



SUBLETHAL EFFECTS OF CHLORDECONE ON HISTOMORPHOLOGY OF THE FRESHWATER CICHLID FISH, *PSEUDETROPLUS MACULATUS* (BLOCH, 1795)

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ABSTRACT

The sublethal toxic effects of chlordecone on histomorphology of tissues such as gill, liver and brain was investigated in the freshwater cichlid fish, *Pseudetroplus maculatus*. Two sublethal concentrations i.e., one-tenth (3.5 µg/L) and one-fifth (7 µg/L) of LC50-96 h of the toxicant was exposed for 96 h. Histological analysis revealed that after exposure to chlordecone caused upliftment of gill epithelium, hyperplasia and absence of secondary lamellae in gill of fish. Liver tissues showed severe damages such as cytoplasmic vacuolization and complete necrosis. Histomorphological changes on brain showed cerebral edema, necrosis of neurofibrillar region, vacuolization and nuclear pyknosis. The results suggests that the degree of morphological alterations in gill, liver and brain was proportional to the concentration, and further confirmed that the sublethal effects of chlordecone could be due to the direct contact of fish to the toxicant.

KEYWORDS : Gill, Liver, Brain, Histology, *Pseudetroplus maculatus*

INTRODUCTION

In the past few decades, pollution in the aquatic environment is a serious concern and has become an international issue. Aquatic pollution is mainly due to the release of several toxicants from industries, agricultural products, sewage effluents and domestic wastes. It could seriously harm aquatic flora and fauna, which lead to bioaccumulation of toxicants in aquatic organisms and bioconcentration in higher vertebrates (Ackerman and Iwama, 2001). Adverse effects of toxicants as a biological response can be assessed by various biomarkers such as physiological, biochemical or histopathological alterations in various tissues or organs. Fish possess natural adaptive mechanisms as detoxification and biotransformation in order to get rid from the exposed environmental contaminants. However, the decline in the fish population may be the result of exposure to low level of numerous toxicants in the aquatic environment. Therefore, the degree of contamination in the aquatic environment can be assessed by detecting warning signs of damage on tissue level through histological analysis.

Histopathology is a cost effective sensitive tool to diagnose the direct toxic effects of any pollutant within the target organs of fish in laboratory condition. The advantage of histological analysis is it proves as the valuable indicator of the health status of fish population and hence reflects the well-being of the entire aquatic ecosystem. The severity of tissue damage also depends on the toxic potential of the compound, resistance of the species exposed, or the concentration and time of exposure. Histological alteration in tissues exposed to sublethal concentration of toxicant serves as a functional response of an organism to that particular compound. Among various tissues, gills are considered as the primary target organ of any pollutant as it is continuously exposed to water and are the site of respiration and osmoregulation (Fernandes and Mazon, 2003). Liver tissue is associated with detoxification and biotransformation of toxicant and hence expected to be the good indicator of environmental pollution (Camargo and Martinez, 2007). Brain is the controlling centre of the body and any change in the architecture of brain tissue reflects the behaviour of the organism (Anam and Maitra, 1995).

The present study focussed on the histological lesions induced by one of the environmental contaminants, chlordecone on gill, liver and brain tissues at sublethal concentrations. Chlordecone was primarily used as an insecticide and has high potential to bioaccumulate in fish and other aquatic organisms (ATSDR, 1995). In recent years, chlordecone has become a matter of concern because of its persistence in air, soil and water and also due to its potential

adverse effects on aquatic organisms. Hence an attempt has been made to investigate the sublethal effects of chlordecone on the histomorphology of gill, liver and brain tissues in the freshwater cichlid fish, *Pseudetroplus maculatus*.

MATERIALS AND METHODS

Animal:

The cichlid fish *P. maculatus*, weighing 7±1 g and length 7±1.5 cm were collected from the local fish farm, KKF Nursery, Manjeri, Vaniyambalam, Malappuram district, Kerala, India. Fish was acclimatized in the laboratory for two weeks prior to the experiment by exposing to good lighting system (12 h light: 12 h dark) in dechlorinated and well-aerated tanks (40 L capacity).

Preliminary tests:

Preliminary test were conducted by using standardized procedures as per American Public Health Association guidelines so that the water temperature was maintained as 28±2°C, oxygen saturation of water (70 and 100 %), and pH 6.5 to 7.5 (APHA, 1998). The standard physico-chemical features were maintained throughout the experiment in both control and treated groups.

Chemical:

Technical grade organophosphate insecticide, chlordecone (Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one, 99.9% pure) was obtained from Supelco, USA. All other chemicals were of analytical grade and obtained from local commercial sources.

