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Histopathological Studies of Chlorpyrifos Toxicity in Catfish

By Nazia Khatun, Taibur Rahman & Rita Mahanta

Gauhati University

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Histopathological Studies of Chlorpyrifos Toxicity in Catfish

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I. Introduction

Pesticides have large impact on the histopathological changes in many fresh water fishes. Large scale production and overall uses of pesticides worldwide has change the bioconcentration of these chemicals which shows its effect in different organs of the fish species. The use of pesticides rises exponentially with the industrial development and agricultural growth. Side by side these pesticides create serious threat to the non-target organisms both in terrestrial as well aquatic ecosystems. Hazardous chemicals from industrial waste water and agricultural runoff are the main cause of water pollution. Aquatic organisms mainly fishes accumulate many contaminants and toxicants directly through their gills and skin and indirectly via their food chain, which may causes diverse alternations in histopathology.

Histopathology showed to be a suitable biomarker in the evaluation of the health of organism exposed to pollutants and can be used as biomonitoring tools for toxicity studies (Meyers and Hendricks, 1985). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this study allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001).

Chlorpyrifos (O, O – diethyl O-3, 5, 6 trichloro-2-pyridyl-phosphorothioate) is used as broad-spectrum chlorinated organophosphate insecticide. Chlorpyrifos (CPF) are widely used in agriculture and against pests. It is used on fruit, grain, nuts, vegetables, livestock, ornamentals, golf courses, buildings, and for treating wood products. It is formulated as liquid, granular, and flowable concentrates, baits, wettable powders and dusts. Different workers at different times reported that exposure of chlorpyrifos to fishes and other aquatic organisms has caused severe damage to liver, kidneys, gills, intestine, etc. Chronic exposure causes significant histopathological changes in liver (Barbhuiya and Dey, 2014)

Hence the present study was undertaken to examine the effect of different sub lethal dose of Chlorpyrifos at different concentrations on histopathological changes in different organs of catfish, *Heteropneutes fossilis* (Bloch).

II. Materials and Method

Healthy live fish *Heteropneustes fossilis* were purchased from the local fish market of Dhubri, Assam. The fishes were acclimatized for one week in the laboratory condition in a glass tank prior to the start of the experiment. The fishes of length 12 ± 1 cm and weight 10 ± 1 gm were selected from the tank and transferred to glass aquaria for experimental procedure.

a) *Experimental design*

Three (3) different groups (each with 5 fishes of both sexes) were used for the experiment. Group-I represent normal control group, Group-II represent sub lethal concentration (1.42 ppm) Chlorpyrifos treated group and Group-III comprises of sub lethal concentration (0.28 ppm) Chlorpyrifos treated group. The experiments were conducted in aerated glass aquaria. The fishes were exposed to two different concentrations of chlorpyrifos for a period of 30 days and one aquarium was left untreated as control group.

b) *Histological procedure*

At the end of exposure period of 30 days, fishes from each group were collected randomly and blotted

Author α: Research Fellow (Ph.D.), Department of Zoology, Cotton College, (Gauhati University), Assam, INDIA.
e-mail: naziakhatun.02@gmail.com
Author σ: Professor, Department of Pathology, College of Veterinary Science, Assam Agriculture University, Assam, INDIA.
e-mail: dr.taibur.rahman@gmail.com
Author ρ: Associate Professor (Retd.), Department of Zoology, Cotton College, Guwahati (Assam), INDIA. e-mail: ritamahanta@yahoo.co.in

dry with soft absorbent paper. Each fish was then sacrificed and dissected to collect the pieces of liver, kidney, intestine and gills. These tissues were then kept in normal saline to remove traces of blood and fixed in 10% formalin for about 24 hours. The specimen were processed for dehydration in graded alcohol, cleared in xylene and finally embedded in molten paraffin wax. Tissues were then sectioned at 4μm thickness using rotary microtome. The tissue sections were stained with haematoxylin and eosin (H & E) and mounted in DPX. Finally the prepared slides were observed under light microscope for histopathological interpretation and microphotographs were taken.

