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Toxicological effects of glyphosate on histopathological parameters of fresh water fish, *Cirrhinus mrigala*

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Abstract
Human population growth and industrial development have been the major causes of water contamination around the world during recent years. The ecological effects of pollutants in aquatic ecosystems and theirbio availability and toxicity are closely related to species distribution, both in the solid and the liquid phase of the aquatic ecosystem. The poisoning by pesticides from agricultural fields is a serious water pollution problem and its environmental long-term effect may result in the incidence of poisoning of fish and other aquatic life forms.

Keywords: Glyphosate, *Cirrhinus mrigala*, Histopathology

Introduction
Pollution of aquatic environment from industrial, domestic and agricultural waste has exposed these important aquatic organisms to contaminants which not only threatened their lives but also eventually enter the food chain leading to serious public health hazards. Unfortunately, the indiscriminate use of these pesticides and herbicides to improve agricultural production and yield may have impacts on non-target organism, especially aquatic life and environment (Nwani *et al*. 2010) [7].
Pesticides and herbicides at high concentration are known to reduce the survival, growth and reproduction of fish (Rahman *et al*., 2002) [10]. Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety. Aquatic bioassay are necessary in water pollution control to determine whether potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa *et al*., 2003) [8].
Fishes are very sensitive to a wide variety of toxicants inwater; various species of fish show uptake and accumulation of many contaminants or toxicants such as pesticides. Histopathology is the microscopic examination of tissue in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides. In contrast, cytopathology examines free cells or tissue fragments.
Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucous secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metals (Nowak, 1992) [6]. Also the liver is a very important organ which breaks down chemicals and as a result, liver, cells are often among those that are damaged by toxic chemicals.

Materials and Methods
Cirrhinus mrigala is also known as the mrigal and white carp. It is a species of ray finned fish in the carp family. Native to the streams and rivers in India, the only surviving wild population is in the Cauvery river, leading to its IUCN rating as vulnerable. Mrigal is popular as a food fish and an important aquacultured fresh water species throughout south Asia. The Indian carps are considered as a delicacy compared to other exotic carp species also cultured in Asia and sell for higher prices. 1 ml of glyphosate is mixed with 1000 ml of water for preparing stock solution (0.1%).

Appropriate narrow range of concentration 2-10 ml was used to find the medium lethal concentration using a minimum of 10 fishes for every 24 hours upto 96 hours. It was found as 6.2 ppm for 72 hours using probit analysis method (Finney 1961). Four groups of fishes were exposed to 0.62 ppm (1/ 10^{th} of 72 hours *LC50* value) concentration of herbicide Glyphosate' for 24, 48, 72, 96hours repectively. Another group was maintained as control. For each experimental study tissue samples were collected from the fish for the analysis of histology of gill, liver, kidney, muscle.

Results and Discussion

Gill Histology

Control

Histological changes noticed at gill tissue of *Cirrhinus mrigala* compared with control group. The gills of control fish showed a row of long thin filaments, the primary lamellae, On its distal and ventral surface were the secondary lamellae. Secondary lamellae consists of an envelope of epithelial cells, usually one layer thick, supported and separated by pillar cells arranged in rows. In this control fish, no recognizable changes were observed in the gills.

Long term exposure

Gills of *Cirrhinus mrigala* exposed for 10 days shows the epithelial disruption and damage and after 20 days of exposure necrosis of epithelium, hyperplasia, desquamation, epithelial lifting, oedema and lamellar fusion were observed. Oedema and epithelial lifting are the results of defence mechanism. As the distance of the lamellar epithelium increase from the secondary gill lamellae, it prevents the direct diffusion of the toxicant present in the water body. These kinds of histological changes in the gills have been observed by researches due to toxic effects of copper (Fernandes *et al*., 2007) [3]. Most common changes like hyperplasia, desquamation, and necrosis of epithelium, epithelium lifting, oedema, lamellar fusion, collapsed secondary lamellae and curling of secondary lamellae have been reported by Velumurugan *et al*., (2009) and Jiraungkoorshul *et al*., (2003) in *Cirrhinus mrigala* and *Oreochromis niloticus* exposed to pesticide and herbicide.

Liver Histology

Control

The histology of control fish liver revealed normal typical paranchymatous appearance. The liver was made up of hepatocytes that were polygonal cells with a central spherical nucleus and densely stained nucleolus. Histological changes noticed at liver tissue of *Cirrhinus mrigala* compared with control group. Liver of control fish composed of large number of polyhedral hepatic cells containing a granular cytoplasm.

