Advances in Bioresearch
Adv. Biores., Vol4 (2) June 2013: 57- 62
©2013 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr/abr.htm
CODEN: ABRDC3

ORIGINAL ARTICLE

Histopathological Alterations in Gill and Liver Anotomy of Fresh Water, Air Breathing Fish *Channa Punctatus* after Pesticide Hilban® (Chlorpyrifos) Treatment

Yogita Devi[a] and Abha Mishra[b]
[ab]Department of Applied Animal Sciences,
Babasaheb Bhimrao Ambedkar University (A Central University),
Lucknow-226025, India.
Email – yogitox@gmail.com

ABSTRACT
Sublethal concentrations of organophosphorous pesticide Chlorpyrifos (0, 0-Diethyl – 0 – 3, 5, 6 – trichloro-2- pyridy phosphorothioate) were investigated with fresh water teleost fish Channa punctatus. Fish were exposed to 1/3rd (1.46 µl/l) and 1/10th (0.538 µl/l) of 24 h LC_{50} for the period 3 days toxicity (short term exposure) and 7 day (long term exposure) through static renewal test. Liver and gill samples were collected after 3 & 7 days of exposure to chlorpyrifos (CPF) and lesions were analyzed by upright microscopy. The main histopathological changes caused by CPF in gills exposed to the highest concentration were edema, lifting of lamellar epithelia and an intense vasodilatation of the lamellar vascular axis. Although less frequent, lamellar fusion caused by the filamentar epithelium proliferation and some lamellar aneurisms were also found. Gill disorders include hypertrophy; hyperplasia and lifting of epithelial cells and fusion of secondary lamellae were pronounced in all treatment. The liver of control group exhibited a quite normal architecture, while the fish exposed to CPF showed vacuolation and necrosis. These hepatic alterations were more evident in fish exposed to higher concentrations and long term exposure tenure. Liver suffered from toxicity as hypertrophy of hepatocytes, infiltration of leukocytes, necrosis and fibrosis. Even low dose of CPF produced severe pathological lesion for long term exposure in gills as well as liver.
Key wards: *Histopathology, Chlorpyrifos, Channa punctatus, gill, liver etc.*

Received: 20/04/2013 Accepted: 01/06/2013

INTRODUCTION

Water pollution is a major problem of this century owing to the addition of various pollutants in water bodies through many ways and they changes their natural qualities of water [27]. In case of inland water contamination pesticides are known commonly, closer to areas of their applications which affect growth, reproduction and nutritional value of fish, when their concentration in water exceeds the critical maximum limit [15]. Among different classes of pesticide organophosphate are more frequently used, because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment.

Chlorpyrifos (0, 0-Diethyl – 0 – 3, 5, 6 – trichloro-2- pyridy phosphorothioate) is an organophosphorous pesticide commonly known as cholinesterase inhibitor and may cause disorders in the physiological by inhibiting the enzyme acetylcholinesterase (AChE), which modulates the amount of neurotransmitter, acetylcholine. Alterations in the cellular morphology of pesticide treated fish [1] and their physiological functions upon exposure to different pesticide concentrations have been observed by Gupta and Saxena, [10]. The investigation of histopathology of various organs may therefore, prove it is cost effective tool to determine health of fish population and reflecting the health of entire aquatic ecosystem [23, 25, and 26]. The study of fish liver is very important in the field of aquaculture induced by many problematic condition and aquatic pollution [2]. It is also well known that gills are among the most sensitive organ, which reacts first in changed environment. Since respiration, osmoregulation and excretion are performed through the gills [12, 22].

To rescue the living world from the harmful effects of these chemical substances as well as to find some alternative ways to control the pests, it is very necessary to carry out works on diversity of organisms by

applying varying concentration of pesticides. However, the reports on the effects of low dose of Chlorpyrifos in fresh water teleost fish *Channa punctatus* are still scanty. In the light of above information and ideas, present investigation is aimed to study the effect of sub-lethal concentration of Chlorpyrifos on liver and gill of the fresh water teleost fish *Channa punctatus.*

MATERIALS AND METHOD

Test chemical and animal model

Hilban® (20 % EC chlorpyrifos) and other chemical of analytical grade were purchased from scientific suppliers locally.

