International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 05
March 2015

Chronic effect of chlorpyrifos on histoarchitectural alterations in gill, liver, kidney and spleen of fresh water fish, *Etroplus maculatus* (Bloch, 1795)

K.P. Raibeemol and K. C. Chitra*
Department of Zoology, University of Calicut, Malappuram District, Kerala, 673 635, India.
e-mail: kcchitra@yahoo.com;

ABSTRACT

Chlorpyrifos is a wide ranging organophosphorus insecticide used to control agricultural and household pests. In the present study the chronic effect of chlorpyrifos at sublethal concentration (0.661 µg/L) was assessed in fish, Etroplus maculatus in order to evaluate the histoarchitectural alteration in various tissues as gill, liver, kidney and spleen. Fish ranging 3.5 ± 0.5 g in weight and 6 ± 0.3 cm length was used in the study and are exposed to chlorpyrifos for 15 days maintaining the control group. Chlorpyrifos exposed tissues exhibited some remarkable histological alterations when compared to respective control tissues. The prominent histological changes observed in gills were fusion of secondary lamellae, epithelial lifting, and vacuolization. Liver tissue showed vacuolization, and degenerative hepatocyte with pyknotic nuclei. Cellular degeneration of tubules, necrosis of tubular epithelium, dilation of renal tubules and pyknotic nuclei were observed in anterior kidney exposed to toxicant. Large number of melanomacrophage centers were observed in spleen after 15 days of chlorpyrifos treatment. The findings of this study clearly illustrates that chronic sublethal exposure to chlorpyrifos could alter the histoarchitecture of various tissues in fish.

Key words: Chlorpyrifos; chronic toxicity; *Etroplus maculatus*; gill; liver; kidney; spleen

1. INTRODUCTION

Chlorpyrifos is one of the most widely used organophosphate insecticides and are reported to be highly toxic in fish. It is a widespread contaminant of fruit and vegetables, and also found in grains, beans, dairy products, meat, fish, tea, and soft drinks. The major pathway of distribution of chlorpyrifos to aquatic ecosystems is through rainfall runoff and air-drift (Xing *et al.*, 2012). Chlorpyrifos is known to moderately persistent in aquatic environments, especially in freshwater and estuaries (Turner, 2003). Therefore, chlorpyrifos when used to control pests are also likely to be exposed to non-target animals including fish. The fishes living in aquatic ecosystems close to agricultural fields are the most important non-target organisms that can be affected by pesticides. Chlorpyrifos is a lipophilic molecule, hence it can easily passes through cell cytoplasm (Uzun *et al.,* 2010). Once inside the cell, chlorpyrifos has been shown to induce damage to the cellular molecules (Ncibi *et al.,* 2008).

Histopathological studies are widely used in ecotoxicology as a valuable tool for assessing the effects of various anthropogenic pollutants, where it reflects on the overall health status of the entire population in the ecosystem. One of the advantages of using histopathological biomarker in environmental biomonitoring is it allows examining the specific target vital organs as gill, liver, kidney and spleen, that are responsible for vital functions such as respiration, metabolism, excretion and immune functions in fish (Wester and Canton, 1991). It also provides a warning signal on the adverse effects of the pollutants and therefore, about the status of aquatic ecosystem and its dwelling organisms. Fish are widely used laboratory model to evaluate the health of aquatic ecosystem and are considered as biologically sensitive organism, due to their ability to respond to changes that occur in the habitat.

The toxicity effect of any pollutants may be either acute or chronic to the exposed animals, where the degree of chronic toxicity could be evaluated by using histopathological biomarkers. The susceptibility of any histoarchitectural alterations in different tissues may vary from animal to animal even at the very low concentrations. Alteration in the normal architecture of tissues in test animal exposed to sub lethal concentration of toxicant are the functional response of the organism, which provides valid information on the effects of pollutant (Bernett *et al.,* 1999).

In the present study an attempt has been made to evaluate the chronic toxicity effect of chlorpyrifos when exposed at sublethal concentration on certain vital tissues as gill, liver, kidney and spleen of freshwater fish, *Etroplus maculatus*.

2. MATERIALS AND METHODS

Freshwater cichlid fish, *Etroplus maculatus*, weighing 3.5 ± 0.5 g and length 6 ± 0.3 cm were collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India. They were transported to laboratory in well aerated polythene bags and acclimatized to the laboratory conditions for two weeks before experiment. During the period of acclimatization, fishes were fed with standard fish pellets and are maintained in well aerated large cement tank (40 L capacity) containing dechlorinated water. The physiochemical features of the tap water were analysed by maintaining water temperature at 28 ± 2°C, dissolved oxygen at 8.5 and pH at 7.6 according to the method as described in APHA (1998).

Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) of technical grate (97%) was obtained commercially from Hikal Chemical Industries, Gujarat, India. Sub lethal concentration of chlorpyifos i.e., 0.661µg/L (one-tenth of LC_{50}-96 h) was chosen for the present study and the animal was exposed to toxicant for 15 days along with the control group. Ten fishes were maintained in each group and after 15 days both control and treated fishes were sacrificed for histopathological study. Gill, liver, kidney and spleen tissues of chlorpyrifos-treated fishes were dissected and fixed in 10% buffered formalin for 24 h and compared with the control tissues.

Tissues were dehydrated in ascending grades of alcohol and were cleared in xylene until they became translucent. Tissues were then transferred to molten paraffin wax for an hour to remove xylene completely and impregnated with wax. The blocks were then cut and sections of thickness 4 to 6 microns were prepared using rotary microtome. The sections were stained with haematoxylin and eosin and mounted in DPX (Roberts and Smail, 2001). The slides were examined and photomicrographs were taken using Cannon shot camera fitted to the Carl Zeiss Axioscope 2 plus Trinocular ResearchMicroscope.

3. RESULTS AND DISCUSSION

The study of histoarchitecture of various tissues exposed to toxicants is increasingly being used as an indicator of aquatic animals on environmental stress since they provide a definite end-point of toxicant exposure (Stentiford *et al.,* 2003). In the present study chlorpyrifos at sub lethal concentration (one-tenth of LC_{50}-96 h) was exposed chronically to freshwater cichlid fish, *Etroplus maculatus*. The alterations in histoarchitecture of tissues as gill, liver, kidney and spleen of fish were examined after 15 days of chlorpyrifos treatment.

a) Histoarchitectural alteration of gill

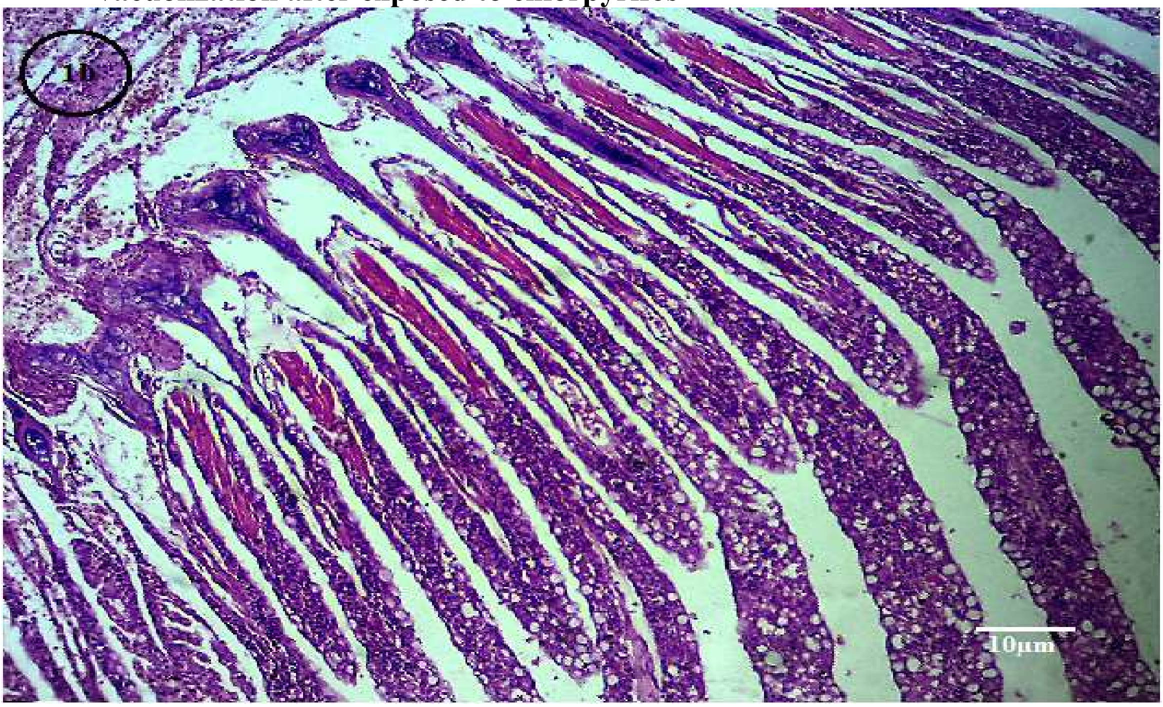
Gills are the primary target of waterborne toxicants because it is the prime organ having constant contact with the water (Mallatt, 1985). In the present study gill of *E. maculatus* in control group showed a normal histoarchitecture. Gill is composed of numerous gill filaments with primary and secondary gill lamellae that run perpendicular to each filament. The lamellae are lined by a squamous epithelium composed of many pillar cells, erythrocytes, several chloride cell, mucous or goblet cell and pavement cells (Fig.1a). In exposure to chlorpyrifos at 0.661 µg/ L showed fusion of secondary lamellae, epithelial lifting, and complete vacuolization at the end of 15 day of treatment (Fig. 1b). In the present study the fusion of secondary lamellae might be due to hyperplasia of epithelial cells and the complete vacuolization of gill could be due to inflammation brought by pesticide toxicity. Therefore, the alteration in histoarchitecture of gill tissue observed in the present study was considered as the direct response of chlorpyrifos. Similar observations were found when chlorpyrifos has been exposed to *Oreochromis mossambicus* and *Etroplus maculatus* for 96 h (Kunjamma *et al.,* 2008; Raibeemol and Chitra, 2015) and in *Cirrhinus mrigala* treated with dichloroves (Velmurugan *et al.,* 2009).