Long term exposure

Liver of *Cirrhinus mrigala* exposed to 10 days shows mildly swollen hepatocytes with numerous lipid vacuoles, and hydropic swelling of hepatocytes in which the nucleus retained a nearly normal shape. And also swelling of hepatocytes of pyknotic nuclei, and large lipid vacuoles were found. After 20 days of exposed liver shows very severe diffuse vacuolution of hepatocytes and disintegration of hepatocytes were observed and the cell outline was no longer discernible. In intact cells, severe necrotic nuclei were observed.

Radhaiah and Jayantha Rao, (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, picnotic nuclei in the liver of *Tilapia mossambica* exposed to fenvalerate. Swelling of the hepatocytes with diffuse necrosis and marked swelling of blood vessels were observed in the liver tissue by Das and Mukherjee, (2000) when *Labeo rohita* was exposed to hexachlorocyclohexane.

Kidney Histology

Control

In the control fish, the kidney was composed of numerous renal corpuscles with well-developed glomeruli and a system of tubules. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei and brush border was reduced or not present. The glomerulus was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border.

Long term exposure

Kidney of *Cirrhinus mrigala* exposed for 10 days shows hydropic swelling of tubules. Lipid vacuoles were accumulated in proximal tubular cells. After 20 days of exposure shows mildly swollen proximal tubular epithelial cells with dilated nuclei were observed. And also kidneys showed sever swelling of tubules.

The kidney of the fish exposed to glyphoste concentrations showed dilation of Bowman's space and accumulation of hyaline droplets in the tubular epithelial cells of the first proximal tubule. Oulmi *et al*., (1995) [9] studied the effects of linuron herbicide on the rainbow trout (*Oncorhynchus mykiss*) and their results showed small cytoplasmic vacuoles, nuclear deformation in the epithelial of the first and the second segments of the proximal tubule. The kidney cells were observed to have been massively destroyed. The renal corpuscles of the kidney were scattered resulting in their disorganization and consequently obstruction to their physiological functions. Some of the kidney cells were found clogging together while they were disintegrated in some tissues of the organ.

Muscle Histology

Control

Muscle of *Cirrhinus mrigala* shows normal structure. Myotomes and myoseptum is seen clearly. The epidermis is seen clearly, appearing normal and individual myptomes are separated by myoseptum this forms the origin and insertion for muscle fibres from adjacent myotomes.

Long term exposure

Muscle of *Cirrhinus mrigala* exposed to 10 days of toxicant shows that in many places the myofibrils are broken and muscle seems to have lost the myoseptum that separates each myotomes. Disintegrated epidermis is also seen in the section of muscle. After 20 days of exposed muscle shows disintegration of myotomes is seen, bundling of myotomes is seen in few places and septum is not distinct. And lesions are observed in many palces. broken myofibrils is also obtained. Bharat *et al*., (2011) [1] studied the histopathology of fish *Cyprinus carpio* exposed to sublethal concentrations

of lead and cadmium. The fish showed marked thickening and separation of muscle bundles with severe intracellular edema. Similar observation has been made by Das and Mukharjie (2000) [2].

Conclusion

This study reveals that the histological status of herbicide treated fish showed various severe alternations in gill, liver, kidney and muscle. Thus the usage of the herbicide should be restricted to have a healthy ecology. Thus the use of herbicide should be avoided or minimal use is recommended.