III. Results and Discussion

a) Histopathological changes

i. *Liver*

The liver of the control fish (Group-I) exhibited a normal architecture with continuous mass of hepatic cells forming a cord like structure (fig.1.A). These cords of hepatocytes were arranged around the central vein. The hepatocytes were large sized, polygonal cells with centrally located nuclei. The sinusoids were seen as communicating channels occupied by blood cells and Kupffer cells. The liver treated with high dose of Chlorpyrifos (Group-II) showed centrilobular necrosis characterized by necrosis of hepatocytes around the congested central vein. Hepatocytes showed increased granularity of cytoplasm with nuclear pyknosis leading to necrosis and complete loss of hepatic parenchyma (fig.1.C). The liver of Group –III fishes treated with low concentration of chlorpyrifos was reflected by disorganization of hepatic cords, nuclear pyknosis, necrosis with complete loss of hepatocytes at many places (fig.1.D). Fine fibrillar structures were present at many places with loss of hepatic cytoplasm, hemorrhage and congestion.

Similar observations were made by Pandey and Dubey, (2015) which showed degeneration and disintegration in most cytoplasmic contents, necrosis along with pyknosis and rupture of hepatocytes on exposure of pentachlorophenol (PCP) to *H.fossilis* for 21 days. Pyknotic nuclei in liver of malathion treated *H.fossilis* were also observed by Deka and Mahanta (2012). Cytoplasmic vacuolation, cellular degeneration, congestion in blood sinusoids has also been reported in the earlier studies after exposure of aluminium in Tilapia zilli for 96 hours (Hadi and Alwan, 2012). This finding is in agreement with Barbhuiya and Dey (2014), Sakr and Lail (2005).

ii. *Kidney*

There was no histological change in the kidney tissue of fish in control group. Histological structure of kidney of control *H.fossilis* showed numerous nephrons, which was composed of renal corpuscles with a well developed glomerulus and renal tubules. Glomerular tuft consists of blood capillaries surrounded by Bowman's capsule (fig.2.A). The tubules were lined with single epithelial cell layer having basal nuclei at the proximal segment while the distal part showed nuclei in central position. The interstices of the tubules were enriched with haematopoietic tissue. Results of the present study demonstrated that sublethal concentration of Chlorpyrifos produces large histopathological alternations in the kidney of treated fishes. Changes observed in Chlorpyrifos group (Group-II) fishes include extensive damage to the renal tubular epithelium with necrosis and complete loss of hematopoietic tissues with presence of some golden brown haemosiderin pigments indicating haemolysis (fig.2.C). The kidneys of Group III chlorpyrifos treated *H.fossilis* exhibited tubular degeneration and the glomerular tufts showed severe damage with vacuolation and loss of glomerular structures (fig.2.D).

Disintegration of glomeruli, increase in Bowman's space, elongation of tubules and damage of haematopoitic tissue in kidney was observed at the end of 21 days treatment of pentachlorophenol (PCP) by Pandey and Dubey, (2015). Similar findings were observed in dieldrin and BHC treated fish *Cyprinus carpio* (Satyanarayan *et al*., 2012). Shrunken glomerulus and conjested to severe degeneration of tubules, vacuolization and dialation of tubules were reported in the study of Tripathi and Srivastava, (2010) with chlorpyrifos in Wister rats. Shrinkage of glomeruli and widening of nephritic tubules was also reported in catfish, *H.fossilis* exposed to chlorpyrifos at a concentration of 2mg per litre (Srivastava *et al.,* 1990).

iii. *Gills*

Normal architecture of gill was observed with intact primary and secondary lamellae, gill arches and gill rays (fig.3.A). Comparing with the control set, it was found that the treated fish with higher concentration of sublethal dose showed swollen gill lamellae with mild congestion. All structures are greatly enlarged as compared to the control group. There was also increase in number of infiltrating cells in both the filaments as well as the lamellar structures (fig.3.C). Degenerative changes were also distinctly observed in experimental fishes with lower concentration of sublethal dose though it is of lower intensity (fig.3.D).

Literature review shows that when Tilapia zilli treated with aluminium, histopathological changes such as cellular hypertrophy or hyperplasia of primary filaments and fusion of secondary lamellae occurred (Hadi and Alwan, 2012). Degenerative epithelium of gill filaments and secondary lamellae accompanied by separation of their epithelium from the lamellar supporting cells was also demonstrated in the experiment of Bhuvaneshwari et al., (2015) on Zebra fish exposed to organo chlorine pesticide and heavy metals. Dilation of blood capillaries, abnormal swellings

epithelium was also observed by Banee et al., (2013) in Rainbow trout exposed to diazinon.

