect control in different countries. It is highly toxic for many fish (Tilak and veeraiah, 2001).

There is considerable evidences indicating that pesticides are responsible for many adverse effects in fishes and other animals from the histochemical and histopathological point of view with altering activity of enzymes to counteracting body metabolism (Shastry and Sharma, 1979, Svoboda, 1971). The present work was conducted to study the effect of the pyrethroid insecticide on the liver of *clarias batrachus* with study of enzyme activity which reveals further disturbances in metabolism and finally affect survival of the fishes.

Materials and Methods

The living fishes were collected from canal side ditch nearby paddy fields treated by insecticide. The fish samples were transported in well aerated containers to large tanks in the laboratory. These tanks were continuously aerated using air pumps. The fish samples were acclimatized to the laboratory condition for about 15 days, various concentrations of untreated and pyrethroid treated effluents were prepared and the fishes were exposed to these concentrations.

The toxicity of the untreated and treated effluents measured directly by LC₅₀ test. The LC₅₀ of cyathrin at 72 hours was found to be 250mg/L as obtained from the lethal curve. The experimental fishes were divided into 3 groups as –

The 1st group: 10 fishes were exposed to 1/10 LC50 of cyathrin for 4 days exposure period in tanks.

The 2nd group: 10 fishes were exposed to 1/10 LC50 of cyathrin for 7 days.

The 3rd group: 10 fishes were used as control fish kept in pure water without any treatment.

At the end of each exposure time, fishes were decapitated and were dissected. The liver was removed and small pieces were fixed in 10% formalin and carnoy's fluid. The fixed samples were dehydrated in ascending series of Ethyl Alcohol, cleared in methyl benzoate and embedded in paraffin wax. 6 micron thick section were cut, mounted and stained with different stains according to the target of investigation. For histopathological examinations, 10% formalin fixed sections were stained with haematoxylin and eosin. For histochemical investigations, materials fixed in carnoy's fluid were stained with periodic acid schiff's (PAS) technique (Hotchkiss, 1948) for demonstration of liver glycogen and mercury bromophenol blue method for demonstration of total protein content (Mazia and others, 1953).

For study for enzyme activity, excised liver washed with 50mm phosphate buffer with 7.4 PH, surface dried with filter paper and homogenized with buffer containing 1mm EDTA, 1mm DTT, 0.15MKCL, 0.01% PMSF. Homogenization was carried out at 4 degree celsius using polytron hand homogenizer and centrifuge at 10,000 g for 20 min at 4 degree celsius. Supernatant was used for biochemical evaluations.

For catalase enzyme 50mm phosphate buffer (PH7.0) and 50mm H₂O₂, the reaction rate was measured at 240 nm. The extinction coefficient of H₂O₂ was 40 m⁻¹ cm⁻¹. One unit of catalase was defined as limit of H₂O₂ degraded min⁻¹ mg⁻¹ protein. For super oxide dismutase (SOD) 10 lil sample and 1 micron litre NBT (Nitro blue tetrazolium) mixed with 200 microne litre of 0.1m EDTA. Phosphate buffer was used to make 3ml of reaction mixture. The tubes of 100 microgram placed in a light box providing uniform light intensity through 40w fluorescent bulb. The tubes was incubated for 5-8 minutes to achieve a standard temperature. At zero and specified time intervals 0.05 ml riboflavin added. The tubes were incubated in the light box for 12 minutes and then at every 3 minutes interval A560 observed for 3 times. By the way % inhibition of NBT reduction was determined and plotted against amount of enzyme activity. The amount of resulting enzyme was determined in one half of maximum inhibition (Halliwell and Gutteridge, 1993).

Results and Discussions

The hepatocytes form a rather cord like arrangement in liver sections of normal fish. These cords are arranged around tributaries of the hepatic vein. The large and polygonal liver cells having homogenous Eosinophilic Cytoplasm and centrally located nuclei (Fig 1). Exposure of *clarias batrachus* to 1/10 LC50 of cyathrin for 4 days causes histopathological changes in liver. The hepatocytes have lost their normal structure with pyknotic nuclei. The extra hepatic blood vessels were dilated and congested with blood causing inflammatory leucocytic infiltrations (Fig 2) with marked cytoplasmic vacuolization in a no. of hapatocytes (fig. 3).

The exposure of 1/10 LC50 after 7 days showing pronounced changes. The liver cells were degenerated with necrosis which appears as focal areas with lymphocytic infiltration (Fig 4) with a large no. of hepatocytes suffered from fatty degeneration (Fig 5).

Histochemical results:

Glycogen: The PAS preparations of the normal liver of *clarias batradil* revealed that glycogen was present in the cytoplasm of the hepatocytes represented as reddish fine granules of different sizes. However, cells of the same specimen exhibit different intensities with PAS reaction (Fig 6). After exposure of fish to 1/10 LC50 of cyclothrin for 4 days, glycogen quantity of the liver cells decreased. This lowering of quantity was quite evidenced in the amount and stain ability (Fig 7). This reduction of glycogen materials became more pronounced after 7 days of exposure with faint stain ability and became hardly detectable (Fig 8).