Animal collection and acclimatization

Live specimens of 36 ± 4g weight & 16 ± 4cm. lengths *Channa punctatus* were collected from local market and water bodies (pond, river, canal etc.) in vicinal area of Lucknow. They were acclimatized in laboratory for 1 week under normal photoperiod (12 h light & dark) and temperature (23 ± 1.5 ^{0}C).

Experimental design

To study the sublethal effect of Hiban® on tissue anatomy (short term), an experiment was conducted in which the experimental fishes were divided in to three groups. In first group: fish were exposed to 1/3rd of LC_{50} (1.46 µl/l) for 3 & 7 days exposure period. The second group: fish were exposed to 1/10th of LC_{50} (0.538 µl/l) for 3 & 7 days and in third group a control set of fish was running with experimental group. The LC_{50} of Hiban® for 24 h was found 5.38 µl/l through the probit analysis. In each set 10 fish were taken. During this period dead fish were removed immediately and the water was changed regularly at 24 h intervals with the same amount of the toxicant. The fish were fed daily. At the end of specified period of experimental exposure, tissues were dissected out for histological process after sacrificing the fish.

Histological study

Tissue (gills and liver) were collected from the treated as well as control fish and preserved in Bouin's fixatives. The method of Bernet, [3] was adopted for processing of tissues for histological studies. Tissue were dehydrated in different graded series of alcohol, they were cleared in xylene and embedded in paraffin. Further the sagittal sections (5 µ thickness) were cut using a rotary microtome and mounted on glass slides. Sections were deparaffinized in xylene, hydratated in ethanol and stained with haematoxylin and alcoholic eosin (H&E) for general histological evaluation. Photomicrographs of stained sections were made using Nikon upright microscope.

RESULTS

In the present study a variety of histopathological changes were observed in the liver and gill of *Channa punctatus*. The severity and frequency of organ lesions was found to be more pronounced in fish treated with higher concentration of Hilban because of alterations were does and duration dependent.

A. Histopathological observations of Gill

The gill is composed of numerous gill filaments which have two rows (primary gill lamellae, PL) and secondary lamellae that run perpendicular to each filament (Fig. 1A). The lamellae are lined by a squamous epithelium composed by pavement (PV) and nondifferentiated epithelial cells. Each lamella is made up of two sheets of epithelium delimited by many pillar cells (PC), which are contractile and separate the blood (capillary) channels (BC) in which one to two erythrocytes are usually recognized within each capillary lumen. Between the lamellae, the filament is lined by a thick stratified epithelium constituted by several cellular types, such as chloride cell (CC), mucous or goblet cell (GC) and pavement cells (Fig.1B). Mucus cells and pavement cells are also present in the epithelium of the filament and at the base of lamellae, but they are smaller than chloride cells the gill filaments are covered with squamous pavement cells showing characteristic concentric patterns of microridges (Fig. 1B).

Fish showed some signs of epithelial lesions when exposed to both sublethal concentration of CPF which was time dependent even treated with low concentration. The main changes observed at long term period (7 day) of CPF exposure were accentuated lifting of the lamellar epithelium (LE), oedema in the filamentary epithelium and an intense lamellar vasodilatation. The section of gills treated with 1.46 µl/l CPF exhibited lamellar fusion in numerous areas of the secondary lamellae with hyperplasia in chloride cell (CC), pillar cell (PL), pavement cell (PV) and mucus secratihg globlet cells (GC) for short term (3 days) exposure time [Fig.1C]. At long term treatment of CPF gill showed lamellar aneurism (lesions of blood vessels), synechiae (adhesion of tip) and congestion & leucocytes infiltration, interlamellare oedema (IO), congestion in secondary lamellae and telengesis (T) were noticed (Fig. 1D). Gills of 0.538 µl/l CPF treated group showed also histopathological changes through the treatment period those were extensive hypertrophy and hyperplasia of epithelial, mucus secreting cell (GC) and chloride cells (CC) due to complete fusion of secondary lamellae and, haemorrhage in cartilaginous core (HC), at the end of 3 day treatment (Fig. 1E). At the long term (7 day) 0.538 µl/l CPF shows hypertrophy and proliferation in the

erythrocytes of cartilaginous core (HPC); complete destruction of the secondary lamellae, obliteration of normal lamellae architecture as lifting of epithelial cells (LE) affecting the apical distal ends of the gill and the distructed blood capillaries aggregate and showed lamellar aneurism (LA) (Fig. 1 F).