Fig. 1a Photomicrograph showing normal architecture of gill in *Etroplus maculatus*

International Journal of Research
Available at https://edupediapublications.org/journals

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 05
March 2015

Fig. 1b **Photomicrograph showing fusion of secondary lamellae, epithelial lifting, and vacuolization after exposed to chlorpyrifos**



b) Histoarchitectural alteration of liver

Liver is the primary target organ that face any pollutant that enter through portal circulation and are considered as more affected tissue to any toxic

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 05
March 2015

compounds (Roberts and Rodger, 2001). The parenchymatous hepatocytes of teleosts perform many important physiological functions as detoxification of endogenous waste products and other externally derived toxic compounds. The control liver tissues showed normal architecture where regularly distributed polygonal hepatocyts with prominent spherical or round nucleus was seen (Fig. 2a). However, in chlorpyrifos exposed group the liver tissue showed structural abnormalities as vacuolization, and degenerative hepatocyte with pyknotic nuclei (Fig. 2b). This finding is similar to one of the observations in which hepatocytes of *Etroplus maculatus* were exposed to chlordecone at sub lethal concentration for 96 h (Asifa *et al.,* 2014).

Fig. 2a Photomicrograph showing normal architecture of liver tissue in *Etroplus maculatus*

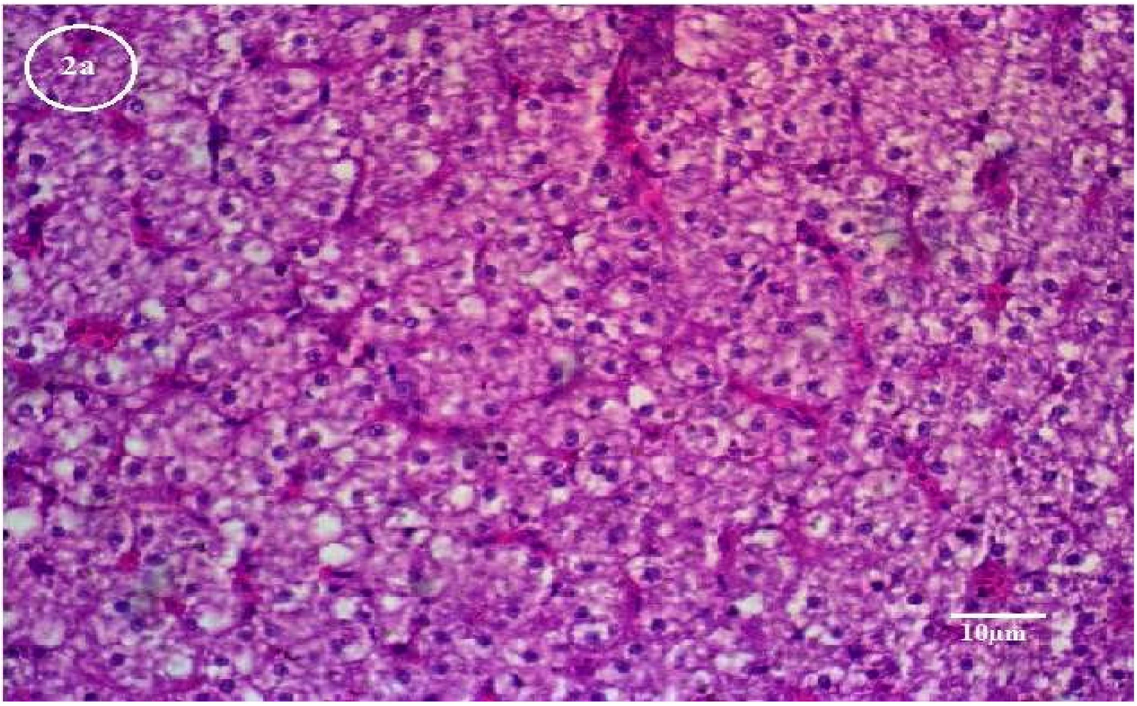
