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ORGANOPHOSPHOROUS PESTICIDE, CHLORPYRIFOS INDUCED CHANGES IN LIVER ANATOMYOF FRESH WATER FISH, *CHANNA PUNCTATA*

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ABSTRACT
The current study examined the poisonous results of an organophosphorous pesticide, chlorpyrifos (CPF) on the liver of *Channa punctata*. The 96-hours LC_{50} value was found to be 0.45µl/L, and two doses- ½ and 1/10 of 96-hours LC_{50} value were selected for sub chronic studies for 28 days. Sublethal concentrations of CPF that is 0.225 µl/L and 0.045 µl/L were run to the fish *Channa punctata* and the histopathological anomalies were observed in the liver tissues under light microscope using Hematoxylin and Eosin stains. The results of this study indicated severe toxic responses in liver exposed to CPF. The alterations in morphology of fish liver showed vacuolar degeneration, fatty degeneration, focal necrosis when exposed to CPF as compared to the control fish that remained healthy throughout the study period. This study recommended that CPF had the potential to cause different histological impairment in fish, developmental toxicity and behavior alterations, supporting environmental watch over aquatic system.

KEYWORDS: chlorpyrifos, fish, histopathology, liver.

INTRODUCTION

Chlorpyrifos (CPF) is an organophosphorous insecticide product usually employed in several countries, including India, on many farming crops and on various trees such as corn, vegetables, several fruits, nuts, soybeans etc to control insect pests (Juberg *et al.* 2013). Due to severe restriction on the use of carbofuran and organochlorine pesticides in the recent past which has encouraged CPF as an alternative preference for managing the pests and crops (Jiang, 2008). It has been reported by many that CPF may spread through aerial spray or due to overspill into surrounding water bodies and gets added in diverse organisms reside in water bodies, mostly fish, harmfully distressing the fishes (Varo *et al.* 2002). Datas are available that these chemicals have indirect effects on fish and this may be able to estimate by using biomarkers as before time caution, which may be shows the unfavorable results on organisms mostly which are not on target (Booth *et al.* 2003). The United States Environmental Protection Agency (EPA) prohibited the domestic use of CPF in United States in 2001 and restricted the use CPF to certain crops only due to the alarming fear over the direct and indirect outcomes of this chemical on the surrounding environment and human beings (Eaton *et al.* 2008).
Reports are there, central and peripheral nervous systems are the principal target organ for CPF toxicity (Eaton *et al.* 2008), as the pesticide CPF restrain the enzymatic action of acetylcholinesterase (Mullar *et al.* 2014). Further, liver being the major organ for detoxification of pesticides suffers both physiological stress and histopathological alterations (Hinton *et al.* 2008). For environmental monitoring, the specific target organs, such as liver, intestine, gills, kidney which in turn are responsible for various vital functions of the body, as digestion, excretion, the process of breathing and etc in the fish, histopathological biomarkers have been widely used which shows remarkable disparity (Au, 2004; Hinton *et al.*, 2008; Stehr *et al.* 2003; Schlenk *et al.*, 2008). Thus, the study here was intended to check over the impact of sublethal doses of the pesticide CPF at 28 days durations on the liver histopathology of *Channa punctata* (Bloch) which is a spotted snakehead teleost fish.

MATERIALS AND METHODS

2.1 Maintenance of fish

Channa punctata weighing 25.02±0.86 g with an average length of 14.25±0.53 cm was bought from a local fishery, which was free from pollutants. The fish were transferred to a large aquarium with chlorine-free tap water and acclimatized for three weeks under laboratory setting with constant aeration. Fish were nourished with commercial fish food twice a day (Tokyu fish pellet obtained from Fish Aquarium Home, Laxmi Nagar, Delhi). Water was changed on a regular basis and dead animals (if any) were eliminated as soon as possible to maintain healthy and clean water in aquarium. In the aquarium the water used was chlorine-free tap water which containing pH 6.4-6.6, dissolved oxygen 6.9-7.2 mgL^{-1}, having temperature 23.4-25.9^{0}C and electric conductivity between 81.9–84.5 µS cm−1.