Architectural distortion of the gill tissue to the chlorpyrifos exposed tadpole larvae of Asian common toad, *Duttaphrynus melanostictus* was reported in the study of Bandara et al., (2012). Hypertrophy of lamellar epithelium, destruction of gill lamellae and blood congestion was reported in cadmium chloride treated fish *Ophiocephalus (Channa) striatus* (Bais and Lokhande, 2012).

iv. *Intestine*

The intestine of normal control fish *H.fossilis* showed lymphoid aggregation at the base of villi. Glandular epithelial layer having cuboidal and longitudinal tissue at the base of villi structure was also observed. The sublethal dose (1.42 ppm) of chlorpyrifos exposure results showed significant changes in the intestine of *H.fossilis*. There was atrophy on the villi structure, degeneration and necrosis of mucosal epithelium of intestine and depletion of lymphoid follicles (fig.4.C).

0.28 ppm of chlorpyrifos exposure for 30 days also showed atrophy and complete disappearance of villi structures in most of the areas. Some of the villi showed focal areas of necrosis (fig. 4.D). The mucosal epithelium was found to be flattened and shrunken.

Various studies at different times reveal that intestinal mucosa and villi structure degenerate upon exposure to different harmful chemicals which ultimately hamper the absorption process. The histopathological alternations observed in the intestinal tissue of the experimental fish are in agreement with those of Mandal and Kulshrestra, (1980) who carried the experiment on *Clarius batracus* treated with submithion; Yildririm *et al*., (2006) showed deleterious effects on tissues on exposure to deltamethrin. Disintegration of the intestinal tissue was observed in the study of cypermethrin administered fish *Oreochromis mossambicus* by Karthigayani *et al*., (2014). Ulceration alongwith erosion of the mucosa, distortion of papillae was demonstrated in flying barb *Esomus danricus* upon exposure to 0.179 μg/l and 1.79 μg/l of malathion respectively for 28 days (Das and Gupta, 2013). Necrosis, degenerative change of mucosal epithelium as cloudy swelling was observed in the intestine of cadmium chloride treated fish, *O.striatus* by Bais and Lokhande, (2012). Flattening of the intestinal folds was well defined in aldrin exposed *Cyprinus carpio* (Satyanarayan et al., 2012).

IV. Conclusion

The present study showed that chlorpyrifos at different sublethal concentration causes significant histomorphological changes in the liver, kidney, gill and intestine of *H.fossilis*. The histopathological changes seen in all the tissues were more pronounced in sublethal concentration at higher dose of chlorpyrifos than lower dose level. Hence the results of the present study are indicative of the related changes in different tissues induced by chlorpyrifos toxicity which was found to be significant at high dose level and chlorpyrifos in sublethal dose of minimum 0.28ppm is found to be effective in causing degenerative changes in tissue architecture.

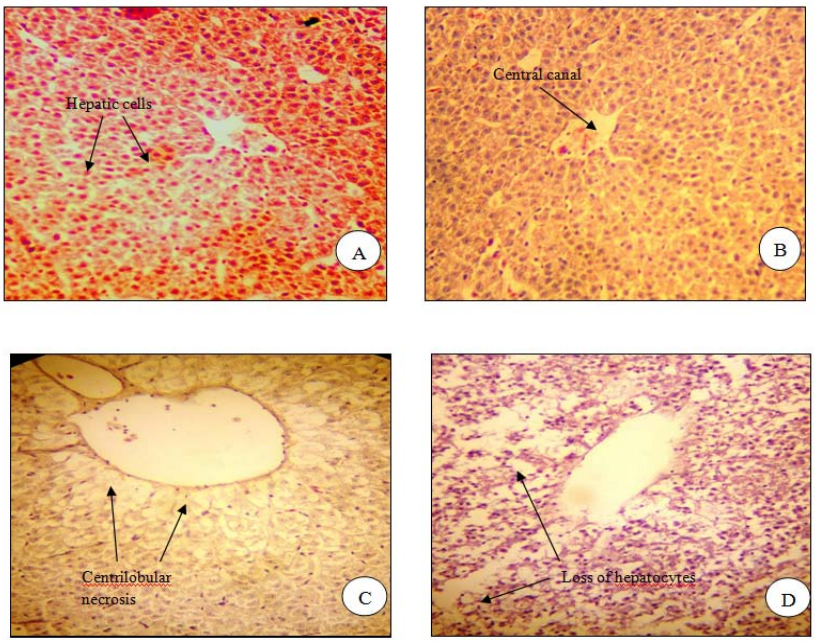

Fig.1: (A) Liver of normal control fish. (Magnification X100)
(B) Liver of normal control fish showing the central canal. (Magnification X100)
(C) 1.42 ppm (1/10th of LC_{50}) Chlorpyrifos treated Liver. (Magnification X400)
(D) 0.28 ppm (1/50th of LC_{50}) Chlorpyrifos treated Liver. (Magnification X100)