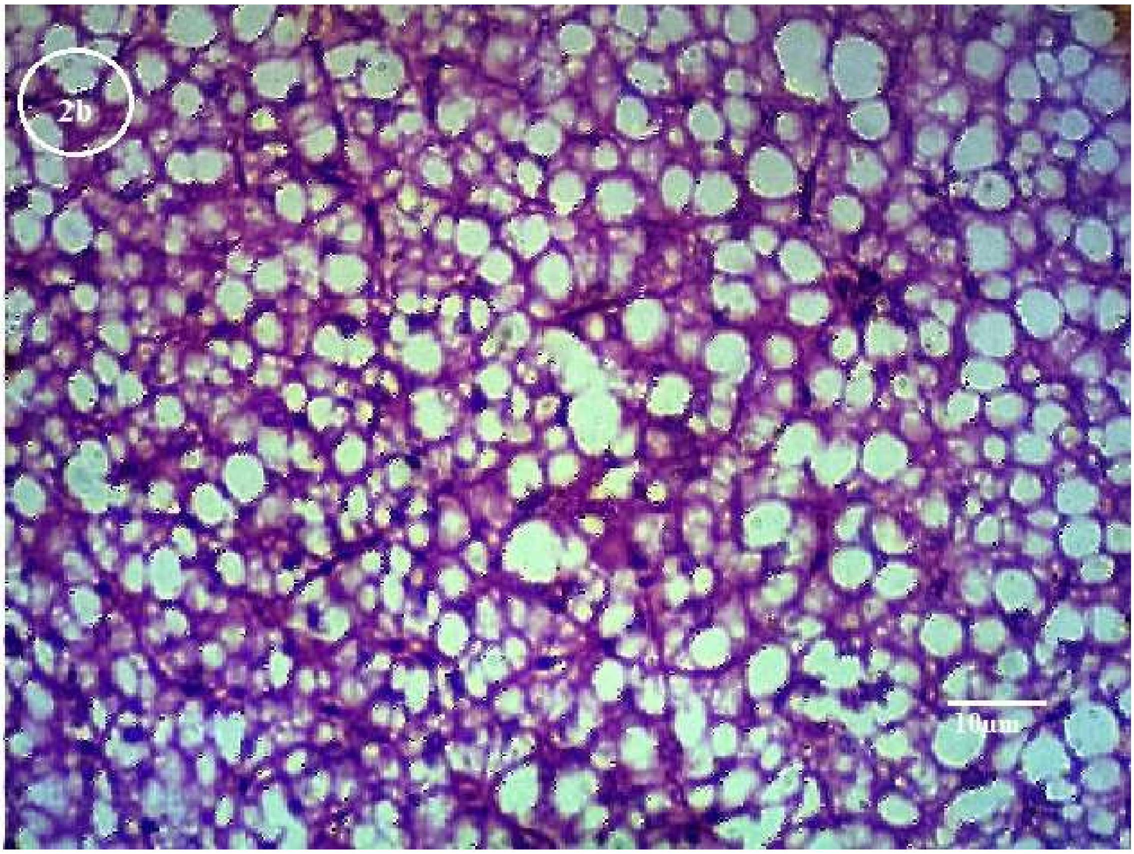


Fig. 2b Photomicrograph showing vacuolization, and degenerative hepatocyte with pyknotic nuclei in *Etroplus maculatus*

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 05
March 2015



c) *Histoarchitectural alteration of kidney*

Kidney is an essential excretory organ that plays a vital role in the maintenance of an organism's internal environment, being the key to the regulation of extracellular fluid volume and composition as well as acid–base balance. It is also a target of toxic chemicals, which can disrupt its functions, and cause temporary or permanent derangement of homeostasis (Iqbal *et al.,* 2004). Therefore, any change in the gross structure of the kidney leads to a corresponding change in the physiological and behavioral parameters (Singla, 2015). Renal lesions may be good indicators of environmental pollution because the fish kidney receives largest portion of post-branchial blood (Ortiz *et al.,* 2003). Kidney of the control group showed normal renal tubules and glomeruli inside Bowman's capsules distributed uniformly in renal interstitial hemopoietic tissue (Fig. 3a). Kidney of 0.661 µg/ L chlorpyrifos treated group showed several pathological changes as cellular degeneration of tubules, necrosis of tubular epithelium, dilation of renal tubules and pyknotic nuclei (Fig. 3b). Similar observations were observed in in the kidney of zebrafish (*Danio rerio*), exposed to sublethal concentration of chlorpyrifos (Scheil *et al.,* 2009).

