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HISTOPATHOLOGICAL BIOMARKERS AS INDICATORS OF AQUATIC POLLUTION

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ABSTRACT

There are a number of studies reporting the pathological changes in fishes exposed to different Organo Chlorine, Organ Phosphorus, Carbamate and Pyrethroid pesticides. The extent of severity of tissue damage of particular compound as toxicant depends on its toxic potentiality in the tissues of organisms. In the present study Gambusia affinis were exposed to 1/10thsub-lethal concentration (1/10thof LC_{50} value is 0.0294 ppm) of technical grade chlorpyrifos, (LC_{50} is 0.294 ppm) for a period of 15, 30 and 45 days. The treated fish groups were compared with the control group for histopathological changes in liver and marked changes were observed. The liver showed acute necrosis of hepatocytes and peribiliary cirrhosis due to Chlorpyrifos, toxicity. The hepatic cellular necrosis was manifested by fibrosis of hepatic tissues. The liver showed biliary proliferative hyperplasia. There was severe biliary profierative hyperplasia and the major ducts were greatly distended.

KEYWORDS: Gambusiaaffinis, Chlorpyrifos, Histopathological, Liver.

INTRODUCTION

Pesticides are used widely all over theworld to control the harmful effects of pestson agriculture productions. The environmental impact of pesticide use has been discussed much due to its widespread use in parallel with the modernization of agricultural operations and indiscriminate permeation of the ecosystem with these pesticides.

It is apparent that human chemical additions have introduced or increased environmental stress for aquatic organisms and fishes, in particular. Pesticides are toxicants capable of affecting all taxonomic groups of biota, including non-target organisms, to varying degrees depending on physiological and ecological factors. Many pesticides are resistant to

environmental degradation so that they persist in treated areas and thus their effectiveness is enhanced. This property promotes their long-term effects in natural ecosystems. Dispersal of pollutants in the atmosphere results in treatment of natural terrestrial areas while water run-off transfers pesticide quantities to fresh water areas, and ultimately the oceans and thus their effect comes in aquatic organisms. The pesticides can be carried by running water to ground.

The organ chlorine pesticides, such as DD Tare highly persistent, bioconcentrate in food chains and can severely affect whole populations or species of wildlife, has led tobans and restrictions in use.[1] Most organophosphorus insecticides are regarded as being non-persistent, but some reports have indicated that residues of organophosphates remain essentially unaltered for extended periods in organic soils and surrounding drainagesystems.[2] The most common termiticidal useof chlorpyrifos involves its application to the soil surrounding building structures as a barrier against termite invasion. At one time chlorpyrifos was applied as a mosquito larvicide, a use that involved application of formulated product directly to bodies of water.[3] The organic pollutants (xenobiotics) are fat soluble and are therefore readily taken up fromthe water, sediment and food sources into the tissues of aquatic organisms.[4] There are a number of studies reporting the pathological changes in fishes exposed to different organ chlorine, organ phosphorus, carbonate and pyrethroid pesticides.

MATERIAL AND METHODS

A teleost fish is considerable importance in experimental work due to its easy availability, economic in rearing and maintenance, hardiness to with stand oscillations, when the fish was exposed to $1/10^{th}$ of sub-lethal concentration (0.0294 ppm) of chlorpyrifos at room temperature for a period of 15, 30 and 45 days. Concentration of chlorpyrifos in fresh water fluctuate both seasonally and within shorter time intervals and in the absence of precise information adversely affected by particular level at certain time of years. In the present work the histopathological effects have been under taken. For histopathological studies fishes were sacrificed after 15, 30 and 45 days of exposure at different concentration of chlorpyrifos. The liver and kidney were dissected out. Tissue must undergo preparatory treatment before microtomy; the slides were stained with eosin for half a minute and then place in 95% alcohol again. The stain sections were place in xylene for 20 minutes, then clean and mounted in D.P.X. The liver of the fish exposed to dose of chlorpyrifos at room temperature showed significant effects, the result of present work showed the pathological symptoms.

OBSERVATIONS

Liver is a large, dense organ and is ventrally located in the cranial region of the general cavity. The size, shape and volume of the organ are adapted to the space available between other visceral organs. It consists of two to three lobes although no lobulation was recognized in Gambusiaaffinis. The colour of the liver usually varies from light brown to dark red. The vascular organization of the liver consists of two afferent blood vessels (hepatic artery and portal vein) and a single efferent vessel (hepatic vein) located at the hilum. According to Takashima and Hibiya,[5] the lobular structure containing a small vein in the centre is present in the liver of higher vertebrates. In fish, however, these structures vary depending on the species and are generally obscure. The arrangement of the hepatocytes can rather be regarded as tubular (hepatic cords).[6]

Fish liver microscopic structure is an integrator of physiological and biochemical function which, when altered, may produce biomarkers of prior exposure to toxicants. The liver has key role in xenobiotic metabolism and excretion, digestion and storage and the production of yolk protein. Thus, alterations in structure are expected under certain toxic conditions.