Treatment:

After two weeks of acclimatization, fishes were kept in different tanks for the experiment maintaining ten animals per group. Chlordecone was dissolved in 1% DMSO and therefore used as a solvent (vehicle) control in the experiment. Earlier studies from our laboratory determined the median lethal concentration (LC50-96 h) of chlordecone in *P. maculatus* by using probit analysis as 35µg/L (Asifa and Chitra, 2015). Two sublethal concentrations of chlordecone such as one-tenth (3.5µg/L) one-fifth (7µg/L) of LC50-96 h was exposed for 96 h maintaining negative and positive controls.

Histological analyses:

At the end of each exposure period, gill, liver and brain of fish were removed and fixed in 10 % buffered formalin for 24 hours. Tissues were dehydrated in ascending grades of alcohol and cleared in xylene until they became translucent. Tissues were then transferred to molten paraffin wax for 1 hour to remove xylene completely and

then impregnated with wax. Then the blocks were cut in a rotary microtome to prepare sections of thickness 4 to 6 microns. The sections were stained with haematoxylin and eosin and mounted in DPx. The structural alterations were observed under light microscope and compared with those of control tissues. were taken using Cannon shot camera fitted to the Carl Zeiss AxioScope 2 Plus Trinocular Research Microscope.

RESULTS

Histological examination revealed that the control tissues of gill, liver and brain are with normal histoarchitecture. Similarly, DMSO (vehicle control)-treated fishes also showed no changes in their histomorphology and it was similar to the negative control tissues. Chlordecone when exposed at both sublethal concentrations for 96 h showed upliftment of gill epithelium, hyperplasia, edema and absence of secondary lamellae in gill of fish (Figure 1). Hepatocytes of chlordecone-treated fish showed severe damages such as cytoplasmic vacuolization and complete necrosis (Figure 2). Histomorphological changes on brain tissue showed cerebral edema, necrosis of neurofibrillar region, vacuolization and nuclear pyknosis (Figure 3). The severity of damage increased to the increase in the concentration of chlordecone.

Figure 1

Photomicrographs of histological sections, stained with H&E in the gill of *Pseudotroplus maculatus*. A-Control; B-DMSO-treated; C and D-Chlordecone-treated for 96 h at 3.5 µg/L and 7 µg/L, respectively, showing upliftment of gill epithelium (*); edema and absence of secondary lamellae (arrow)

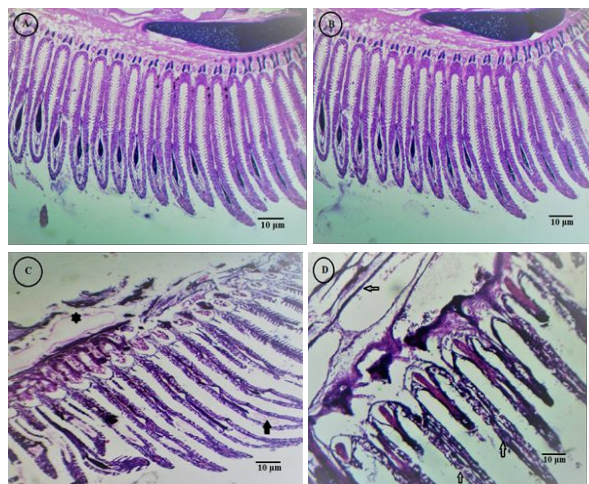


Figure 2

Photomicrographs of histological sections, stained with H&E in the hepatocytes of *Pseudotroplus maculatus*. A-Control; B-DMSO-treated; C and D-Chlordecone-treated for 96 h showing vacuolization and complete necrosis at 3.5 µg/L and 7 µg/L, respectively

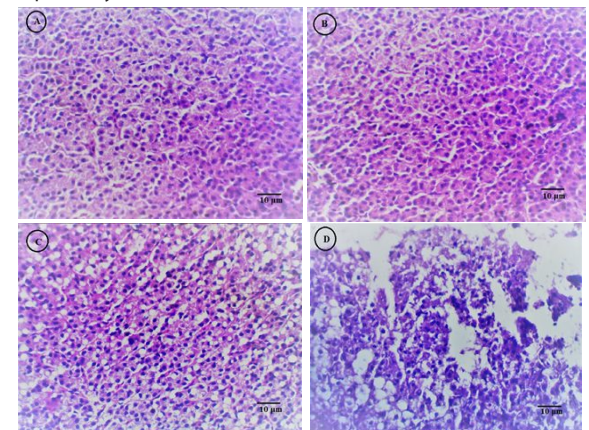
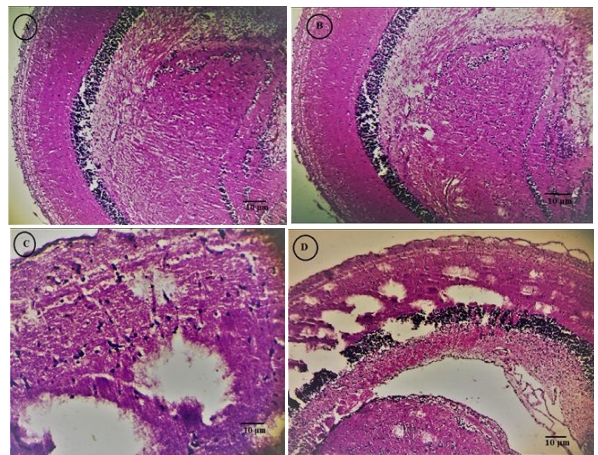