Fig. 3a Photomicrograph showing normal architecture of kidney tubules in *Etroplus maculatus*

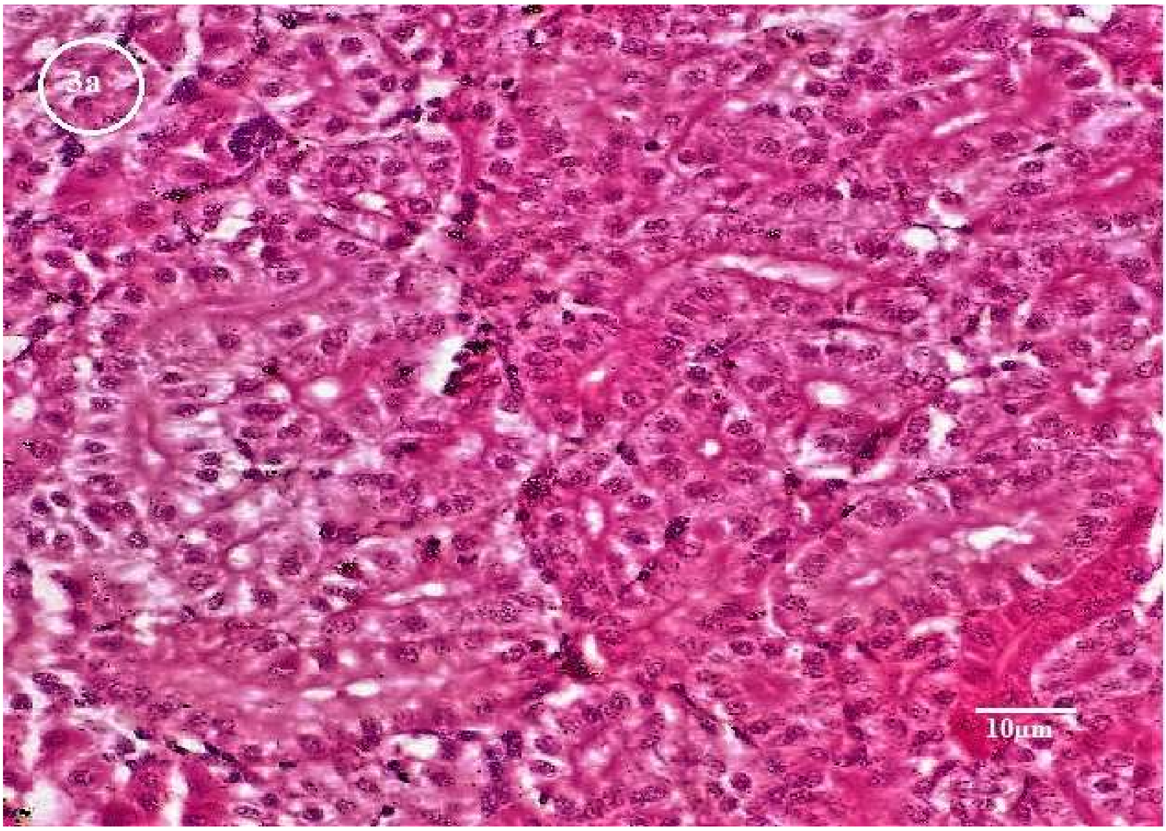
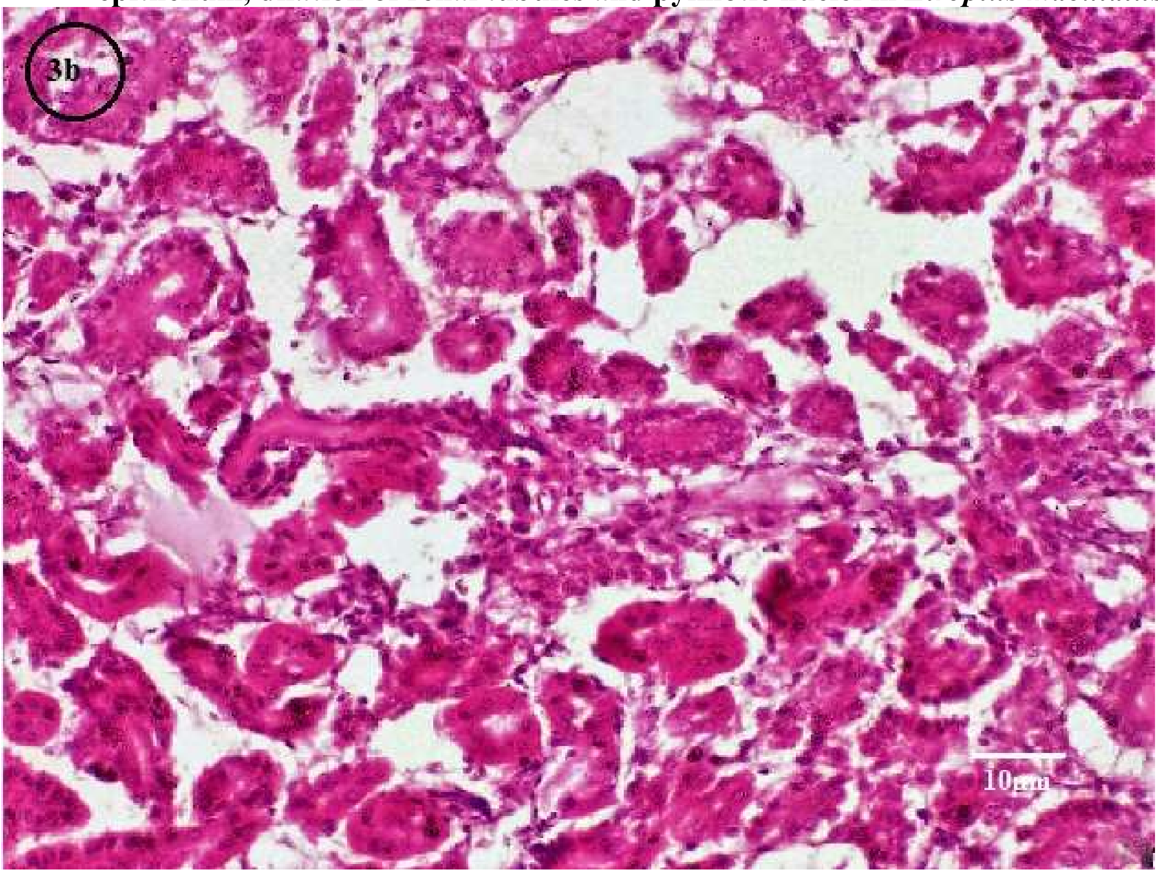