Histopathological examinations of liver of fish Gambusiaaffinis, presented in Plate1 for control fish liver, Plate 2 for 15 days of exposure, Plate 3 for 30 days exposure and Plate 4 for 45 day of exposure at 0.0284 ppm ($1/10^{th}$ of LD_{50}) concentration of chlorpyrfos.

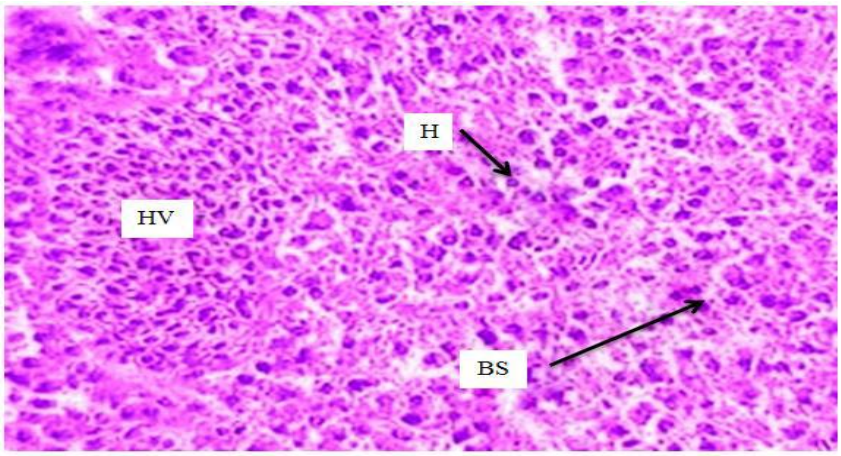

Plate 1: T.S. of Control Liver showing BS: Blood sinusoids, H: Hepatocyte, HV: Hepatic vein.

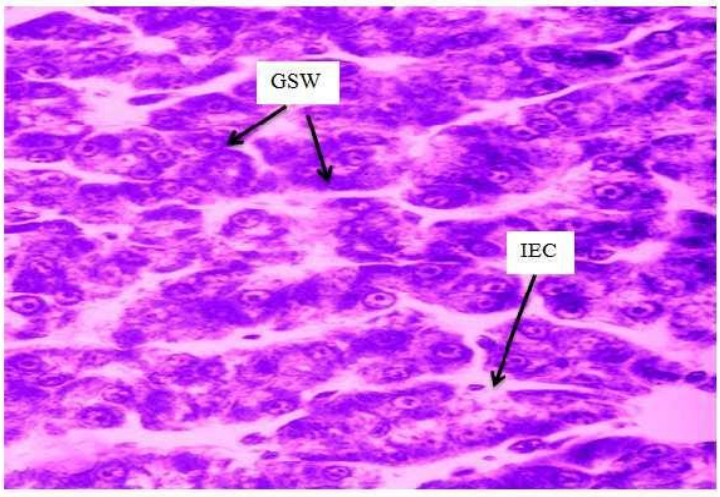

Plate 2: The liver showed generalized swelling (GSW) & Incomplete Epithelial cells (IEC) on 15 days of exposure at 0.0284 ppm concentration of chlorpyrifos.

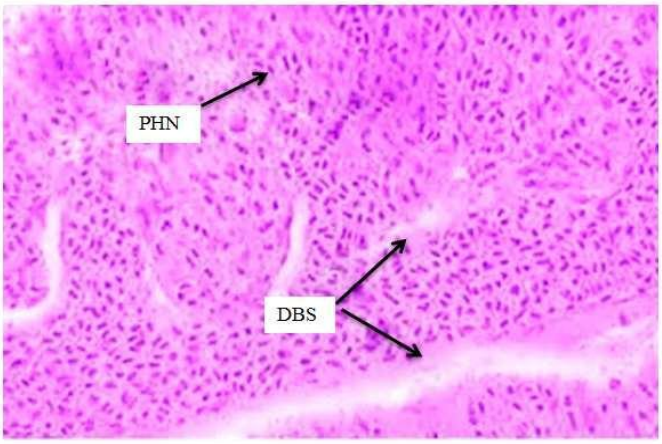

Plate 3: Beginning of pyknosis of haepatocyte nuclei (PHN) & dilation of blood sinusoid (DBS) on 30 days of exposure at 0.0284 ppm concentration.

