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Research Article

HISTOPATHOLOGICAL EFFECT OF CHLORPYRIFOS PESTICIDE ON LIVER AND KIDNEY OF FRESH WATER FISH *CATLA CATLA*

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ABSTRACT

All the natural water bodies are continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Chlorpyrifos is one of the most widely used organophosphate pesticides. Excessive application of pesticides from agricultural fields contaminate aquatic medium, resulting in serious damage to non-target species, including fish. The fish as a bioindicator of aquatic medium plays an important role in the monitoring of water pollution. Histopathology is an important tool for evaluating the action of any toxicant at tissue level. The focus of the present study is to measure the histological changes in liver and kidney of the fresh water fish *Catla catla* exposed to sub lethal concentrations of chlorpyrifos $1/15^{th}$ of the 96 hour LC_{50} values for the period of 10 and 20 days. The fish exposed to chlorpyrifos showed mild vacuolar hepatocytes, dilation of central vein, vaculor degenerative changes, mild hyperaemia and severe hyperaemia of central vein in the liver tissue slide. The changes observed in kidney slide due to the impact of chlorpyrifos toxicity are hemorrhage, degenerative changes in between tubules, constriction of primary tubule, vacuolar degenerative changes, necrosis in epithelial lining of proximal tubules, chlorpyrifos compound induced.

Keywords: Histopathology, *Catla catla*, Chlorpyrifos, Liver, kidney.

1. INTRODUCTION

The aquatic environment has always been subjected to different types of pollutants. The problems of environmental pollution and its harmful effect on fish is receiving focus during the last few decades. The pollution of freshwater ecosystem by chemical pesticides has become one of the most critical environmental problems [1].

Aquatic ecosystems that run through agricultural or industrial areas have highly contaminated by the hazardous chemicals. Among the pesticides, organophosphorus pesticides are the most commonly used pesticides in the world owing to their high insecticidal property [2]. Contamination of fresh water habitats is the main reason for the decline of fish population. Fishes have been the most popular test organisms because they are presumed to be the best understood organisms in the aquatic environment [3].

Histopathology is one of the methods for assessing both short term and long term xenobiotic effects. Various cytological and cytochemical techniques have been employed in investigating pollution induced alternations in cellular structure as well as function. As such, histopathology offers a great deal to aquatic toxicological studies [4]. Histopathological approach should be obligatory component of environmental assessment and may be used to formulate monitoring systems. According to Hinton et al. [5], it is essential that routine histopathological studies discriminate between toxicant induced lesions and normal variations in cellular structure.

One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allow examining specific target organs, including gills, liver and kidney that are responsible for vital functions, such as respiration, excretion, accumulation, and biotransformation of xenobiotics in the fish [6].

Chlorpyrifos is also widely used for the agricultural activities in Andhra Pradesh. Small amounts of chlorpyrifos can have significant effect due to its nature, because it cannot be mixed well with water, and therefore, remain on the surface of the water with air and evaporate [7].

The present investigation aimed to see how the pesticide toxicity has its impact on histology of liver and kidney at short term and long term durations.

2. MATERIAL AND METHOD

The fish *catla catla* having mean weight of (20-25 gms) were purchased from Sri Mahalakshmi Hatchery, Palakoderu, Bhimavarm, W, G(DT), AP, and acclimatised to laboratory conditions. They were given the treatment of 0.1% $KMNO_4$ solution and then kept in cement tank for acclimatization for a period of two weeks. They were fed on oil cake and rice bran daily in calculated ratio. Chlorpyrifos technical grade insecticide with 97.5% purity was obtained from Nagarjuna, Agri Chem Limited, Ethakote, East Godavari (DT), AP, India. LC_{50} was found out for 96h (0.5211ppm) and 1/15th of the LC_{50} value 0.035ppm was taken as sublethal concentrations for this study.