B. Histopathological observations of Liver

The liver of untreated fish was exhibited parenchymatous appearance and mainly consisted of polygonal shaped hepatocytes (H) with their central nuclei. Sinusoids (S), are irregularly distributed between the polygonal hepatocytes. Hepatopancreatic alveoli of the exocrine pancreas were seen to be placed in the parenchyma and the melanomacrophase centres stained light brown in colour, were located closed to the hepatopancrease. Some of the hepatocyte nearby the hepatopancrease was slightly vacuolated it could be due to storage of glycogen and lipid (Fig.2 A).

The fish exposed to both sublethal concentrations showed moderate to severe changes. The degree of histopathological lesions was seemed to be related to the increasing exposure period of CPF treatment. The main alteration found in the liver was: nuclear hypertrophy (NH), nuclear vacuolation (V), granular cytoplasm, bile stagnation (BS) as brownish yellow granules in the cytoplasm and necrosis (N). The intensive hepatocelluar vacuolization i.e. steatosis (S) was raised from 3 day (Fig. 2B) exposure of 1.46 µl/l CPF and more pronounced on 7th day treatment such as granular cytoplasm and degeneration of hepatocytes (DH) with dialation of sinusoids (DS) lumen (Fig. 2C). Cellular breakdowns and cytoplasmic vacuolation (CV) were distinguished in combination with pyknosis (P) and karyokinesis (K) was observed in the liver of 0.538 µl/l exposed CPF for 3 day (Fig.2D). In the hepatopancreatic region of liver section of fish exposed to 0.538 µl/l CPF for 7 day showed damage which was characterised by widen extracellular spaces and loss of contact between hepatocytes and pancreocytes (Pn), and lysis of pancreatic membrane (M) was observed (Fig. 2E).