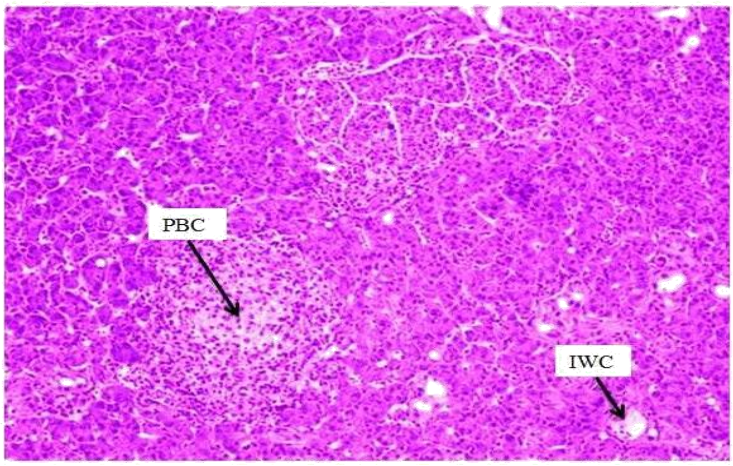

Plate 5.4: Showing Peribiliary cirrhosis (PBC) & Infiltration of white cells (IWC) on 45 days of exposure at 0.0294 ppm concentration of chlorpyrifos.

RESULT AND DISSCUSSION

Liver of teleost is a bilobed gland comprising of two tissue compartments, the parenchyma and stroma. The parenchyma comprising of hepatocytes and the stroma comprising of hepatopancreas, bile duct, blood vessels and connective tissue.s. Hepatocytes are polygonal cells with a prominent spherical central nucleolus and densely stained nucleolus. In the present study liver histopathological observations of the control fish showed a normal architecture with hepatocytes were located among the sinusoids forming cordlike structures known as hepatic cell cords. The hepatocyte has a polyhedral cell body with a central core containing generally one spherical nucleolus (Plate1). The liver of fish Gambusiaaffinis exposed to 0.0284 ppm concentration of chlorpyrifos for 15 days showed generalized swelling. Liver in fish surrounded by connective tissue called capsule, inside it there is a central vein and parenchyma which consist of hepatocytes arranged as two rows leaving capillaries between them called sinusoids lining with incomplete epithelial cells (Plate2). The liver of fish Gambusiaaffinis exposed to 0.0284 ppm concentration of chlorpyrifos for 30 days showed beginning of pyknosis of haepatocyte nuclei and dilation of blood sinusoid (Plate 3). The liver of Gambusiaaffinis exposed to 0.0284 ppm concentration of chlorpyrifos for 45 days showed peribiliary cirrhosis and Infiltration of white cells were seen in the liver (Plate 4). On prolonged exposure to chlorpyrifos, vacuolated areas seen might be indicating fatty changes. This may subsequently lead to fibrotic changes. Hyperplasia observed at certain regions might be the feature of regenerating hepatic tissue. This might be the reason for slightly elevated ALT and ALP levels in hepatic tissue after 45 days of exposure to chlorpyrifos. These damages can result from a wide range of stimuli, from long-standing biliary obstruction, heavy metal or pesticide poisoning. In aquatic system, fish liver ultrastructure has proved to be particularly susceptible to low levels of environmental contaminants.[7] Khan, S. studied effect of cadmium chloride on histopathology of kidney of Lebistes reticulates and observed vacuolated epithelial cells in the renal tubule.[8] The Kidney showed shrinkage of uriniferous tubules and beginning of degeneration in haemopoietic tissue. The haemopoietic tissue showed necrosis and fibrosis. Kunjammaet al. studied the histopathological effects on Oreochromismossambicus (Tilapia) when exposed to sub lethal concentration of Chlorpyrifos and reported swollen hepatocytes with granular cytoplasm and pancreatic acini. Currently chlorpyrifos is used for different purposes. Organophosphate insecticide chlorpyrifos caused kidney damage, and a combination of vitamins E and C reduced partially this damage. Jacob et al. studied Histopathologic study as indicators of aquatic pollution in Mystusgulio (Hamilton).

CONCLUSION

The present study revealed that theorganophosphorus insecticide chlorpyrifos ispotent to cause toxic responses, even structuralalterations, in aquatic organism like fish. Anumber of studies have elucidated the aquatic toxicity of different kinds of pesticides. Thesereports bring discussions on the deteriorating nature and the lethal effects of the pesticideson ecosystem. Pesticides, especially the nondegradableones, even in minute levels, arecausing a stress to aquatic organisms. The toxic responses are reflected by the behavioral and pathological changes. But anagricultural effort reducing the use of pesticidesand implementing natural remedies for pestencroachmentcan becomes one solution for pesticide pollution.

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