Thirty fish were selected and divided into 3 groups of 10 each. The first group was maintained in free from chlorpyrifos and served as the control (Group-I). The other group (Group-II) was exposed to 1/15th of sub lethal concentration of chlorpyrifos (0.035ppml) in 10 litre capacity aquaria for 10 days and the third group was exposed to 1/15th of sub lethal concentration of chlorpyrifos for 20 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for histological studies. To examine the extent of cellular changes by chlorpyrifos in the liver and kidney of the control and treated tissues of 10 days and 20 days exposed groups were fixed in 5% formalin for 24 hrs. The fixative was removed by washing through running tap water. After dehydrating through a graded series of alcohols, the tissues were cleared in Methyl benzoate, embedded in paraffin wax. Sections were cut at 6 µ thickness and stained with hematoxylin (Harris,1900) and counter signed with eosin (dissolved in 95% alcohol). After dehydrating and clearing sections were mounted with DPX and observed under microscope.

2.1. Physicochemical properties of chlorpyrifos

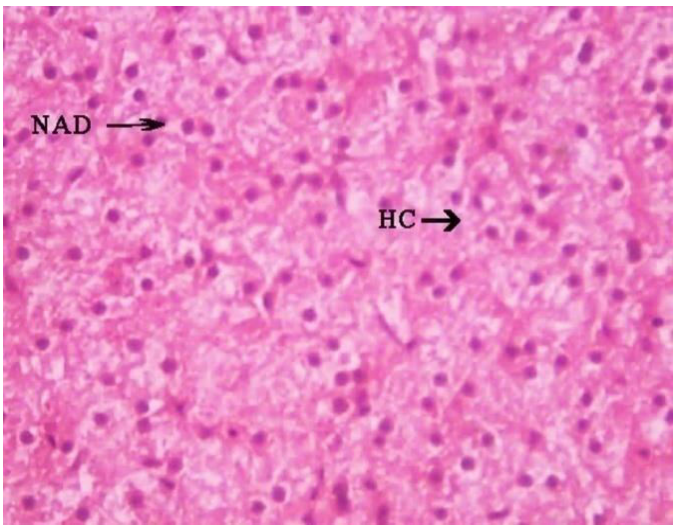
- Chemical Formula - $C_9H_{11}Cl_3NO_3PS$
- IUPAC Name: diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-lambda5-phosphane
- Chlorpyrifos is a colorless to white crystalline solid.
- Chlorpyrifos has the smell of sulfur compounds found in rotten eggs, onions, garlic and skunks.
- Molecular weight: 350.6 g/mol
- Solubility (water): 0.0014g/L (1.4 mg/L) at 25°C

Water quality parameters of source water taken during entire experiment period is as follows-

The experiment was conducted under natural photoperiod and temperature. Water quality was measured as per (APHA,2005) The temperature of the experimental water was 23 ± 1.5° C, pH was 7.2 ± 0.4, Dissolved oxygen was 7.2 ± 0.6mgl^{-1}, and free carbon dioxide was 6.2 ± 0.4 mgl^{-1} and total hardness as calcium carbonate was 176 ± 3.2 mgl^{-1}.

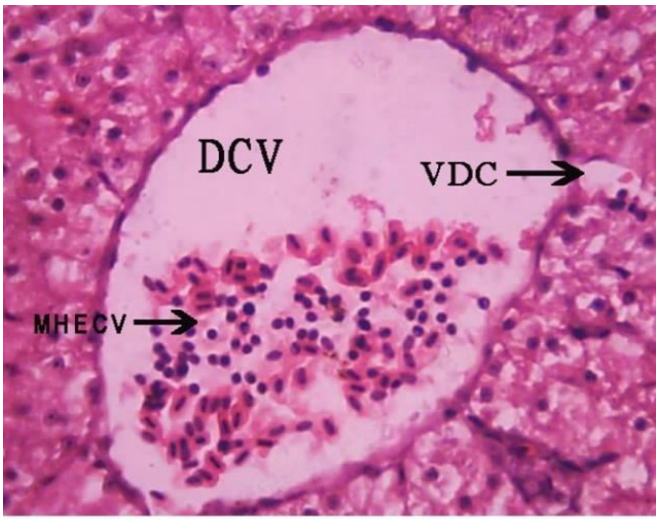
3. RESULTS

The structure of the normal liver of the fish consists of a continuous mass of large hexagonal cells. The hepatocytes are large in size with homogenous granular cytoplasm and either centrally located distinct nuclei (Fig. 1). In group -I control fishes, no changes are observed in liver tissue slide. However, in group-II fishes exposed to chlorpyrifos for 10 days, has shown necrotic changes, dilated central vein, mild degenerative changes (Fig. 2). In group-III fishes, the impact of chlorpyrifos is more and we can see hepatocytes with widespread vacuoles, clear necrotic changes and picnotic nuclei of cells, severe hyperremia of central vein. (Fig.3). In the liver of fishes exposed to the toxicant for 20 days resulted in degenerative hepatocytes, changes in central vein with congestion. This reflects that with the increase of exposure period the pesticide has resulted in heavy change in the architecture of liver.