Histology of gill of *Cirrhinus mrigala*

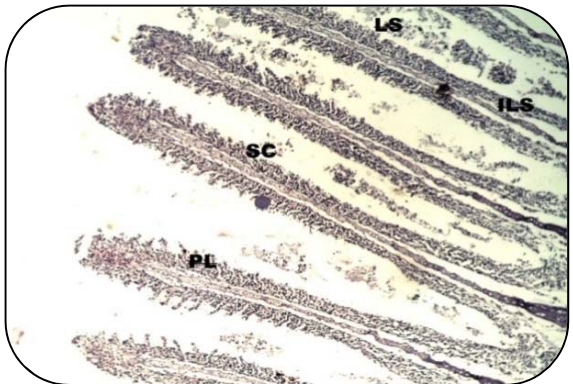

Fig 1: Control gill section of fish

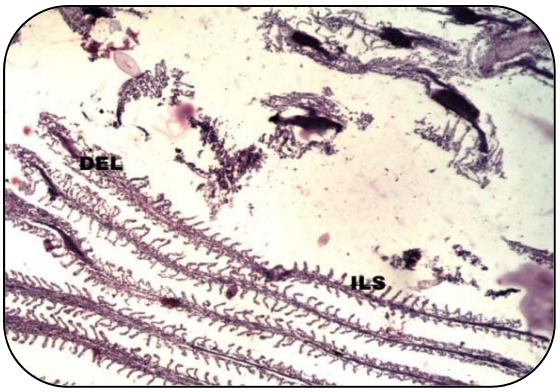

Fig 2: Gill section of fish exposed to 10 days of herbicide

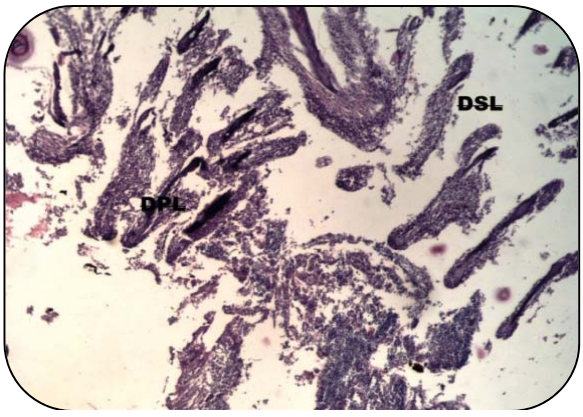

Fig 3: Gill section of fish exposed to 20 days of herbicide

PL- primary lamellae, SL- secondary lamellae, LS- lamellar spaces, ILS- inter lamellar spaces. DEL- degeneration of lamellae, DPL- degradation of pimary lamellae, DSL- degeneration of secondary lamellae. DEL- degeneration of epithelial lining.

Histology of liver of *Cirrhinus mrigala*

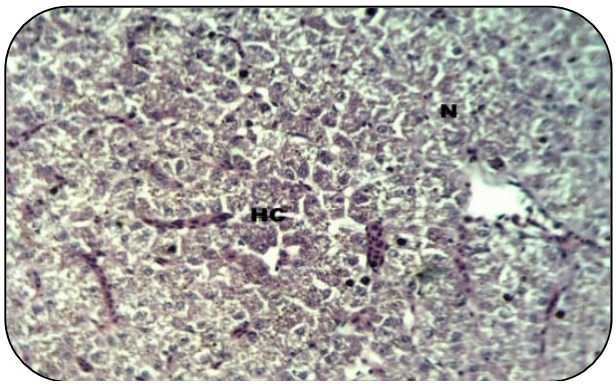

Fig 4: Control liver section of fish

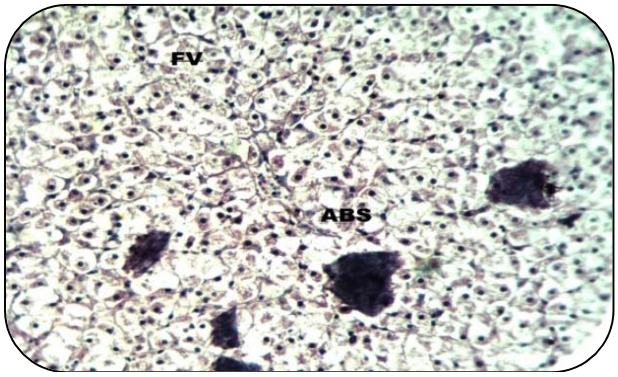

Fig 5: Liver section of fish exposed to 10 days of herbicide

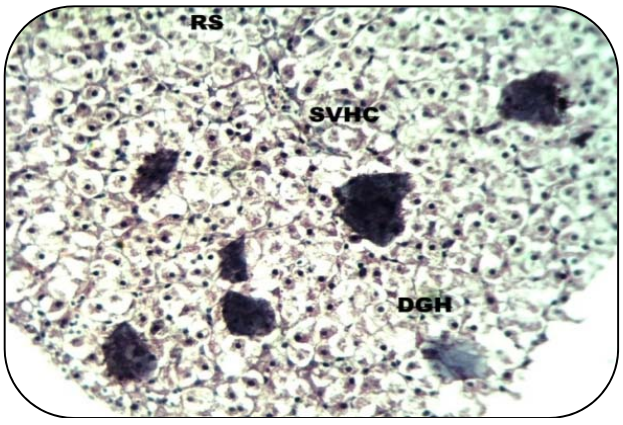

Fig 6: Liver section of fish exposed to 20 days of herbicide

HC- hepatocytes cells, VC- vacuoles, BV- blood vessels, Ec- erythrocytes N- nucleus, showing hydropic swelling of hepatocytes and numerous large lipid vacuole accumulation, SVHC- severe vacuolation of hepatocyte cells, ABS- appearance of blood streaks, DHC- degradation of hepatocyte cells, RS- rupure of sinusoids.