Fig. 3b **Photomicrograph showing cellular degeneration of tubules, necrosis of tubular epithelium, dilation of renal tubules and pyknotic nuclei in *Etroplus maculatus***



p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 05
March 2015

d) Histoarchitectural alteration of spleen

In teleost fish the only lymph-node organ is spleen and as there is no medullary cavity in the bone, spleen also function as primary hemopoeitic organ (Roberts, 2001). Melanomacrophage centers are the important physiological features noted in the fish spleen (Agius and Roberts, 2003). Melanomacrophage centers contain four types of brown pigments namely melanin, lipofuscin, ceroid and hemosiderin (Couillard *et al.,* 1999). In the present study the control group showed normal spleen histoarchitecture consisting of two general components, the lymphopoeitic white pulp and the hemopoeitic red pulp, enclosed by the capsule (Fig. 4a). In chlorpyrifos treated group large number of melanomacrophage centers were observed in spleen tissue. Several toxicological studies have reported tremendous increase in the number of spleen melanomacrophages occur during stressful condition, probably when exposed to toxicants (Montero *et al.,* 1999). Therefore the increase in melanomacrophages might be due to the stress caused due to chlorpyrifos exposure. Similar observation was reported when *Oreochromis niloticus* was dosed with azathioprine (Gogal *et al.,* 1999).

Fig. 4a Photomicrograph showing normal architecture of spleen with white and red pulp in *Etroplus maculatus*