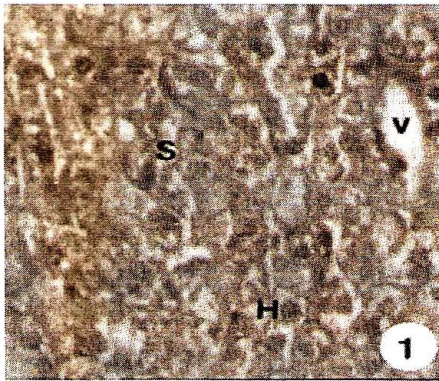


Fig. 1: section in the liver of a control Fish, S: sinusoidal lumen, H: hepatocytes, V: vein X 320



Fig. 2: section in the liver of a fish exposed to 1/10 LC50 of cyclothrin for 4 days showing V:congested vein and inflammatory leucocytic infiltration (Li) x 320



Fig. 3: section in the liver of a treated fish showing cytoplasmic vacuolization of the hepatocytes (arrow) x 320



Fig. 4: section in the liver of a fish with 7 days cyclothrin exposure showing necrosis area (N) with leucocytic infiltration x 320

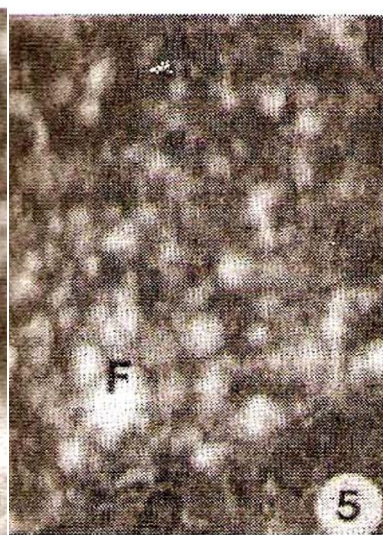


Fig. 5: section in the liver of a treated fish showing lipid infiltration (F) x 320

Total Proteins: The total proteins appeared as intensely dark blue colored inclusions in the cytoplasm of hepatocytes in normal fish. Chromatin bodies and nucleoli exhibited a deep coloration with bromophenol blue (Fig 9). Total proteins were found to exhibit a clear decrease in cytoplasm and nucleus of the hepatocytes of *clarias batrachus* exposed to pesticide for 4 days (Fig 10). The liver cells of fishes exposed for 7 days showed a clear reduction in the protein quantity and their traces were mainly located at the peripheries of the hepatocytes which showed severe cytoplasmic vacuolization.

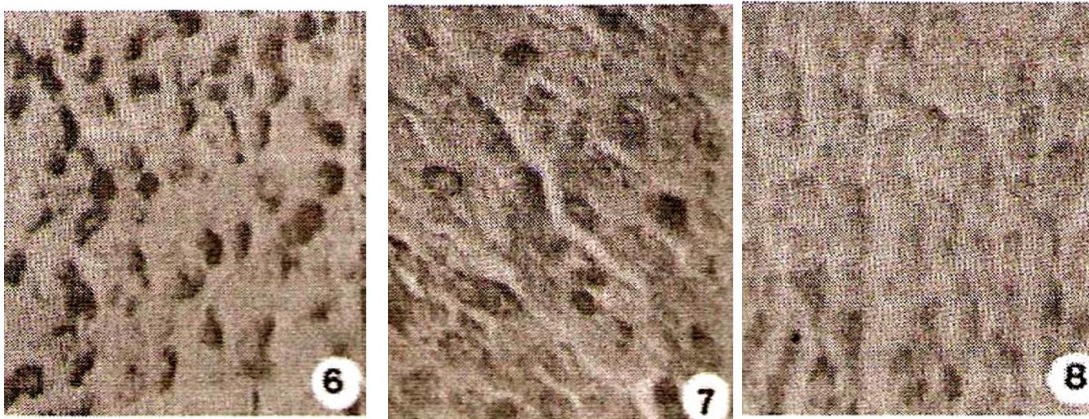


Fig 6: sections in the liver of a control fish stained with PAS technique showing distribution of glycogen in hepatocytes x 320

Fig 7: Hepatocytes with reduction of glycogen after 4 days exposure to fenvalerate x 320