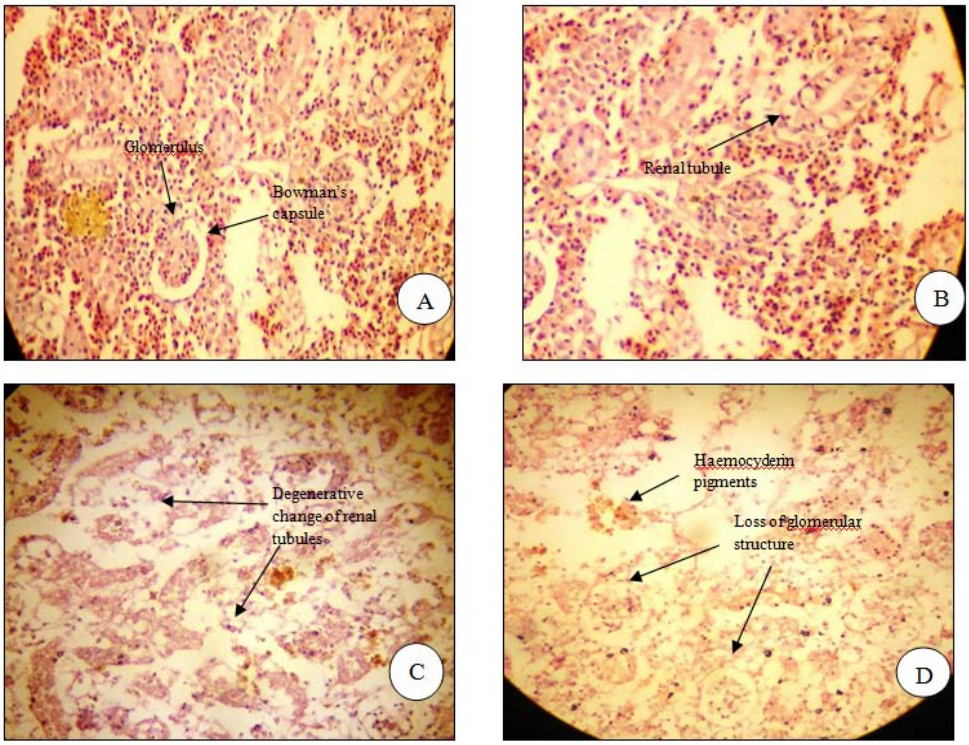

Fig. 2: (A) Kidney of normal control fish. (Magnification X100)
(B) Kidney of normal control fish showing the renal tubules. (Magnification X100)
(C) 1.42 ppm (1/10th of LC_{50}) Chlorpyrifos treated Kidney. (Magnification X100)
(D) 0.28 ppm (1/50th of LC_{50}) Chlorpyrifos treated Kidney. (Magnification X100)