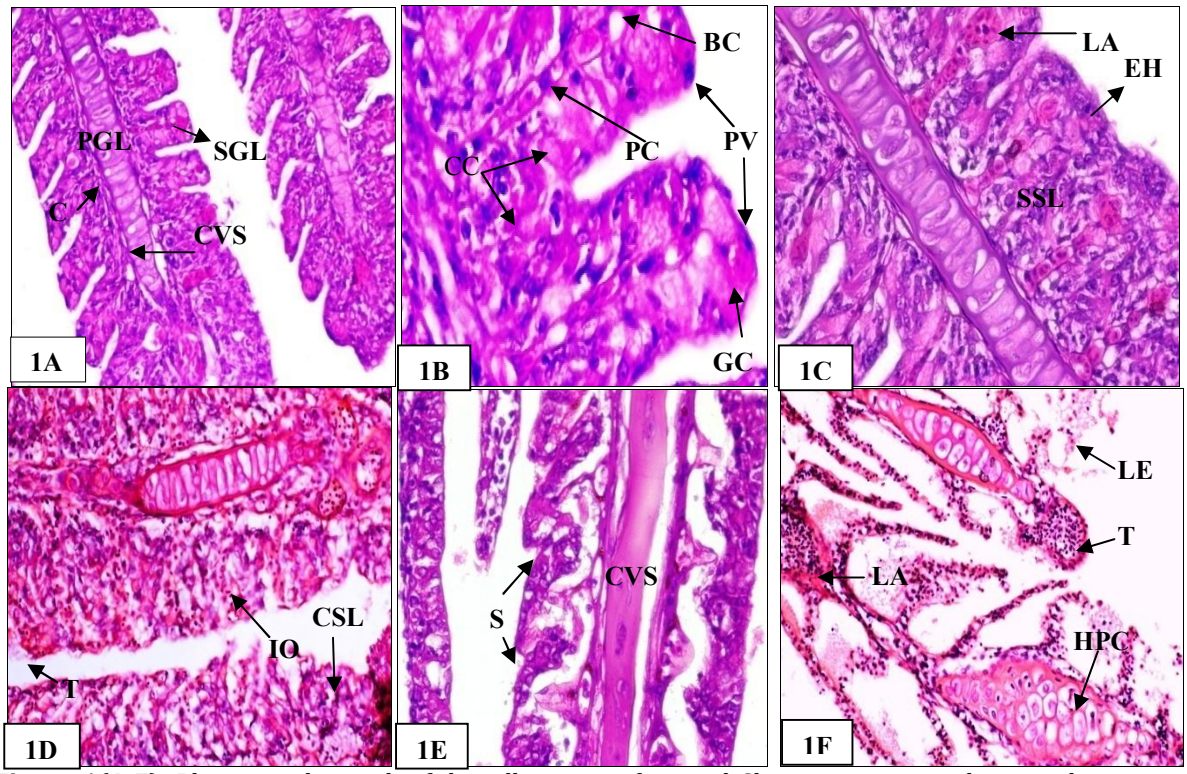

Figure 1(A-F): Photomarphograph of the gill section of control Channa punctatus showing the primary (PGL), with cartilaginous core (C) with erythrocytes cells (EC), [1A, 20X] and in magnified part of Fig 1A of secondary gill lamellae (SGL), showing epithelial cells - pillar cells (PC) separated by blood channels (BC), pavement cells (PV), chloride cells (CC), goblet cells (GC) [1B, 40X]. Photomarphograph of the gill section treated with 1.46 µl/l for 3 day showing epithelial hyperplasia (EH) and sloughing of secondary lamellae (SSL): chloride cells, pillar cell and goblet cells [1C] and for 7 day showing intraepithelial oedema (IO), congestion of entire secondary lamellae (CSL) and telengiectiasis (T) [1D]. Photomarphograph of gill section treated with 0.538 µl/l for 3 and 7 day [1E&F] marked epithelial hyperplasia with leukocyte infiltration and lamellar synehiae (S), sever telengiectiasis (T), haemorrhage in the central venous sinus (CVS) of cartilaginous core (HC) and hypertrophy and proliferation in the erythrocytes of cartilaginous core (HPC); complete destruction of the secondary lamellae, obliteration of normal lamellae architecture as lifting of epithelial cells (LE) affecting the apical distal ends of the gill.

