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Research paper

Histopathological Changes in Liver and Kidney of *Clarias batrachus* (Mangur) on Chlorpyrifos Exposure

Nitu Singh [a] *

[a] *Research Scholar, Jai Prakash University, Chapra, Bihar, India*

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***Corresponding author**
Nitu Singh
✉ **Email**
nitu3146@gmail.com

ABSTRACT

Aquatic organisms are inadvertently exposed to runoff water carrying pesticides and chemical fertilizers from agricultural land. Chlorpyrifos is one of the most commonly used organophosphate insecticides applied in agriculture for insect control. Effect of chlorpyrifos on kidney and liver in *Heteropneustes fossilis* (Bloch) was studied after exposing the fish for short term-24h, 48h, 72h and 96h. Healthy adult fish were exposed to sub-lethal concentration 1.56mgL^{-1} (75% of 2.08mgL^{-1} 96h LC). Histopathological changes in liver and kidney were examined at given time intervals after sacrificing fishes and tissue fixation followed by histological examination of Haematoxylin and Eosin (H/E) stained sections of tissues under light microscope. Fishes exposed to chlorpyrifos exhibited histomorphological alterations in kidney and liver while fishes in control were normal with no observation of any damage in these organs. Liver cells in exposed fishes showed necrosis and vacuolization together with random observation of denucleated cells and eosinophilic material in the cytoplasm. In kidney necrotic lesions were observed with presence of pyknotic nuclei and dilation of renal-tubules. In this study it was observed that chlorpyrifos was highly toxic for the fish *Heteropneustes fossilis* and has profound adverse effect of one of the most vital organs, the liver and kidney.

1. Introduction

Pesticides are used for protection of crops from insect pests all over world. The sale of all categories of pesticides has increase from 850 million US dollar in 1960 to 31191 million dollar in 2005 (Zhang *et al.* 2011). Per capita use of pesticides has also witnessed an increase in recent years. Currently, use of pesticides in China is 13.1 kg, Japan 11.8 kg, Italy 2.5 kg and UK 3.2 kg, USA 2.5 kg and India 0.3 kg per hectare has been reported (URL 1). Many pesticides do not biodegrade as fast as introduced in the ecosystem and a large quantity persists in soil, water, and atmosphere and also in biotic system, often in the form of their metabolites. These pesticides and metabolic forms of pesticides undergo biotransformation and bio-magnification in food chain becoming more harmful for organisms at top of the food chain. Fishes, especially carnivorous and omnivorous such as *H. fossilis*, are the most affected organisms in aquatic environment contaminated with pesticides and other chemicals. In India as much as70% of the chemical formulations used for agriculture practices are believed to affect non-target organism and to find their way to freshwater fish (Bhatnagar *et al.* 1992).

Chlorpyrifos, a broad-spectrum chlorinated organophosphate insecticide is the most used pesticide in world to control a variety of insect pests

in agriculture, orchards and households. Chlorpyrifos is highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms (USEPA, 1989). Cholinesterase inhibition property of chlorpyrifos was observed in acute toxicity tests of fishes exposed to very low concentration (Vasudeva *et al.* 2005). Fish may be used natural bio- indicator of aquatic pollution caused by chemicals like pesticides, herbicides, fertilizers etc. (Sinaie *et al.* 2010; Joseph and Raj, 2011).

Alteration in the cellular morphology of pesticide treated fish (Abhilash and Prakasama, 2005) and their physiological functions upon exposure to different pesticide concentration have been observed by Gupta and Saxena (2006). The study of fish liver is very important in the field of aquaculture induced by many problematic condition and aquatic pollution.

The study of fish liver and kidney is very useful in assessing the health of fishes exposed to pesticides. Histopathology of liver and kidney provides indication of chemical toxicity and a useful way to study the effects of exposure of toxicants on aquatic animals. Liver is considered as one of the most important organ responsible for detoxification and bioaccumulation process. Pesticides entering in body are eventually transported in the liver, bio-transformed and detoxified in the form of water soluble substances and finally excreted out (Hodgson and Goldstein, 2001). Fish liver histopathology is an indicator of chemical toxicity and a useful way to study the effects of toxins in exposed aquatic animals (Deka and Rita, 2012). Liver is also one of the organs used as reliable biomarker of toxic injury and contaminant exposure (Stentiford *et al.* 2003).