Fig 8: Marked reduction of glycogen after 7 days exposure period x 320

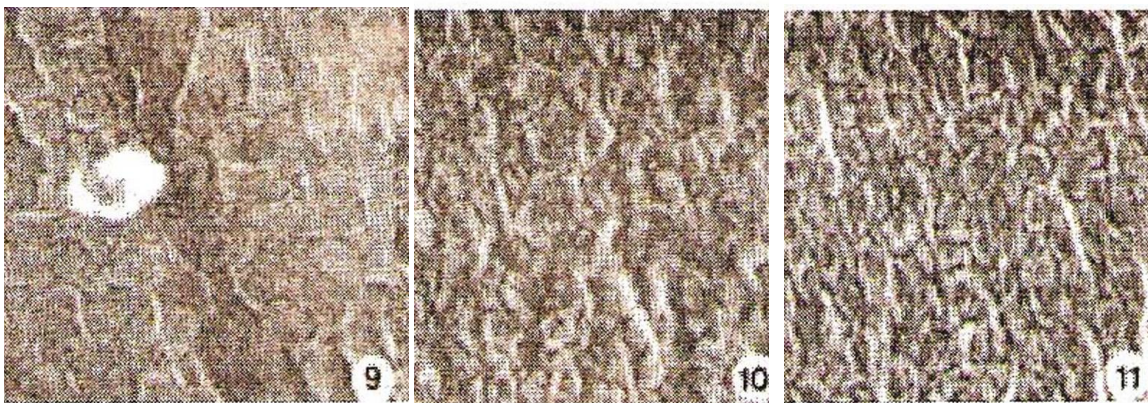


Fig 9: sections in the liver of control fish stained with mercury tromphenol blue for total proteins x 320

Fig 10: Total proteins in liver of a fish exposed to cyathrin for 4 days x 320

Fig 11: Reduction of total proteins in hepatocytes after 7 days exposure period to cyathrin x 320.

The present results showed that cyathrin induced many histopathological changes in the liver of cat fish *Clarias batrachus*. These lesions included cytoplasmic vacuolization of the hepatocytes, inflammatory leucocytic infiltrations, congestion of blood vessels, necrosis and lipid infiltrations. Similarly, Teh et al. (2005) found that

exposing 7 day old larvae of the fish sarcomento splittail to sublethal concentrations of fenvalerate for 1 week includes vacuolar degeneration and cell necrosis in the liver.

The effect of insecticides on the liver of different fish species were studied by many investigators. Mandal and Kulshrestha (1980) studied the effects of sublethal concentrations of submition of liver, kidneys and intestine of *clarias batrochus*. They observed liver necrosis, vacuolization and breakdown of the cell membranes. They also observed vacuolization of epithelial cell of uriniferous tubules and degeneration of the glomeruli in the kidney, lesion formation in the villi with enlargement of mucous cells in the intestine. Histological changes in the liver of *tilapia mossambica* after exposure to the organophosphate monocrotophos were reported by Desai et al. (1984). At the initial stage of intoxication necrosis and vacuolization of hepatocytes were recorded, while lipid degeneration later on. Elezabi et al. (2001) studied the effect of malathion on the fish *oreochromis niloticus* and result showed induction of histopathological changes in the liver and gills of the fishes. These changes were hemorrhage, necrosis and lipidosis in the liver. Their results showed histopathological changes in the liver represented by disarrangement of liver cells, cytoplasmic vacuolization of the hepatocytes, damage and congestion of blood vessels with inflammatory leucocytic infiltrations.

It was found that cyclethrin induced marked reduction in glycogen quantity in the hepatocytes of *clarias batrachus* is in agreement with Reddy et al. (1991) reported effect of fenvalerate in liver and muscles of *cyprinus carpio*. Exposure of fresh water fish *Mystus Vittatus* to sublethal concentrations of the two pesticides thiothox and dichlorous for one month was found to induce marked depletion in both liver and muscle glycogen. The activity levels of succinate dehydrogenase (SDH) and glucose-6 phosphate dehydrogenase (G6PD) were studied by Redy et al. (1991) indicated decreased SDH activity through SDH-inhibition at level of mitochondria and increased G6PD through alternative pathway of carbohydrate metabolism viz. Hexose monophosphate shunt (HMP) or pentose phosphate pathway as a biochemical adaptation to overcome toxic effect of different pyrethroids after 72 hour exposure.

Total protein quantity of the liver of *clarias batrachus* showed a obvious decrease after exposure of cyclethrin is with agreement of Reddy et al. (1991) reported reduction of total structural and soluble proteins, whereas enhancement of free amino acids and the increased activities of proteolytic and aminolytic enzymes viz. protease, aspartate, Aminotransferase and Alanine Aminotransferase in fenvalerate exposed fish *Cyprinus Carpio*. The protein quantity reduction in liver of fanvalerate exposed fish might be due to either arrested metabolism in the liver or to use it to build up new cells or enzymes to reduce the stress in fishes.

The study reveals SOD and CAT activity increases in digestive gland and gills is stress specific response of *clarias batrachus*. Parihar et al. (1997) reported that SOD helps in inhibiting the oxygen radical accumulation thereby reducing the oxidative damage to hepatocytes. The catalase helps to eliminate hydrogen peroxide in body. Since both the enzymes are involved in detoxification of hydrogen peroxides, the increased activity indicated their protection up to their life end.

By the way, the present study proved that cyclethrin affected the structural and histo chemical quantity of the liver of *clarias batrachus* and also enhance activity of antioxidant enzymes which protect fish for their survival. The study also showed that

affect of pesticide is time dependant. The stress of pesticide affect also depend upon developmental stage as larva were found to be more susceptible than matured fish.

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