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a. Pesticide

A commercial grade chlorpyrifos, TRICEL, (CPF effective concentration 20%, obtained from Excel Crop Care Limited, Maharashtra, India) was obtained from agrochemical retailer. Stock solutions were prepared by dissolving the one mililiter of insecticide in one liter of tap water. To get the test concentrations in aquariums the stock solutions were further thinned out. LC_{50} values for 96 h of CPF to *Channa punctatus* were found to be 0.45 μL^{-1} in a prior study.

b. Experimental design

Two experimental doses of CPF, and a control set were maintained for 28 days under constant aeration and renewal of entire test waters every alternative day. For each set, there were three replicates and each replicate had 3 fish. Food was provided to all the three sets twice daily, *ad libitum*. The three sets included, no pesticide control (in chlorine free tap water), ½ of 96-h LC_{50} of CPF (0.225 µL/ L, designated as ½ CPF), 1/10th of 96-h LC_{50} of CPF (0.045 µL/L, designated as 1/10 CPF). All the experiments were performed as per the Assam University Bioethics guidelines.

c. Histopathological analysis

After 28 days of exposure, the fish were anaesthetized with 1mg/L 2-phenoxyethanol (Sigma, India) and liver tissue was collected both from treated and control sets and were set in 10% formaldehyde and for light microscopic study further fixed in paraffin wax. Various sections (5µ) were obtained and as per standard protocol (Das and Gupta, 2012) further stained with Hematoxylin and Eosin and observed under a light microscope at 400× magnification (Olympus Microscope, model CX41RF with Olympus digital Camera, model: E-420). A study was carefully carried out to check for the alterations of liver tissue.

RESULTS

Various range of histopathological alterations were spotted in the liver of *Channa punctata* in the current study. The fishes which were treated with higher doses of CPF, the organ lesions were noticed to be more marked and severe which implied that the alterations were dose dependent.

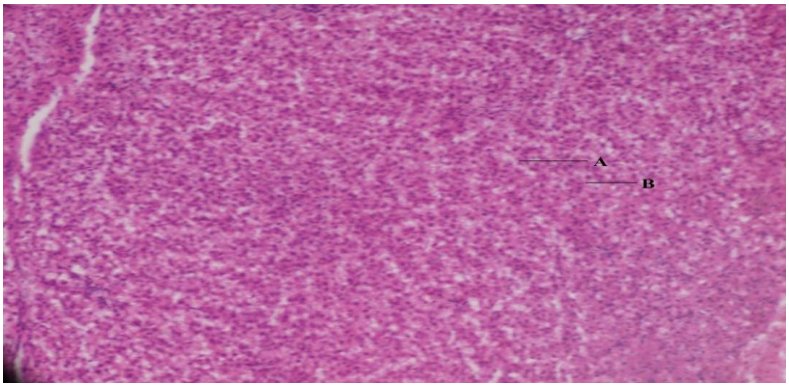

Fig 1: TS of liver of control *Channa punctata* after 28 days (× 400): A= sinusoid, B=hepatocyte.

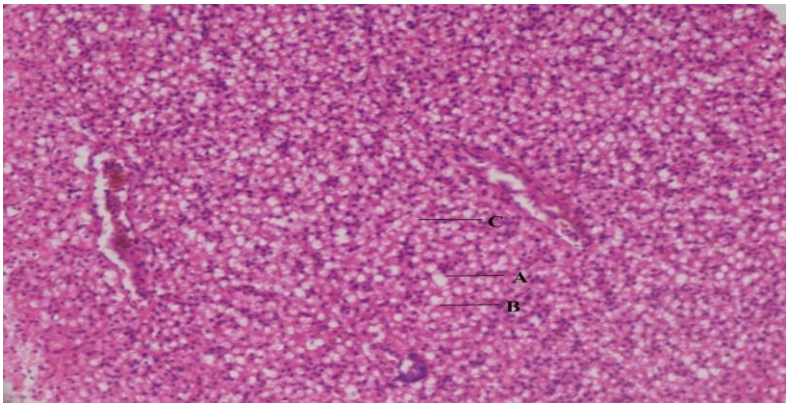

Fig (2): T.S of liver of Channa punctata after 28 days of exposed to 0.045µl/L chlorpyrifos (X400): (A)= vacuolar degeneration, (B)= Fatty degeneration, (C)= Focal necrosis.

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No such histopathological variation was found in the liver of the untreated or control fish and they showed normal architecture throughout the observation period (Fig.1). The histopathological results indicated that liver tissues were affected adversely by exposed to 0.225 µl/L and 0.045 µl/L of CPF for 28 days. The most remarkable changes the liver showed was fatty degeneration, vacuolar degeneration, focal necrosis and hyperplasia of hepatocytes. With increase in dose (0.225 µl/L), the severity intensified with extensive vacuolar degeneration, focal necrosis along with hyperplasia of hepatocytes, fatty degeneration (Fig.3) as compared to dose (.045 µl/L) with less severe impacts (Fig.2).

