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Histopathological modulations in liver tissue of commercially edible fish, *Catla catla* treated with lihocin

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Abstract

In the present study the histomorphological changes in liver was observed for a period of 45 days exposed to an organochlorine pesticide Lihocin, caused alterations in liver. Liver architecture indicates compactly arranged hepatocytes, with a strong cytoplasmic vacuolization. With increased basophilion suggestive of chronic toxicity with eccentric or Pkynotic nuclei. The liver is a large vital organ of fish. Liver has a wide range of functions, including detoxification, protein synthesis, and production of enzymes necessary for digestion. Liver has a central role in the metabolism and it is an important organ for the analysis of stress conditions and diseases. Hepatocellular necrosis with parenchymal vacuolization, breakdown of cell boundaries, swelling and degeneration of the endothelial lining cells. Damage of central veins are also observed, with increase in the duration of exposure to Lihocin. The sub-lethal concentration of Lihocin in fish caused to increase oxidative stress that causing additional increase of hepatic connective tissue and also caused formation of number of Melano-macrophage aggregations. The liver showed swelling and Pyknosis of hepatocyte nuclei. The Lihocin caused extensive pathological modulations in liver hepatocytes. The liver as day's prolonged under lihocin showed advanced stage of hepatocyte damage. The lihocin showed extensive change in architecture of hepatocytes.

Key words: *Catla catla*. Lihocin, Liver, Hepatocytes, Nucleus, Histopathology, Melanomacrophage centres (MMC's).

Introduction

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Organochlorine pesticides are one of the major classes of toxic substances used in India for control of pest in agricultural lands and insect vectors cause human diseases. The runoff water from pesticide treated areas enters the river and aquaculture ponds, cause adverse effects on fish (Begum, 2004). Fishes are sensitive to different concentrations of pesticides and their tissues are prone to pathological effects. Toxicants impair the metabolic and physiological activities of an organism, but such studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Hence it is useful to have insight in to histological analysis. The importance of study of the histopathological modulations.There are few reports on effect of different pesticides in the different organs of fish, have been well documented (Richmonds and Dutta, 2001; Tilak et al., 2001; Janardana Reddy, 2012).

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Histopathological biomarkers are closely related to stress, since many pollutants either toxic or non toxic have to undergo metabolic activation in order to be able to culminate cellular change in the affected organism. The mechanism of action of several xenobiotics could initiate the formation of a specific enzyme activity that causes changes in metabolism, further leading to cellular intoxication and finally death. These manifests as necrosis, as well as from chemical insult, lesions may arise and other degenerative alterations to which the organism responds with an inflammatory, defensive mechanism (Roganivic - Zafirova et al., 2003; Velkova - Jordanska, 2005).

The liver is an organ notable for its sensitivity to a grade variety of environmental factual. It is biomarker of environmental pollution. The liver is a large vital organ of fish. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion. Liver has central role in the metabolism and it is an important organ for the analysis of stress conditions and diseases. The liver synthesizes a variety of organic compounds like albumin, fibrinogen, urea, uric acid, prothrombin, lipoprotein

transferring glycoprotein, hippuric acid, cholesterol and other lipids.

Glucose stores in the liver as glycogen and is return to the blood circulation when the glucose level depletes other than glycogen. The liver stores fat soluble vitamins (A, D, E and K) and vitamin B12. The excretory and detoxifying function of the liver are evident from the process of removal of free bilirubin, the product of haemoglobin breakdown. Many toxic substances are similarly removed from blood circulation through the bile duct and many of these substances are insoluble in water and hence cannot follow the kidney route. Diagnostic test results for the assessment of liver function includes increase of serum transaminase activity.

Histopathological studies have been conducted to help for establishment exposure and other various biological responses. These investigations have also been proved to be a sensitive tool to detect the direct effects of chemical compounds with in target organs of fish in laboratory experiments (Schwaiger et al., 1996; Machado and Fanta, 2003; Sakr and Jamal Allail, 2005). Such analysis appears to be very sensitive parameters and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads (Dutta, 1996). Investigation of such nature may therefore prove to be a cost effective tool to determine the nature of fish populations, hence reflecting the well being of an entire aquatic system.

The advantage of histopathology as a biomarker lies in its intermediate location with regard to the level of biological organization. Histological changes appear as a medium term response to sub lethal stresses and histology provides a rapid method to detect the effects of irritants, especially chronic once, in various tissues all once gill, liver, kidney and muscle are suitable organs for histological examination in order to determine the effect of pollution.