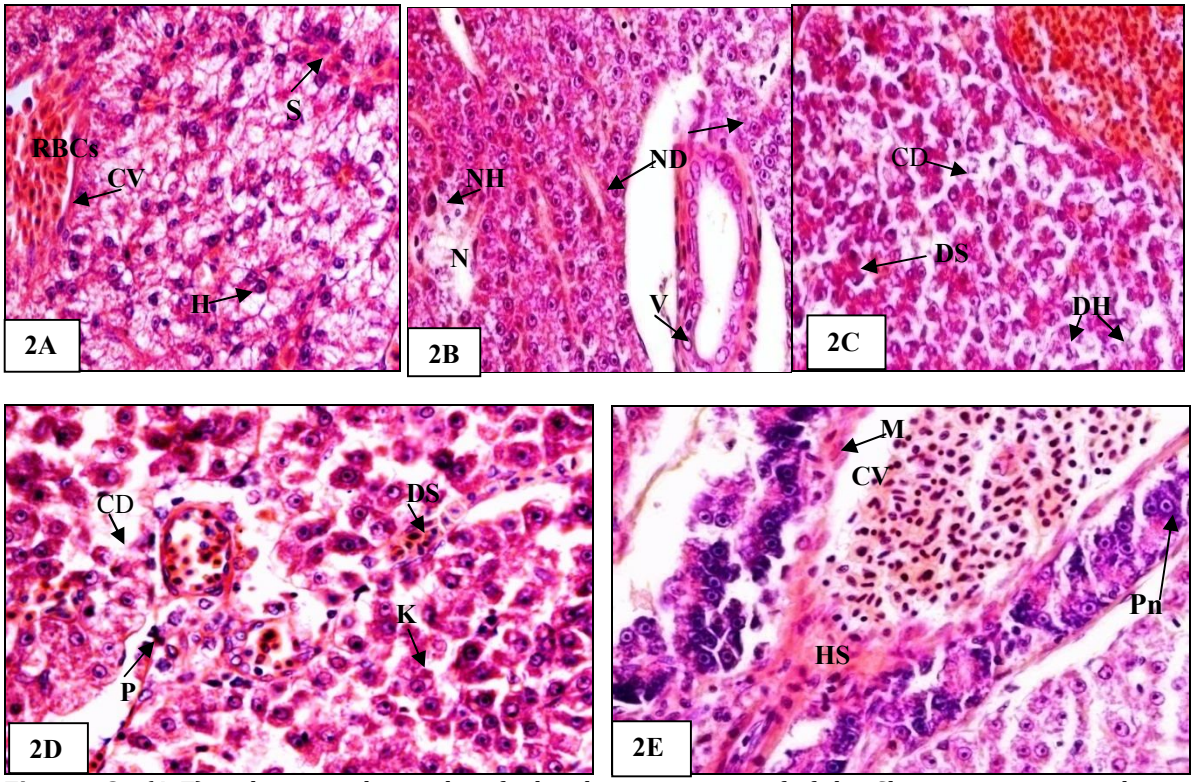

Figure 2 (A-E): photomarphograph of the liver section of fish *Channa punctatus* showing parenchymatous structure in which sinusoids (S) are arranged with polygonal hepatocytes (H) and a central vein (CV) arranged RBC, in between. And there was no lesion (2A). Photomorphograph of liver section of fish treated with 1.46 ul/l of CPF for 3 day (short term), showing necrosis and dilatation in the sinusoids (ND), necrosis in hepatpcytes (N) hepatocytes vacuolation (V) and central vein with no RBC (2B). Photomorphograph of liver section of fish treated with 1.46 ul/l of CPF for 7 day (long term), showing dilatation in sinusoid (DS), degeneration of hepatocytes (DH) and cytoplasmic (CD) (2C). Photomorphograph of liver section of fish treated with 0.538 ul/l of CPF for 3 (short term) & 7 day (long term), showing cytoplasmic vacuolation (CV), severe dilation in sinusoids (DS), pyknosis (P), karyokinesis (K) (2D) and widen extracellular spaces and loss of contact between hepatocytes and pancreocytes (Pn), and lysis of pancreatic membrane (M) (2E).

DISCUSSION

Histopathological changes have been used as important biomarkers in environmental monitoring that allows examining specific target organs. The histological results observed in all the tissues of *C. punctatus* in the present study indicate that sub lethal concentrations caused moderate to severe alteration in gill and liver architecture, which are an important organs performing vital function like detoxification, respiration, osmoregulation, acidbase balance, etc. Furthermore the alternations found in these organs are normally easier to identify than functional ones [8] and sever as warning signs of damage to animal health. Various lesions in gill were recorded in the present study such as epithelial hyperplasia and hypertrophy, sloughing of secondary lamellar epithelial cells: chloride cells, pillar cell and goblet cells, intraepithelial oedema congestion of entire secondary lamellae, telengiectiasis, leukocyte infiltration and lamellar synehiae, haemorrhage in cartilaginous core, hypertrophy and proliferation in the erythrocytes of cartilaginous core, lamellar aneurism and obliteration of normal lamellae architecture as lifting of epithelial cells affecting whole structure of the gill. The different concentrations of CPF used in the present study as well as the different exposure periods showed different degrees of pathological changes. These result were recorded similarly in the freshwater fish (*Puntius gonionotus*), *Oreochromis niloticus*), exposed to pesticides paraquat, and dimethoate [6, 7] respectively. In the present study, the epithelial hyperplasia and hypertrophy could be a consequence of the epithelial detachment [13] fusion of the lamellar epithelial cells and the adhesion of the lamellar tips, seen as synechiae [14]. Lesions associated with the lamellar aneurysm were observed in the prevalence in all treatment could be due to the disturbance of blood flow in the blood channels. Hypertrophy and hyperplasia, total fusion of the secondary lamellae, dilation of capillaries of secondary lamellae and lifting up the gill epithelium in the respiratory area observed in the current work, were considered to be of the first degree of gill lesions

while lamellar aneurysms It was that extensive lamellar aneurysm (telagiectasis) takes considerably longer time to resolve than the hyperplastic lesions of the gill [4]. Leucocytic infiltration in the gills was also noticed it may be due to inflammatory reaction by which, fish fight constantly against the osmotic influx of water in the toxic environment that occurs across the gill during respiration as studied by Neskovic, [17] in carp fish *Cyprinus carpio* treated with glyphosate. Epithelium lifting (chloride cells, pavement cells) increases the distance through which the toxicant reach to the blood stream that caused oxygen uptake is impaired [11] and lamellar fusion could be protective as it diminishes the amount of vulnerable gill surface area [18]. These epithelial lifting reactions could result in dysfunctional or even non- functional gills, and eventually asphyxiate the fish. These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants thorough the gill surface and probably due to increased capillary permeability [19].