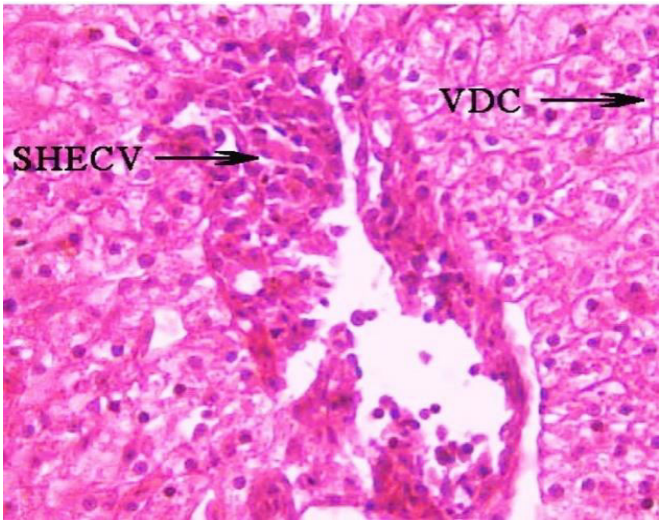
(NAD-Normal Architectural Details, HC-Hepatocytes

Fig. 1: Histological image of fish liver unexposed to chlorpyrifos (Group-I)



DCV-Dilated Central Vein, VDC-Vacuolar degenrative changes, MHECV-Mild Hyperemia of Central Vein

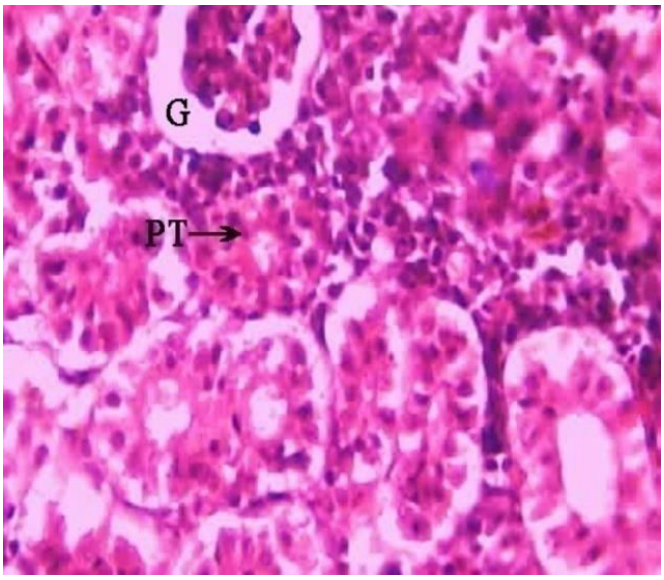
Fig. 2: Histological image of fish liver exposed to chlorpyrifos for 10 days (Group-II)



(VDC-Vacuolar Degenerative chnages,SHECV- Severe Hyperemia Of Centarl Vein)

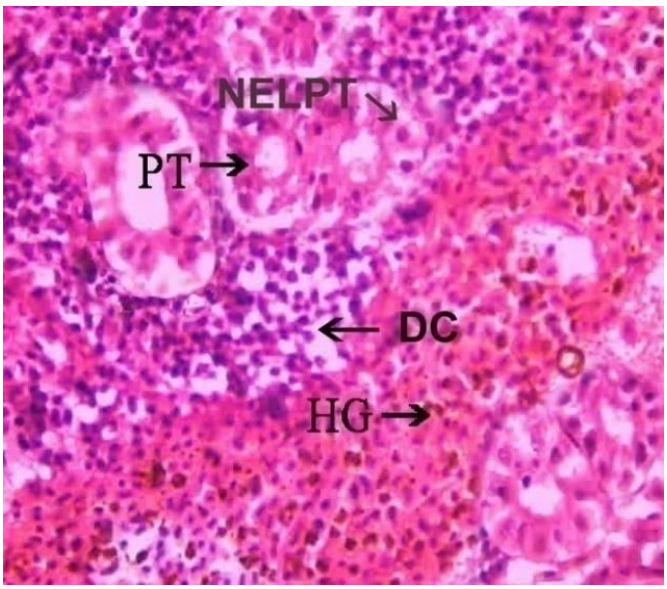
Fig. 3: Histological image of fish liver exposed to chlorpyrifos for 20 days (Group-III)