A review on available literature of fish and environmental pollutant indicate that the sub lethal doses of most of the pesticides cause varying extent of histopathological injuries to different organs in fishes, the amount of damages are usually dependent on those duration of exposure and type of pesticide (Oliviera Ribeiro et al., 2005; Camargo and Claudia, 2007; Advait Bhadgade, 2012).

Hence an attempt has been made to study the histopathological modulations in the tissues of liver of fresh water fish, *Catla catla* exposed to sub lethal (LC_{50}) Concentration of Lihocin, for 45 days exposure period.

Material and Methods

LC_{50} of Lihocin to *Catla catla* was identified by the method of Finney (1971) as 0.447 mg/L. After some trail experiments, the 1/5th sub lethal concentration was identified as 0.0894 mg/L.

The fish *Catla catla* (15 ±3g) were exposed to 1/5th sub lethal concentration of Lihocin, an Organochlorine insecticide for 3, 7, 15, 30 and 45 days were sacrificed and liver tissue was quickly isolated and washed in fish ringer solution (Ekberg, 1958). Histopathology of the tissues was studied by the method of Clayden (1962).

The physiological saline solution (0.75% Nacl) was used to rinse and clean the tissues. They were fixed in aqueous Bouins fluid for 48 hours, processed through different series of alcohol, cleared in xylene and embedded in paraffin wax. Sections were cut at 6µ thick, stained with Ehrlich hematoxylin / eosin, dissolved in 70% alcohol and were mounted in Canada balsam. Tissue damage at cellular level caused by the Lihocin is examined and the change in the individual cells are visualized to ultimately arrive at a conclusive diagnosis by employing microscopic examination of tissue, in which the tissue is sectioned to single cell thickness and stained to differentiate the individual tissue elements. The tissues are then transferred to the blick marker. The tissue are embedded in paraffin was (58-60^{0}c) blocks. Sections were cut of 4-6 µ thickness stained with Haematoxylin – Eosin (dissolved in 70% alcohol) (Humason, 1972).

If deeper shade of red is desired in staining, add 5cc of glacial acetic acid to each 100cc of strain. After staining, the slides were made in to permanent slides using DPX mountant Canada balsam.Histopathological lesions were examined and photographed with the help of Pentium QX3 computer attached microscope under 400X lens.

Results & Discussion

Fish are extensively used to assess the health of aquatic ecosystems in their physiological changes serve as biomarkers to monitor the environmental pollution. (Kock et al., 1996). In this study the liver and kidney was the prime target for the evaluation of pesticide Lihocin accumulation in *Catla catla* fish. The present study reviewed that the fish *Catla catla* treated with pesticides manifest histopathological changes in liver.

General histology of Liver

The surface of liver is covered with serous membrane and some connective tissue extends inward into parenchyma. It is composed of parenchymal cells (hepatic cells) (HC) and lattice

fibres, which support the former. Hepatic cells are roundish polygonal, containing clear spherical nucleus (N). They are located among sinusoids forming cord like structures known as hepatic cell cords. In fish, these structures are generally obscure. Bile canaliculus (BC) is centrally located in each cord. Fairly large quantities of lipid glycogen granules (LGG) are also observed in the cytoplasm of fish hepatic cells (Figure: 1). Hepatic cells have many vital functions. Other than the secretion of bile, they play an important role in protein, lipid and carbohydrate metabolism. They serve as storage sites for some nutrients and detoxification is an another function attributed to them.

In the present investigation liver of a control fish exhibited a normal architecture with hepatocytes presenting a homogenous cytoplasm in a large spherical nucleus (Fig-1). Hepatocytes were located among blood capillaries called Sinusoids (SS) forming cord like structures.

The liver of fishes is a dense organ centrally located in the cranial region of the general body cavity. Its shape, size and volume are adapted to the space available between other visceral organs. Liver cells formed more than 80% of the liver parenchyma and in fish liver they are set around the capillary space 'sinusoids' between sinusoid endothelium and the membranes of the hepatocytes there is very sinusoidal space (or) space of disso of varying width. There is no basal membrane under epithelium of the liver sinusoids of fish (Cicik and Engine, 2005). Hepatic cells may have many vital functions other than the secretions of the bile they play an important role in protein, lipid and carbohydrate metabolism. They serve as storage site for some nutrients. Detoxification is another important function.