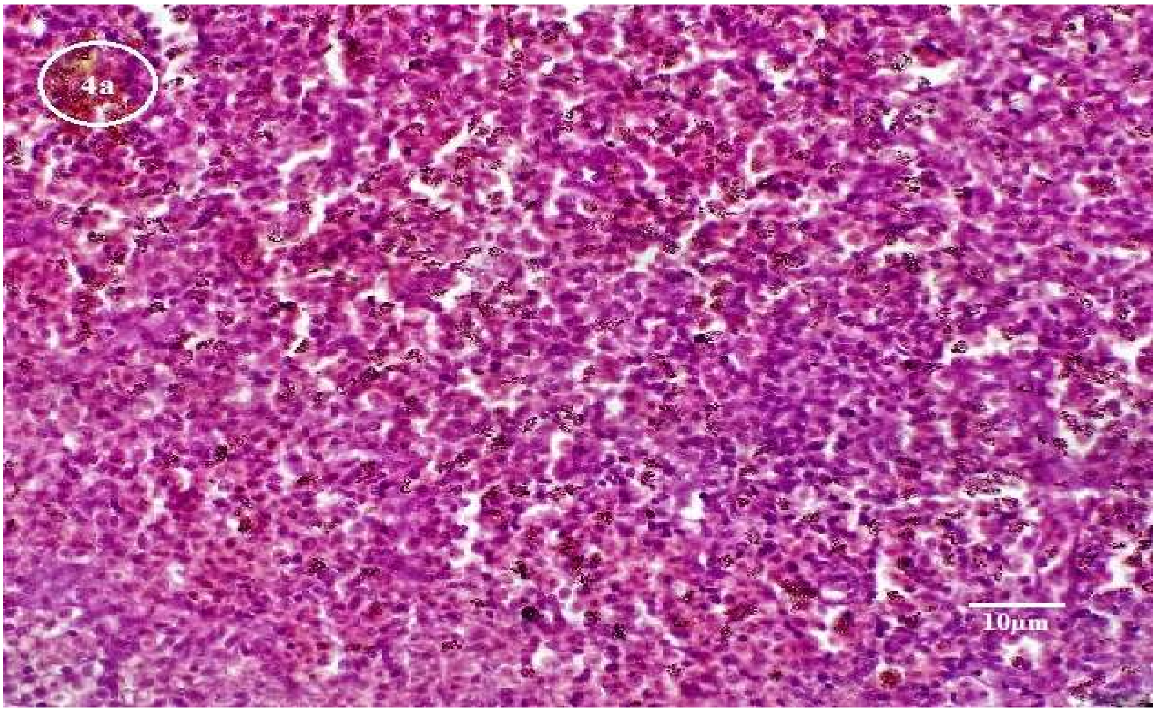
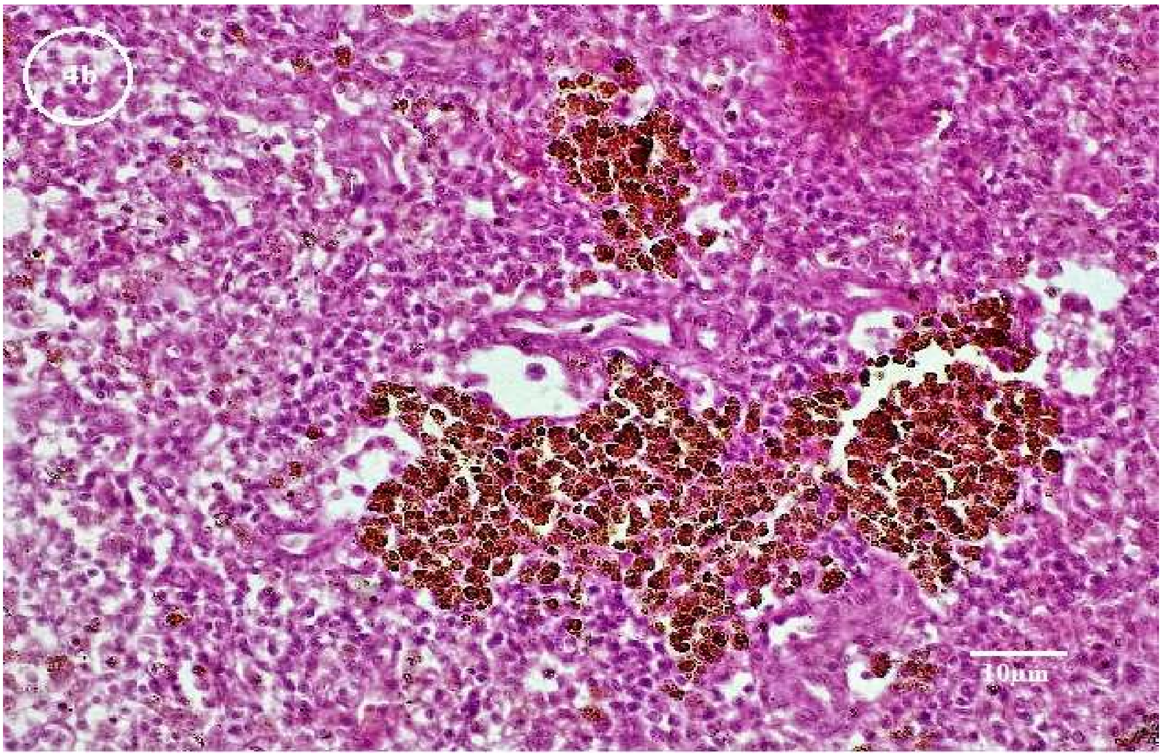


Fig. 4b Photomicrograph showing large number of melanomacrophage centers in *Etroplus maculatus*



4. CONCLUSION

The study clearly indicated the effects of chlorpyrifos on the histoarchitecture of various vital tissues. Therefore, chronic exposure to chlorpyrifos at sub lethal concentration could result in cellular toxicity in freshwater cichlid fish *Etroplus maculatus*. Hence safety measures must be undertaken in the aquatic ecosystem that are likely to be polluted to the organophosphorus pesticide, chlorpyrifos.

Acknowledgment

Authors gratefully acknowledge the financial support provided by Moulana Azad National Fellowship – University Grants Commission (UGC-MANF), Government of India.

5. REFERENCES

[1.] Agius C. and Roberts RJ. (2003). Melano-macrophage centres and their role in fish pathology. *J. Fish Dis.* 26: 499–509.

[2.] APHA. 1998. Standard methods for the examination of water and waste water, 20th Edition, Washington, DC.