Kidney plays a vital role in the maintenance of homeostasis in an organism being the key to regulate of extracellular fluid volume of composition as well as acid –base balance. It is also a target of toxic chemical, which can disrupt its function and cause temporary or permanent disruption of homeostasis. Fish kidney is composed of two parts head and body. In *Heteropneustes fossilis*, head kidney is devoid of most of the functional structures like glomeruli and consists mostly of a vascular lymphoid mass. The posterior trunk kidney is functional and contains many uriniferous tubules for excretion. Histopathological changes in the kidney due to exposure of various organic and inorganic chemical compounds have been reported in several studies (Banaee *et al.* 2011; Katuli *et al.* 2014 Millar 2002; Ghasemzadeh *et al.* 2015).

2. Materials and Methods

Heteropneustes fossilis, a freshwater bottom dweller catfish; weight 22.5±1.75 gram and length 16.53±1.07 cm, were collected from local ponds in Saran district of Bihar. These fishes were fed with standard fish food pellets during acclimatization. Fish were maintained in well aerated, large plastic tank containing non-chlorinated water and acclimatized under natural photoperiods (11h light and 13h dark) for 10 days.

The physicochemical feature of the normal water was maintained according to the methods described by APHA, (1998); temperature-22±1.5 °C, dissolved oxygen-8.5±0.41mL^{-1}, CaCO-3 154.66±5.37 mgL^{-1} and pH - 7.5.

Healthy acclimatized fishes were selected and transferred to 15L glassware tanks for conducting experiment. Fish were divided in control and test groups. Test group was further divided in four groups depicted as 24h, 48h, 72h and 96h, and five fishes in each were introduced for further treatment. Fishes in test group were subjected to exposure of 1.56 mgL^{-1} (75% of 96h LC_{50}) concentration of chlorpyrifos. Neither control nor test group fishes were given any food 24h before and after commencement of the test.

Fish were sacrificed at different durations (24h, 48h, 72h and 96h) by spraying MS-222 (tricaine-S-methane sulfonate) solution and organs- liver and kidneys were extricated carefully. Isolated tissues were immediately preserved in Bouin's solution for further processing and histological analysis. Tissues were dehydrated and embedded in paraffin. Sagittal sections (5µ thickness) were cut using microtome. Slides were by double staining method using aqueous Haemotoxylin and alcoholic eosin stains. Photomicrographs of stained slides were made using light microscope and observed under 100xresolutions for analysis of cellular deformity.

3. Results and Observations

In the present research a variety of histological changes were observed in the liver and kidney of *Heteropneustes fossilis* after exposure to chlorpyrifos at 1.56mgL^{-1}(75% of 96h LC_{50}). LC_{50}value was predetermined using SPSS- 20 computer program for Probit analysis.

Liver: The liver of control fish exhibited normal parenchymal texture generally formed of polygonal hepatocytes between which sinusoids are irregularly distributed. Hepatocytes were uniform morphologically and no vacuolization was observed. No apparent degenerative changes in sinusoids and blood vessels were marked (Fig. 1).