The microscopic studies of normal liver of fish revealed that the normal liver histomorphology. The liver tissue was composed was of large hexagonal or pentagonal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. The hepatocytes are embedded in tribiculus running radiantly from the central vein and the spaces between the cell cords called Blood sinusoids which converged towards the central vein and lined by Kpuffer cells. The cords extended between central and portal zones.

The hepatocytes are also ranged in regular pattern and contain a large spherical nucleus which is a district mark nucleolus and a peripheral chromatin distribution, however some cells may have two nuclei (Fig- 1). It is obvious from the results the sub lethal concentration of Lihocin disrupted structural integrity of the liver tissue of fresh water fish *Catla catla*.

The histopathological changes in fishes on day 3 treated with sub lethal concentration of Lihocin indicate compactly arranged hepatocytes but with a strong cytoplasmic vacuolization with increased basophilion suggestive of chronic toxicity with eccentric (or) Pkynotic nuclei. Hepatocellular necrosis with parenchymal vacuolization breakdown of cell boundaries swelling and degeneration of the endothelial lining cells leading to the damage of central veins are also observed, with increase in the duration of exposure to Lihocin.

The histopathological modulations in fishes on day 7 treated with sub lethal concentration of Lihocin revealed loss of basic architecture of liver moderately vacuolated hepatocytes (Fig - 3). The sections of the liver of *Catla catla* on day7 showing the appearance of Melano macrophage centres and blood lacunae.

The sub-lethal concentration of Lihocin on day 15, (Fig - 4) in fish caused to increase oxidative stress that causing additional increase in hepatic connective tissue also formation of number of Melano-macrophages (Fig - 4 to 6). The liver showed swelling and Pyknosis of hepatocyte nuclei. The liver showed extensive changes in hepatocytes, the liver as exposure period prolonged showed advanced stage of hepatocyte damage.

Several works have reported degenerative changes in hepatic tissue subjected to pollution by various pesticides and insecticides (Gill et al., 1990; Pandey et al., 1996; Tilak et al., 2001; Sakar and Jamal Al lail, 2005; Janardana Reddy, 2012). All the histopathological observation indicated that exposure to sub lethal concentration of Lihocin caused destructive effect in the liver tissue of *Catla catla*.

Conclusion

In the present study we conclude that main alterations found in the liver showed altered tissue architecture, nuclear vacuolization, hepatocyte damage and presence of Melano-macrophage aggregations cytoplasmic and nuclear degenerations were also observed. The tissues were slightly too moderately damaged as is evident by altered in histomorphological structure and cytoplasmic degeneration.

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Reddy *et al.*, 8(6): June, 2017:5531-5536]
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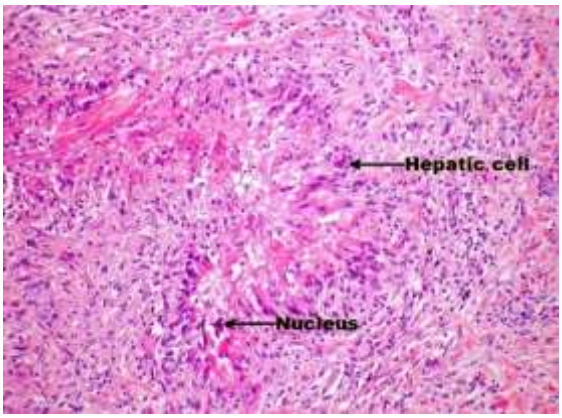

FIGURE-1: Section of the Liver of *Catla catla* (Control): Showing large number of hepatocytes and Nucleus.

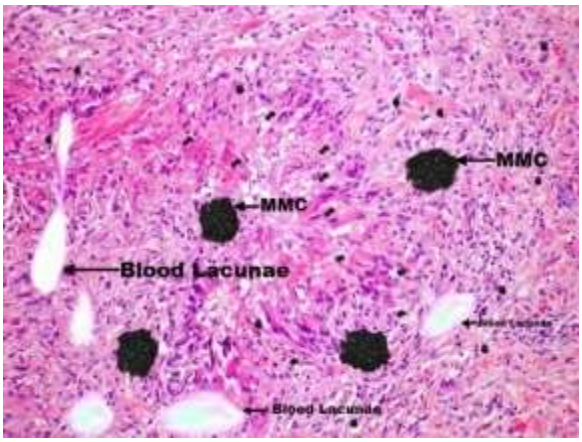

FIGURE-3: Section of the Liver of *Catla catla* (Experimental -7 days): Showing the appearance of Melano-macrophage centres and Blood lacunae exposed to Lihocin.