The present study also demonstrates that the kidney of control fish exhibits a normal architecture and there were no pathological abnormalities. No marked changes were observed in the kidney of *catla catla* control group I fishes (fig. 4). But chlorpyrifos induced mild pathological changes in group II fishes exposed for 10 days (fig. 5) such as constriction of primary tubule, swelling and exfoliation of tubules, necrosis in epithelial lining of proximal tubule. And the group III fishes (fig. 6), exposed to the toxicant for 20 days have shown haemorrhage, degenerative changes and architectural damage, tubular degeneration and necrosis. Because of the important role of kidney in the excretion of harmful materials, the present study proved occurrence of several histological alterations in the kidney resulting from chlorpyrifos toxicity and reflect participation of kidney in excreting chlorpyrifos toxicant from body.



(PT- Nornal Proximal Tubule,G-Glomeruli)

Fig. 4: Histological image of fish Kidney unexposed to chlorpyrifos (Group-I)



(CPT-Constriction Of Primary Tubule, NELPT- Necrosis in Epithelial lining of proximal Tubules, DGT- Degenrative Changs InBetween Tubules)

Fig. 5: Histological image of fish Kidney Exposed to chlorpyrifos For 10 days (Group-II)

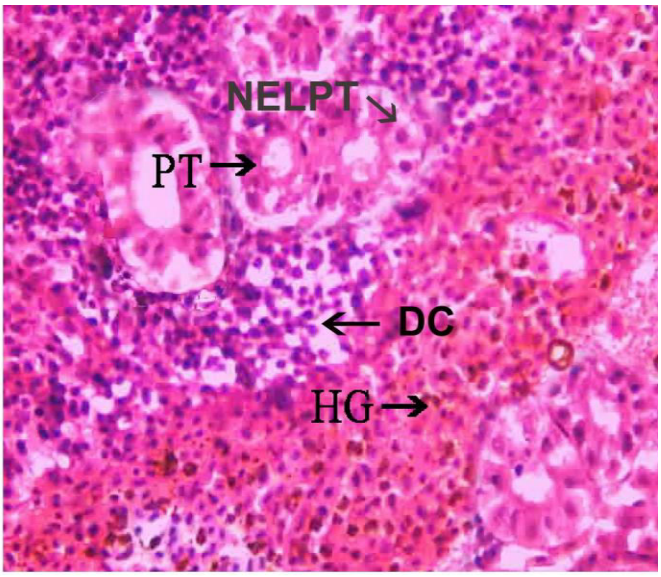


Fig. 6: Histological image of fish KidneyExposed to chlorpyrifos fro 20 days (Group-III)

(HG - Haemorrhage, PT - Proximal Tubule, DC - Degenerative Changes,NELPT - Necrosis in Epithelial Lining of Proximal Tubules) HG = Haemorrhage

4. DISCUSSION

Histopathological examination of fish tissues exposed to toxicants indicates signs of prolonged damage in cells, tissues and organs [8]. It can be used as a useful tool to visualize the stress induced structural alterations in the cells and tissues. Histological changes in the liver could be attributed to the fact that, the liver is the major site of detoxification according to Nagei et al [9]. It is expected that the toxicant insecticide would reach there in abundance for detoxification and disposal as investigated by Mushigeri and Fenvalerate M. [10]. In teleosts the liver is the primary organ for biotransformation of organism xenobiotics and probably also for the excretion of harmful trace elements, food digestion and storage, metabolism of sex hormones [11].