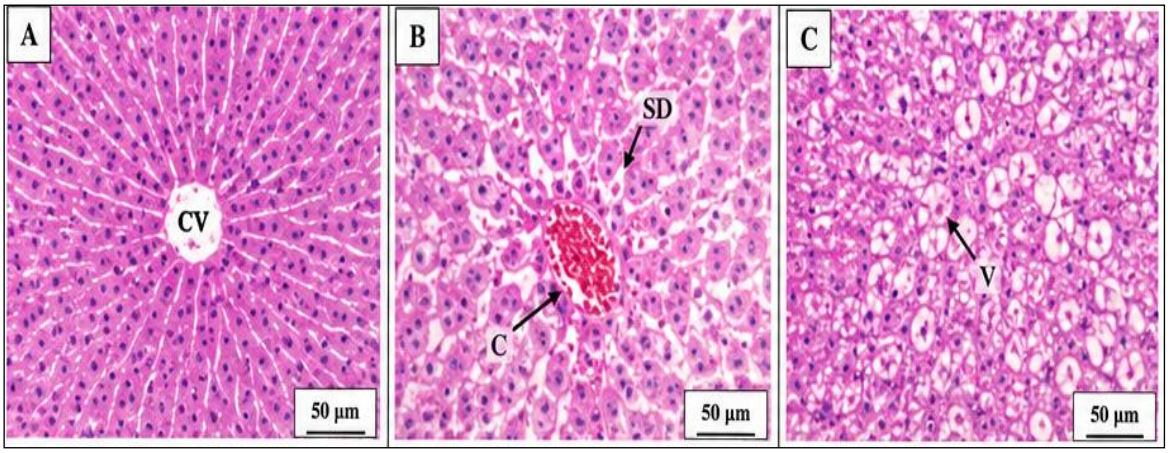


Fig. 1 **A.** Control fish; **B.** 0.25 mg/l **C.** 0.50 mg/l treatment effect on liver

Fishes in test group exposed to sub-lethal concentration showed moderate to severe histological changes. The degree of degenerative changes in tissues may be related with the increasing duration of chlorpyrifos exposed fishes.

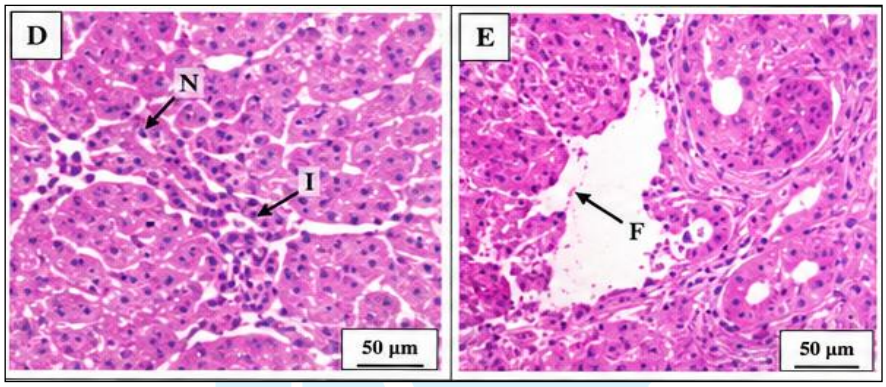


Fig. 1 Chlorpyriphos exposure **D.** 0.75 mg/l; **E.** 1.00 mg/l effect on liver

After 0.25mg/l (Fig. 1B) vacuolization randomly observed becomes highly prominent after 0.50mg/l (Fig. 1C). Cellular degeneration of hepatocytes became more pronounced after 0.75mg/l (Fig. 1D), showing dilation in blood vessels and enlargement of sinusoidal spaces. Liver tissue exposed for 1mg/l (Fig. 1E) exhibited extensive tissue damage characterized by hepatocyte degeneration, delamination and vacuolization.

Kidney: In *H. fossilis*, kidney may be distinguished in two regions, the anterior head kidney and posterior body kidney. Head kidney consists of lymphoid tissues and has no known role in excretion. The interstitial tissue is the main part hematopoietic tissue in the posterior body kidney and has functional nephrons. Each nephron consists of two parts, the glomerulus and the urinary tubules. Exposure of the *Heteropneustes fossilis* to chlorpyrifos for 96h at sub-lethal concentration (75% of 96h LC50 =1.56 mgL^{-1}) affected kidney as observed in histo-morphological details.