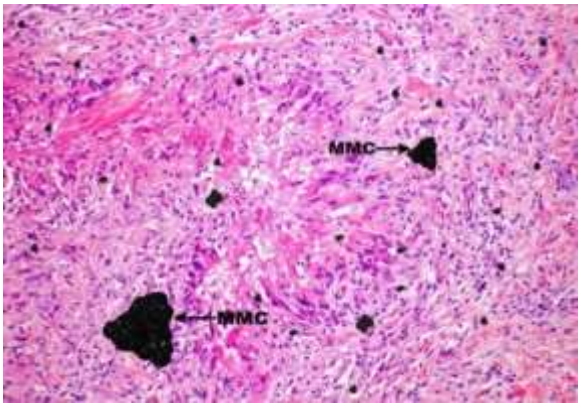

FIGURE-2: Section of the Liver of *Catla catla* (Experimental - 3 days): Showing the appearance of Melano-macrophage centres exposed to Lihocin.

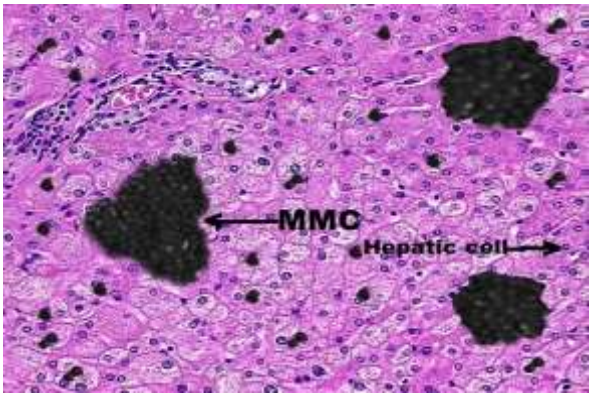

FIGURE-4: Section of the Liver of *Catla catla* (Experimental -15days): Showing the appearance of Melano-macrophage centres and Hepatic cells.

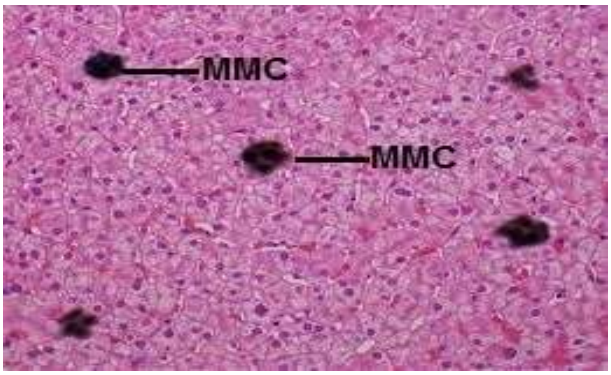

FIGURE-5: Section of the Liver of *Catla catla* (Experimental - 30 days): Showing the aggregation of Melano-macrophage centres exposed to Lihocin.

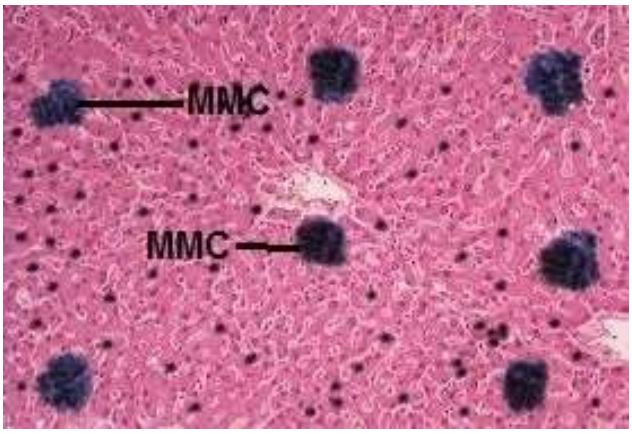

FIGURE- 6: Section of the Liver of *Catla catla* (Experimental - 45days): Showing the aggregation of Melano-macrophage centres exposed to Lihocin.

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