Figure 3

Photomicrographs of histological sections, stained with H&E in the brain of *Pseudotroplus maculatus*. A-Control; B-DMSO-treated; C and D-Chlordecone-treated for 96 h showing cerebral edema, necrosis of neurofibrillar region, vacuolization and nuclear pyknosis at 3.5 µg/L and 7 µg/L, respectively



DISCUSSION

Histological studies are considered as an indicator of environmental pollutants, which represents a useful tool for the diagnosis of degree of pollution particularly for sublethal concentration. In the present study chlordecone was exposed at two sublethal concentrations, one-tenth and one-fifth of median lethal concentration for 96 h, in the cichlid fish, *Pseudotroplus maculatus*. Gill is the most sensitive and the first exposed tissue to any toxicant, where the important physiological functions as respiration, osmoregulation and excretion take place (Perry and Laurent, 1993). In the present study, control gill tissues, negative and positive controls, showed normal architecture having gill arches on either side of buccal cavity. Each gill arch contains numerous gill filaments with primary and secondary lamellae having chloride cells, erythrocytes, mucous cells etc. Chlordecone treatment showed the upliftment and separation of gill epithelium from the gill lamellae and hyperplasia in the gill arches was also noticed. The changes were observed at 3.5 µg/L concentration of chlordecone exposure and are considered as the first line of defensive mechanism of fish in order to escape from the toxicant, as the lifting of gill epithelium provide distance across the waterborne pollutant to diffuse into the blood stream (Arellano et al., 1999; Raibeemol and Chitra, 2015). When exposed to 7 µg/L of chlordecone showed edema in the primary lamellae and absence of secondary lamellae which affect the free gas exchange across the gill lamellae and are associated with disturbance of blood flow, and therefore affect the vital functions of gill tissue as respiration, osmoregulation and excretion. The results are in agreement with the toxic effects of one of the nanoparticles, fullerene C60 in the fish, *Pseudotroplus maculatus* (Sumi and Chitra, 2017).

The present study showed morphological alterations in hepatocytes of fish treated with two sublethal concentrations of chlordecone and the degree of pathology increased in dose-dependent manner. The degenerative changes noted in hepatocytes after one-tenth of sublethal concentration of chlordecone showed decrease in the surface area of liver cells thus resulting in gradual degeneration of syncytial arrangement of hepatocytes. Cytoplasmic vacuolization was also observed in fish liver as a result of exposure to chlordecone, which are related to the altered metabolic functions of the liver (Asifa et al., 2014; Asifa and Chitra, 2017b). Vacuolization is the common response of hepatocytes owing to liver injury and are likely due to accumulation of glycogen in hepatocytes (Westerman and Canton, 1986). Exposure of chlordecone at 7 µg/L concentration showed complete necrosis of hepatocytes. Chlordecone stress could have disorganized hepatocytes and this may be due to the depletion of glycogen because it serves as a glucose reserve and are supplied at high energy demand (Hinton and Laurén, 1990). The

study reveals that the histological alterations increase with increase in the level of concentration.

Chlordecone exposure induced pathological changes in the brain of exposed fish showing cerebral edema, necrosis of neurofibrillar region, vacuolization and nuclear pyknosis in concentration-dependent manner. All these changes could be correlated to the possible reduction in cholinergic activity of brain on exposure to chlordecone. It is evidenced from our previous study that the decrease in the activity of acetylcholinesterase in brain after chlordecone exposure are associated to the histological changes observed in the brain (Asifa and Chitra, 2017a).

Thus the histological changes proved in the present study reveal the defensive mechanism of the fish against chlordecone toxicity. It is further assumed that if the duration of toxicant exposure is prolonged then it could cause severe destruction in the organ structures. The structural modifications in the gill, liver, and brain of the fish observed at 96 h exposure to chlordecone strongly support the inability of the fish to resist the sublethal stress.

CONCLUSION

As a safety measure to the aquatic ecosystem and food chain, the continual release of such toxicants can be prevented by treating the effluents before they are discharged into the aquatic environment.

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