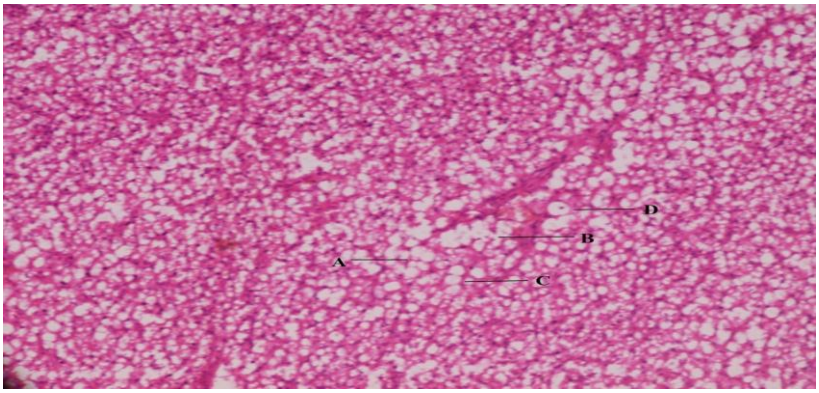

Fig (3). T.S of liver of Channa punctata after 28 days of exposed to 0.225 µl/L (1/2 of 96 hr LC_{50} chlorpyrifos (×400): (A)= extensive vacuolar degeneration, (B)= focal necrosis, (C)=fatty degeneration and (D)= hyperplasia of hepatocyte.

DISCUSSION

The process of releasing toxins takes place in the liver tissue which is the most important organ though this process is not always managed and destruction may occur to the host tissue. The various alterations in architecture of liver including degenerative necrotic condition were found due to the toxic contact (Arellano *et al.* 1999; Olojo *et al.* 2005; Figueiredo-Fernandes *et al.* 2007). Aquatic species, especially fish may face serious ecological problems due to toxic effect of CPF. Earlier studies had pointed that CPF induced various alterations, in normal functions of living organisms, biochemical processes and histopathological changes in many tissues of aquatic animals (Kavitha and Rao, 2008; Ali *et al.* 2009; Xing *et al.* 2012). Similar results have been found in *C. punctatus* that points to the fact that CPF is highly toxic even in low dose. We observed histological variations in liver tissue on exposure to CPF which included focal necrosis, vacuolar degeneration, fatty degeneration, and hyperplasia of hepatocytes. Cengiz *et al.* (2001) also examined several histopathological alterations in the anatomy of liver of mosquito fish, *Gambusia affinis* due to thiodan. Reports on apoptosis (Caughlan *et al.* 2004) due to CPF in human beings and in diverse species of animals have been accounted (Gupta *et al.* 2010; Li *et al.* 2009). Reports were also made on *Clarias gariepinus* an African catfish, which on acute treatment by a pesticide, glyphosate shows alterations in the liver as leukocyte infiltration, nuclear necrosis and diffused necrotic tissues etc (Ayoola, 2008).

Ultrastructural alterations were reported (Szarek *et al.* 2000) in the hepatocytes in *Cyprinus carpio* due to exposure to herbicide Roundup. Sub-lethal treatment of CPF in tilapia, *Oreochromis mossambicus*, showed swollen hepatocytes with granular cytoplasm and pancreatic acini (Kunjamma *et al.* 2008). In eel, *Anguilla anguilla* the liver parenchyma showed mark of degeneration, both cytoplasmic and nuclear, as well as hepatocitic vacuolation (Pacheco and Santos, 2002) due to contaminants. The anomalies which found in the current study due to CPF contaminated water might arise because of metabolic impairment. Xenobiotics initiate precise enzymatic activity to facilitate metabolic transformations and intoxicity of cells, and which finally leading to death. Such histopathological manifestations, as lesions or degenerative alterations, arising from the chemical insult, are predominantly defensive reaction of the organism (Roganovic- Zafirova *et al.* 2003).

However, normal metabolism of the organisms may get upset with these reactions in the tissues such as the hepatocytes, thereby inducing diseases or even death. Such types of structural changes in liver are indicative of toxicity, which might subsequently compromise the functions of liver.

CONCLUSION

The current study concluded that CPF, even in highly diluted form, is highly toxic to fish producing hepatic damage at cellular level. CPF, which is an organophosphate chemical most prevalently, exercised insecticides in India and other various countries of the world. It has a potential bio-hazardous and vulnerable impact on aquatic animals and is established as a very toxic for aquatic organisms, mostly to fish. As food source, fish interfere with food chain impacting human's life quality. CPF, as in this study, has the potential to stimulate toxic effects in the liver tissues of fish, which is indicative of serious toxicity. Therefore, chlorpyrifos have got to be applied cautiously.

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