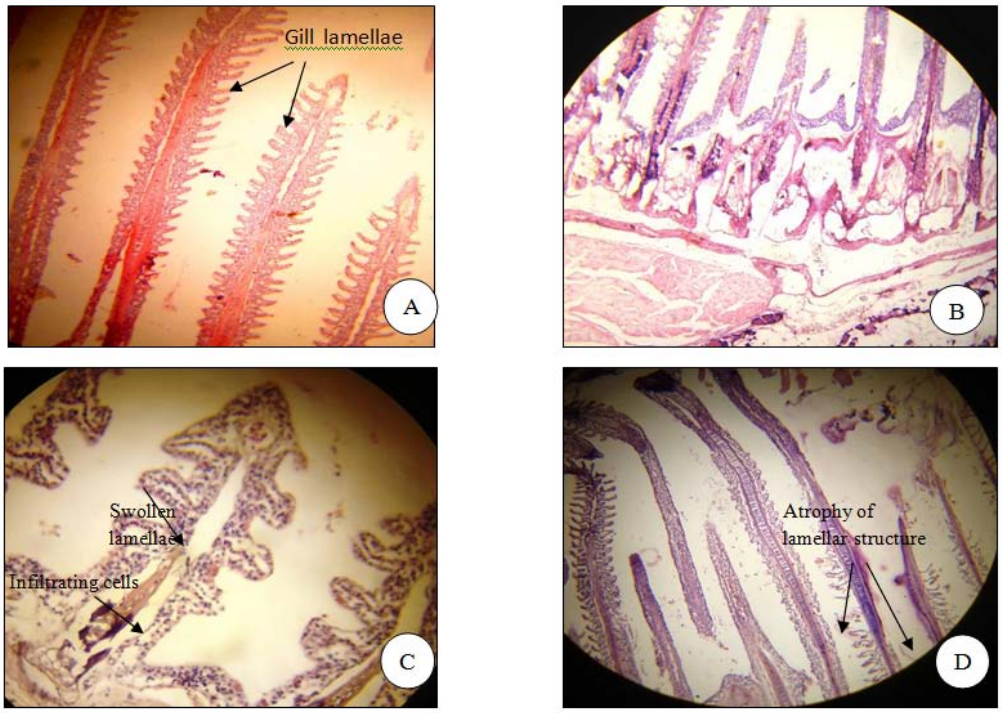

Fig. 3: (A) Gill structure of normal control fish showing the gill lamellae. (Magnification X400)
(B) Gill structure of normal control fish. (Magnification X100)
(C) 1.42 ppm (1/10th of LC_{50}) Chlorpyrifos treated gill structure. (Magnification X400)
(D) 0.28 ppm (1/50th of LC_{50}) Chlorpyrifos treated gill structure. (Magnification X100)

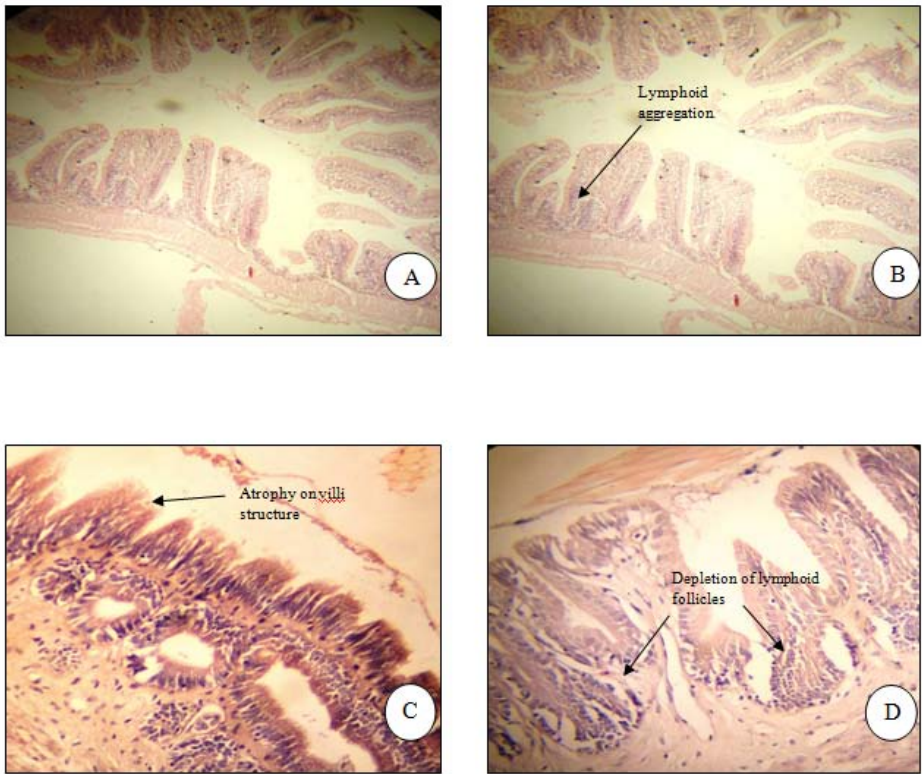

Fig. 4: (A) Intestine of normal control fish. (Magnification X100)
(B) Intestine of normal control fish showing the lymphoid aggregation. (Magnification X100)
(C) 1.42 ppm (1/10th of LC_{50}) Chlorpyrifos treated Intestine. (Magnification X100)
(D) 0.28 ppm (1/50th of LC_{50}) Chlorpyrifos treated Intestine. (Magnification X100)

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