The organ most associated with the detoxification and accumulation process is liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water [5] it also plays a prominent role in fish physiology, both in anabolism (protein, lipid, carbohydrate) and catabolism (glycogenolysis, detoxification) and it acts as storage center for many substances, mainly glycogen. The liver of the fish exposed to both low as well as high dose showed vacuolar degeneration, hypertrophy in the hepatocytes with nuclear pyknosis and karyopyknosis [22] due to apoptosis and fragmentation of the nucleus. These changes may be attributed to direct toxic effects of pollutants on hepatocyte as found in pesticide toxicity [16], because of it is the site of detoxification of all type of toxins and chemicals. The liver parenchyma showed sign of degeneration (cytoplasmic and nuclear), vacuolation of the hepatocytes [21], probably due to metabolic damage related to exposure with CPF contaminated water. In the current observations the bile stagnation in the liver characterized by remains of the bile in the form of brownish yellow granules in the cytoplasm of the hepatocytes indicates that the bile is not being released from the liver. This accumulation of bile indicates possible damage to the hepatic metabolism [8].

CONCLUSION

The current study concluded that CPF induced highly toxic to fish Channa punctatus as even low dose by producing hepatic and respiratory toxicity through damage in cellular morphology of gill and liver tissues. As food source, fish interferes food chain including human's life quality.

ACKNOWLEDGMENT

Yogita Devi is thankful to Department of Applied Animal Sciences, BBA University for financial assistance as UGC research fellowship.

REFERENCES

1. Abhilash, R. and Prakasam, V.R., (2005). Toxic, physico-morphological and behavioural responses *Oreochromis mossambicus* exposed to commercial grade endosulfan. Envirn. Ecol., 54(2): 234-238.
2. Bertolucci, B., Vicentini, C.A., Vicentini, I.B.F. and Bombonato, M.T.S., (2008). Light microscopy and ultrastructure of the liver of Astyanax altiparanae (Garutti and Britski, 2000) (Teleostei, Characidae) Mari. 30:73-76.
3. Bernet, D., Schmidt, H., Meir, W., Burkhardt-Holm, P. and Wahli, T., (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22:25-34.
4. Campagna, A., Eler, M., Fracacio, R., Rodrigues, B. and Verani N., (2007). The toxic potential of aldrin and heptachlor on *Danio rerio* juveniles (Cypriniformes, Cyprinidae). Ecotoxicol., 16: 289–298.
5. Camargo, M. M. and Martinez, C. B., (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotro. Ichthyol. 5:327-336.
6. Cengiz, E. and Unlu, E., (2006). Sublethal effects of fish histology-normal and pathological features commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: A microscopic study. Environ. Toxicol. Pharmacol., 21:246-253.
7. Elezaby, M. M., El-Serafy S., Heckmann R., K. Sharf Eldeen, Seddek, M. M., (2001). Effect of some toxicants on the fresh water fish *Oreochromis niloticus*. J. Egy. Gen. Soci. Zool., 36: 407-434.
8. Fanta, E., Rios, F. S., Romao, S., Vianna, A. C. C. and Freiberger, S., (2003) "Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food." Ecotoxicology and Environmental Safety, 54(2), pp. 119-130.
9. Fernandes, M. N. and Mazon, A. F. (2003). Environmental pollution and fish gill morphology. In:Val, A.L. and B.G. Kapoor (Eds.). Fish adaptation. Enfield, Science Publishers. pp. 203-231.
10. Gupta, P. and Saxena, P.G., (2006). Biochemical and haematological studies in freshwater fish *Channa Punctatus* exposed to synthetic pyrethroids. Poll. Res., 25(3): 499-502.
11. Kumar, M., Prasad, M. R., Srivastva, K., Tripathi, S., and Srivastva, A. K., (2010) "Branchial histopathological study of Catfish Heteropneustes fossilis following exposure to purified neem extract, Azadirachtin." World Journal of Zoology, 5(4), pp.239-243.