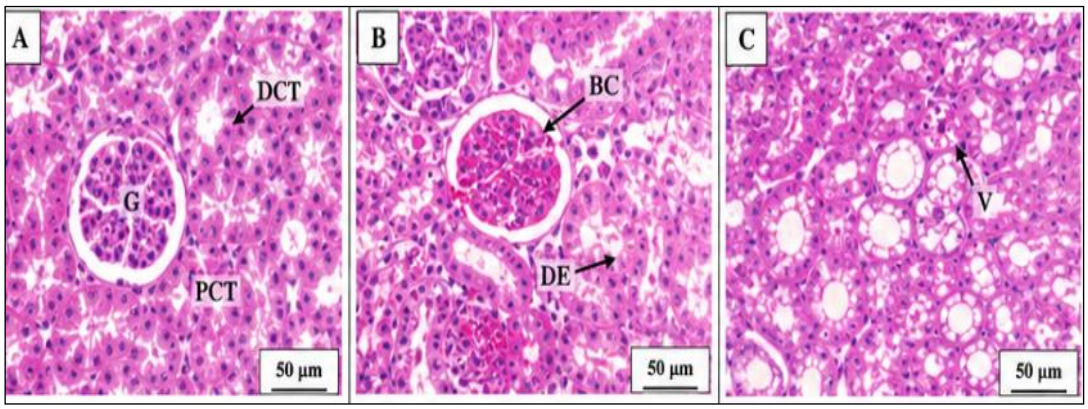


Fig. 2 **A.** Control fish; **B.** 0.25 mg/l; **C.** 0.50 mg/l Chlorpyriphos effect on kidney

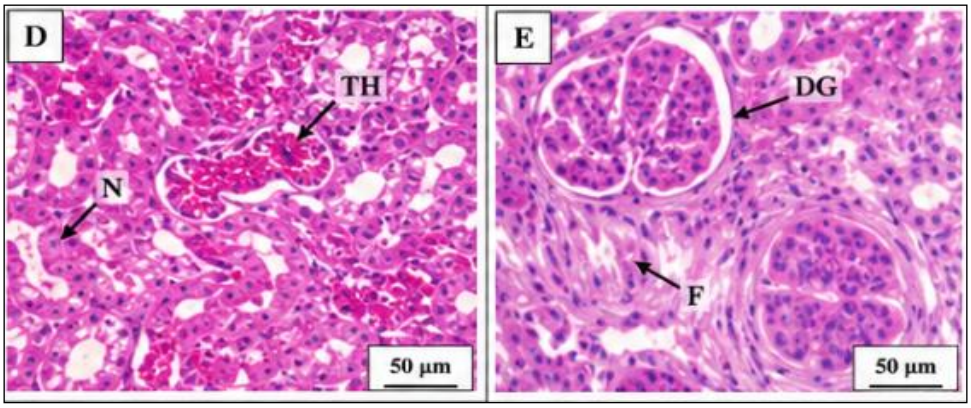


Fig. 2 **D.** 0.75mg/l; **E.** 1.0mg/l chlorpyriphos effect on kidney

Kidneys in untreated control group fishes exhibited normal histological texture (Fig. 2A). In treated group (0.25mg/l), degenerative changes in kidney were marked by enlargement of glomerulus and Bowman's capsule as well as increase in intercellular spaces (Fig. 2B) when compared to control.

After 0.50mg/l treatment (Fig. 2C), loss of nuclear material was observed in few cells associated with cellular degeneration. There were marked atrophy, shrinkage and dilation in renal tubules after 0.50mg/l treatment (Fig. 2D). The damage in renal tissues gradually spreads through the glomeruli, hematopoietic tissues, and cells of distal and collecting tubules. At 96h, highly degenerative changes in haematopoitic tissues associated with severe necrosis, cellular hypertrophy and swelling in renal tubules were observed. The interstitial tissues exhibited vacuolization along with deformed and undistinguished cellular boundaries (Fig. 2E).

4. Discussion

Contamination of aquatic ecosystem by pesticides has adverse effects on various non-target organisms, especially the fish. Numerous studies have reported chlorpyrifos toxicity causing alterations in biochemical parameters and histological damage in vital organs like liver, kidney etc. (Bernet *et al.* 1999; Ghorashi *et al.* 2013).