[3.] Asifa KP, Balakrishnan V. and Chitra KC. (2014). Toxicity evaluation of chlordecone and its effect on oxidative imbalance in the Cichlid fish, *Etroplus maculatus* (Bloch). *Int. J. Zool. Res.* 4(2): 1-20.

[4.] Bemet D, Schmidt H, Meier W, Burkhardt-Hol P. and Wahli T. (1999). Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *J. Fish Disease.* 22: 25-34.

[5.] Couillard CM, Williams PJ, Courtenay SC. and Rawn GP. (1999). Histopathological evaluation of Atlantic tomcod (*Microgadus tomcod*) collected at estuarine sites receiving pulp and paper mill effluent. *Aquat. Toxicol*. 44: 263-278.

[6.] Gogal RM, Smith BJ, Robertson JL. Smith SA. and Holladay SD. (1999). Tilapia (*Oreochromis niloticus*) dosed with azathioprine display immune effects similar to those seen in mammals, including apoptosis. *Vet. Immunol. Immunopathol.* 68: 209-227.

[7.] Iqbal F, Qureshi IZ. and Ali M. (2004). Histopathological changes in the kidney of commoncarp, *Cyprinus carpio* following nitrate exposure. *J.Res.Sci.* 15: 411-418.

[8.] Kunjamma A, Philip B, Bhanu S. and Jose J. (2008). Histopathological effects on *Oreochromis mossambicus* (Tilapia) exposed to chlorpyrifos. *J E R D.* 2(4): 553-559.

[9.] Mallatt J. (1985). Fish gill structural changes induced bytoxicants and other irritants: a statistical review. *Canadian J. Fisheries Aquat. Sci.* 42: 630-648.

[10.] Montero D, Blazer VS, Socorro J, Izquierdo MS. and Tort L. (1999). Dietary and culture influences on macrophage aggregate parameters in gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture* 179: 523-534.

[11.] Ncibi S, Ben Othman M, Akacha A, Krifi MN. and Zourgui L. (2008). Opuntia ficus indica extract protects against chlorpyrifos-induced damage on mice liver. *Food Chem. Toxicol.* 46(2): 797-802.

[12.] Ortiz J, De Canales M. and Sarasquete C. (2003). Histopathological changes induced by Lindane (γ- HCH) in various organs of fishes. *Scientia Marina* 67: 53–61.

[13.] Raibeemol KP. and Chitra KC. (2015). Histopathological alterations on gill, liver, kidney and spleen of cichlid fish, *Etroplus maculatus* exposed to chlorpyrifos. *Int. J. Recent Sci. Res.* 6(10): 6892-6896.

[14.] Roberts R. (2001). Fish pathology, 3rd Edition, (Ed. W. B. Saunders), Churchill Livingstone, USA, pp. 1-492

[15.] Roberts RJ. and Rodger HD. (2001). The pathophysiology and systematic pathology In: Ronald J. Roberts, *Fish pathology, 3rd Edn*., Harcourt publishers limited, 88.

[16.] Roberts RJ. and Smail DA. (2001). Laboratory methods In: Ronald J. Roberts, Fish pathology, 3rd Edn., Harcourt publishers limited, pp 380-390.

[17.] Scheil V, Zürn A, Köhler H. and Triebskorn R. (2009). Embryo development, stress protein (Hsp70) responses, and histopathology in Zebrafish (*Danio rerio*) following exposure to nickel

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 05
March 2015

chloride, chlorpyrifos, and binary mixtures of them. *Environ. Toxicol.* 25(1): 83-93.

[18.] Singla N. (2015). Histological changes induced by monocrotophos in the kidney of *Ctenopharyngodon idellus* (Cuvier and Valenciennes). *Int. J. Res. Fish. Aqua.* 5(2): 54-59.

[19.] Stentiford GD, Longshaw M, Lyons BP, Jones G, Green M. and Feist SW. (2003). Histopathological biomarkers in estuarine fish species for the assessment of biological effects of contaminants. *Mar. Environ. Res*. 55: 137-159.

[20.] Turner L. (2003). Chlorpyrifos analysis of risks to endangered and threatened salmon and steelhead. Environmental field branch Office of Pesticide Programs. 134 p.

[21.] Uzun FG, Demir F, Kalender S, Bas H. and Kalender Y. (2010). Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. *Food Chem. Toxicol.* 48(6): 1714-1720.

[22.] Velmurugan B, Selvanayagam M, Cengiz EI. and Unlu E. (2009). Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos., *Brazilian Arch. Biol. Technol.* 52(5): 1291-1296.

[23.] Wester PW. and Canton JH. (1991). The usefulness of histopathology in aquatic toxicity studies. *Comp. Biochem. Physiol.* 100: 115-117.

[24.] Xing H, Li S, Wang Z, Gao X, Xu S. and Wang X. (2012). Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and Chlorpyrifos. *Chemosphere* 88: 377-383.