Damage is well demonstrated histologically as the normal histoarchitecture of the hepatocytes observed in liver of control fish is disrupted in the groups exposed to chlorpyrifos. Changes in the liver tissue were time dependent. In 20 days treated group, most of the hepatocytes were degenerated as indicated by heavy vacuolization. Similar changes in the liver of *Channa punctatus* were observed which include degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles and ruptured blood vessels, necrosis and disappearance of hepatocyte cell membrane disposition [12]. Singh et al [13] in their investigation says that Hepatic cords are found to be decreased in size and nucleus became pyknotic, changes in the liver of *Channa punctatus*, include cytoplasmolysis, nuclear pyknosis and necrosis leading to complete exhaustion and disintegration of hepatocyte. Histopathological alterations in the snake head fish, *Channa gachua* exposed to dichlorvos included vacuolation in the cytoplasm, degeneration in the hepatic sinusoids, ruptured blood vessels, necrosis and hepatocytes atrophy [14]. Present study confirms that the liver is the major site of detoxification. The recorded results in the present study were similar to those as observed by Tilak et al and Kunjamma et al [15] who recorded pyknotic nucleus, protein precipitation, pancreatic acini appeared with the loss normal structure and necrosis of the hepatic and pancreatic tissue in freshwater fish *Catla catla* and *Oreochromis mossambicus* treated with chlorpyrifos.

In the present study, the appearance of pycnotic nuclei indicated that the cells became hypofunctional. Focal necrosis were also observed in the liver of the fishes *Heteropneustes fossilis* and *Brachydanio* were exposed to organophosphate insecticide malathion and dimethoate 500 [16]. Focal necrosis is probably due to the involvement of liver cells in the metabolic transformation of the insecticide, causing functional and structural changes to the cells [17]. In common carp, 40-day exposure of CPF (1.16 to 116μg/L) and atrazine (4.28 to 428 μg/L) exhibited pathological lesions in the liver such as hydropic degeneration, vacuolization, pyknotic nuclei, and fatty infiltration investigation [18].

Kidneys are important organs to maintain osmotic balance. Thophon et al [19] specifies that teleostean kidney is one of the first organs to be affected by contaminants in the water. Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman′s space is investigated by Takashima and Hibiya T [20]. They also send many metabolites outside which reach body in the form of xenobiotics. Kidneys are continuously exposed to toxic chemicals, so the risk of effects is high [21]. In the present study, histopathological alterations in the kidney were hydropic swelling and exfoliation of tubules, vacuoles accumulation in tubular cells, and many necrotic areas. Tubular degeneration and necrosis were also observed. The severity of the damaged increased when the days of exposure increased. The pathological changes are in agreement with those

observations made in *Channa punctata* by Gupta and Srivastav N. [22]. Their results showed small cytoplasmic vacuoles, nuclear deformation and cellular hemolysis in the proximal tubules. A similar study was also reported by Camargo and Martinez I [23] in case of *Prochliodus lineatus* due to anthropogenic activities of agricultural, industrial and domestic effluents to Apertados stream. Necrosis of tubular epithelium might be due to toxin, injury and infection, which led to unregulated cell death.

In the present study, it has been found that the pesticide induced alterations in the structure of liver and kidney tissues of the fish *Catla catla*. Histological alterations in the organs of experimental fish are increased with increase in exposure of the pesticide time period. The increased severity of the lesion of the tissues was high in group III treated fish as compared to group II, which throws light that the impact of pesticide increases with increase in duration of exposure.

5. CONCLUSION

Organophosphorus insecticide chlorpyrifos induced histological alterations in the tissues like liver and kidney of *Catla catla* freshwater fish. The histopathological changes in the liver and kidney tissues are time dependent alterations. The findings of the present study indicate that sublethal concentrations of chlorpyrifos was toxic to fish population and it will lead to the damage of internal organs of fish which may ultimately lead to the reduction in the population of fish species. The present study also shows that the histological studies serve as biomarkers for assessing pesticide toxicity in aquatic environments. These findings will be helpful in assessing environmental monitoring and plan strategies to alleviate the eco-toxicological impacts of pollution magnifying in fresh water, aquatic fauna particular to fish and indirectly to human populations.

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Conflict of interest

No any conflict of interest.

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