Liver: In present study, short term (96h) chlorpyrifos exposure has shown deleterious effects on liver and kidney. Histological studies are useful tool to assess sub-lethal and chronic exposure of fishes to aquatic pollutants. Liver is the primary site of detoxification and one of the most important organs affected by pesticide exposure. Most common adverse effects observed in liver due to pesticide exposure include, cellular deformity associated with nuclear hypertrophy and vacuolization, sinusoidal enlargement, necrosis and congestion of sinusoidal spaces by WBCs infiltration. The exposure effects become moderate to severe with increasing exposure duration as revealed by the results (Fig. 1-4).

Liver in chlorpyrifos exposed *H. fossilis* after 24h (Fig. 2) in this study exhibited randomly located vacuolization becoming highly prominent after 72h (Fig. 3) of exposure. Besides these changes, other histopathological conditions observed in liver at 96h were, cellular degeneration of hepatocytes, dilation in blood vessels, enlargement of sinusoidal spaces, extensive tissue damage characterized by hepatocyte degeneration, necrosis and delamination of cellular boundaries were also observed in exposed fishes (Fig. 4). These findings are in agreement with that of Manjunatha and Philip (2015) and Barbhuiya and Dey (2014) who reported similar affects in *Heteropneustes fossilis* on exposure to sub lethal concentration of 0.12 µl/L (1/10thofLC_{50}) chlorpyrifos.

Devi and Mishra (2013) have also made similar observations in *Channa punctatus* after hilban (chlorpyrifos) treatment. However, different authors observed different toxicological effects in the liver of fish after exposure to different toxicants. Kaoud *et al* (2010) have reported congestion of central vein in fish liver. Degenerative changes in hepatocytes like necrosis, vacuolization and delamination of cellular boundaries reported by Velisek *et al* (2009) and Abdel-Hameid (2009) are in agreement with findings of this study. In another study, Indirabai *et al* (2010) have observed same results in malathion treated *Heteropneustes fossilis* as found in this study. Rahman *et al.* (2002) also reported similar effect of diazinonin *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* exposed at sub-lethal concentration of the pesticide for 7 days.

Kidney: The kidney of fish receives the largest proportion of post branchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution. It has been found that the sub-lethal doses of chlorpyriphos induced alterations in the structure of kidney of *Clarias batrachus.* These results reveal that the histopathological changes are dose and duration dependent. The proximal tubules were the first to be affected resulting in the reduction of nuclear material in some cells. The control fish showed normal kidney cells with normal veins (Fig. 2A). The damage in kidney tissues further advances gradually through the glomeruli, hematopoietic

tissues, and cells of distal and collecting tubules in that order (Fig. 2B-2D). Renal tubules proliferation and dilation, necrosis of tubular epithelium and pyknotic nucleus were seen in the tissue treated with chlorpyrifos in this study (Fig. 2E). These observations are in agreement with Subburaj *et al.* (2020) in the kidney of *Oreochromis mossambicus* exposed to chlorpyrifos (50%EC) for 96h. Raibeemol and Chitra (2018) have also reported reduced kidney functions and cellular disorganization in a freshwater fish, *Pseudetroplus maculates* when exposed to chlorpyrifos.

In agreement to the results of this study Banik *et al.* (2016) have reported pyknosis, necrosis of tubular and hematopoietic cells in *G.giuris* after diazinon exposure. Ghasemzadeh *et al*. (2015) have reported injury the kidney of spotted scat fish, *Scatophagus argus* when exposed to diazinon for short-term (96h). Diazinon induced damage in kidney has also been reported by Khosravi *et al.* (2014). Similar results were observed in kidney of *Gambusia afinis* exposed to chlorpyrifos (Sabiha and Neela, 2013).Kidney lesions consisting of dilation of Bowman's space has been reported by Wannee *et al.* (2002) which in agreement to our observation.

5. Conclusion

The histopatholgical alterations observed in liver and kidney are due to acute toxic effect of chlorpyrifos in *Heteropneustes fossilis*. The study is useful in understanding harmful effects of pesticides of aquatic life.

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