12. Lease, H. M., Hansen, J. A., Bergman, H.L., Meyer, J.S., (2003). Structural changes in gills of Lost River suckers exposed to elevated pH and ammonia concentrations Comp. Biochem. Physiol., 134:491-500.
13. Machado, M. and Fanta, E., (2003). Effects of the organophorous methyl parathion on the branchial epithelium of a freshwater fish *Metynnis eoosevelti"*. Brazi. Arch. Biol. Technol., 46(3):361-372.
14. Morrison, R, Nowak, B. and Carson, J., (2001). The histopathological effects of a levamisole-adjuvanted *Vibrio anguillarum* vaccine on Atlantic salmon (*Salmo salar* L.) Aquaculture, 195:23-33.
15. Moore, A. and Waring, C.P., (2001). The effect of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon *Salmo salar* L. Aqu. Toxicol. 52(1):1-12.
16. Mohamed, F. A. (2009). Histopathological studies on Tilapia zillii and Solea vulguris from Lake Qarun, Egypt. World J. Fish and Mari. Sci., 1(1):29-39.
17. Neskovic, N., Poleksic, V., Elezovic, I., Karan, V. and Budimir, M. (1996). Biochemical and histopathological effects of glyphosate on carp, *Cyprinus carpio* L. Bull. Environ. Contam. Toxicol., 56:295-302.
18. Ortiz, J., De Canales, M. and Sarasquete, C. (2003). Histopathological changes induced by lindane (γ-HCH) in various organs of fishes. Sci. Mar., 67 (1):53–61.
19. Olurin, K., Olojo, E. Mbaka, G. and Akindele, A., (2006). Histopathological responses of the gill and liver tissues of Clarias gariepinus fingerlings to the herbicide, glyphosate." Afr. J. Biotechnol., 5:2480-2487.
20. Poleksic, V. and Mitrovic-T. V., (1994). Fish gills as monitor of sublethal chronic effects of pollution. In: Muller R. and Lloyd R. Sublethal and chronic effects of Pollutants on Freshwater fish. United Nations Fishing New Books, 339-352.
21. Pacheco, M. & Santos, M. A. (2002). Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). Ecotoxicol. Environ. Saf., 53(3):331-347.
22. Ramah, K., (2011). Histopathological study on the effect of rice herbicides on grass carp (Ctenopharynggodan idella). Afr. j. Biotechn., 10(7):1112-1116.
23. Rodrigues, E. L. and Fanta, E., (1998). Liver histopathology of the fish Brachydanio Rerio Hamilton-Buchman after acute exposure to sublethal levels of the organophosphate Dimethoate 500. Revta bras. Zool. 15(2):441-450.
24. Sridhar, K., and Joice, P. E., (2012). Carbendazim induced histopathological and histochemical changes in liver tissues of common carp *Cyprinus carpio*. Int. J. adv. sci., 5(1):65-70.
25. Thophon, S., Kruatrachue, M., Upathan, E. S., Pokethitiyook, P., Sahaphong, S., Jarikhuan, S., (2003). Histopathological alterations of white seabass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environ. Poll., 121: 307-320.
26. Vinodhini, R. and Narayanan, M., (2009). Heavy Metal Induced histopathological alterations in selected organs of the Cyprinus carpio L. (Common Carp).' Int. I. Environ. Res., 3(1):95-100.
27. Voltz, M., Louchart, X., Andrieux, P. and Lennartz, B., (2005). Process of water contamination by pesticides at catchment scale in mediteranean areas. Geophy. Res., 7:106 -134.

How to Cite This Article
Yogita D. and A. Mishra. (2013). Histopathological Alterations in Gill and Liver Anotomy of Freh Water, Air Breathing Fish *Channa Punctatus* after Pesticide Hilban® (Chlorpyrifos) Treatment. Adv. Biores. 4 (2): 57-62.