Histology of kidney of *Cirrhinus mrigala*

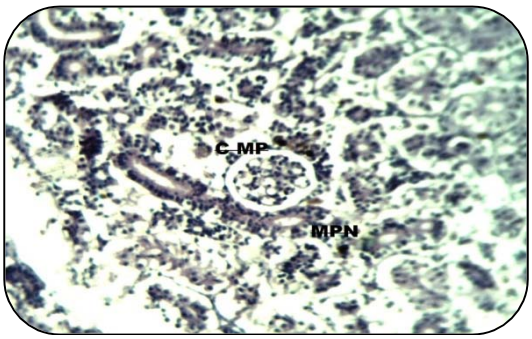

Fig 7: Control kidney section of fish

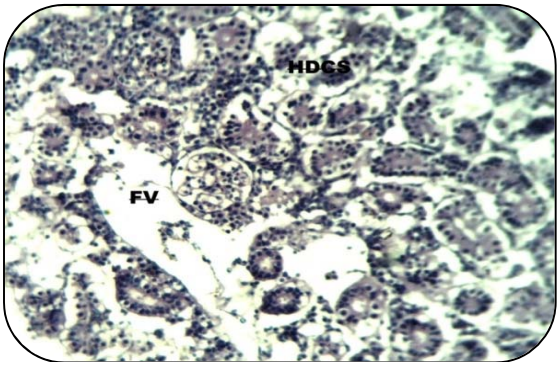

Fig 8: Kidney of fish exposed to 10 days of herbicide

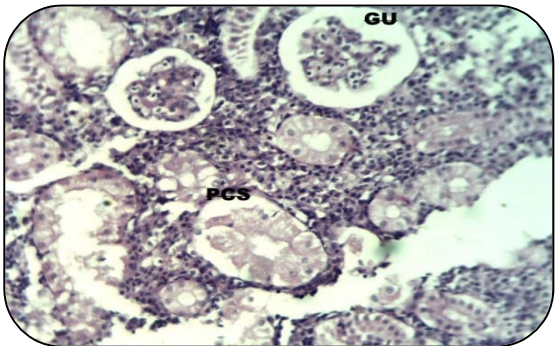

Fig 9: Kidney of fish exposed to 20 days of herbicide

U- ureter, G- glomerulus, PCS- proximal convoluted segment, DCS- distal convoluted segment, FV- formation of vacuoles, HDCS- hypertrophy of distal convoluted segment, CMP- congestion and mild pyknosis.

Histology of muscle of *Cirrhinus mrigala*

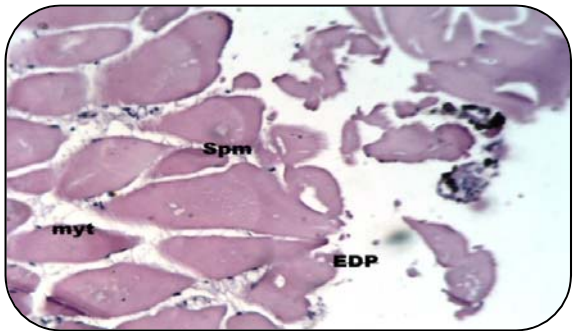

Fig 10: Control muscle section of fish

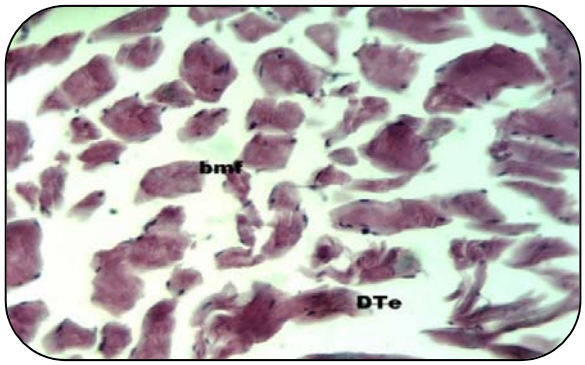

Fig 11: Muscle section of fish exposed to 10 days of herbicide

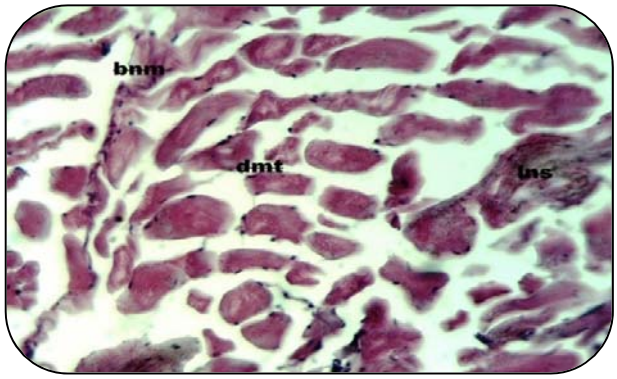

Fig 12: Muscle section of fish exposed to 20 days of herbicide

Spm- septum, Myt- myotomes, Edp- epidermis layer, DMT- disintegrated myotomes, DTE- disintegrated epidermis, BMF- broken myofibrils, BNM- bundling of myotomes, Lns- lesions.

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