BIOLOGIA (LAHORE)
December, 2019, 65 (II), 189-198

PKISSN 0006 – 3096 (Print)
ISSN 2313 – 206X (On-Line)

Histological responses in Intestine, Kidney and Liver tissues of *Labeo rohita* during acute and chronic exposure to Pesticide, Chlorpyrifos

ADEEBA SYED[1], DILAWAR HUSSAIN[2], UZMA RAFI[1] & SUMAIRA MAZHAR[1*]

[1]*Department of Biology, Lahore Garrison University, Phase-VI, DHA, Lahore Pakistan*
[2]*Department of Zoology, Government College University, Lahore-54000 Pakistan*

ARTICLE INFORMAION

Received: 27-07-2018
Received in revised form: 26-07-2019
Accepted: 18-09-2019

***Corresponding Author:**

Sumaira Mazhar:
smz.mmg@gmail.com

Original Research Article

ABSTRACT

Aim of the present study was to examine the acute and chronic exposure of pesticide, chlorpyrifos (CPF) to the fresh water fish *Labeo rohita*. During acute exposure, fish were exposed to different concentrations of CPF ranging from 0, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L for 96 hrs in glass aquaria. The 96 hrs LC_{50} value of CPF for *Labeo rohita* was found to be 0.01 mg/L. During chronic exposure fish were subjected to $1/3^{rd}$, $1/5^{th}$, $1/7^{th}$ and $1/9^{th}$ of LC_{50} for 30 days. At the end of the trials, tissues from various organs like intestine, liver and kidney were collected and sections were examined under digital microscope. Pronounced histological changes like necrosis, infiltration, atrophy, shrinkage and degeneration of intestine was observed for different CPF concentrations. Kidney sections of *Labeo rohita* under different CPF concentrations exhibited nuclear hypertrophy, vacuolar degeneration of glomeruli, and occlusion of tubular lumen, cloudy swelling degeneration and hyaline droplets degeneration. In the liver tissue prominent histological changes observed including hepatic cell degeneration, nuclear hypertrophy, bile stagnation, irregular shaped cells, degeneration in the liver parenchymal cells, nuclear and cytoplasmic degeneration. Therefore, we here conclude that Chlorpyrifos adversely affects the major organs of *Labeo rohita* (Rohu).

Keywords: *Labeo rohita*, Chlorpyrifos, Acute, Chronic, Histology

INTRODUCTION

Chlorpyrifos is extensively used, second largest and highest selling organophosphate pesticide, used to control pests, which cause severe damage to crops for more than ten years (Rao *et al.,* 2003). Extensive use of CPF boosts the toxicity level in aquatic life thereby has severe effects on fish. Previous studies showed slight and chronic effects of CPF for different species, e.g., *Channa punctatus, Cyprinus carpio, Oreochromis mossambicus, Cirrhinus mrigala* (Ali *et al.,* 2012; Banaee *et al.,* 2013; Padmanabha *et al.,* 2015; Anita *et al.,* 2016). Day-by-day uses of pesticides increase due to the high demand; pesticides are used widely in agriculture, forestry, and public health and in veterinary practices. Hence, it is essential to study the instant and chronic effects of pesticides on fish, which supply the protein-source, an essential part of human diet (Ali *et al.,* 2009). It has been reported that fish are highly sensitive to aquatic pollution and showed strict physiological changes when they are exposed to sub lethal concentrations of toxicants (Ufodike & Omoregie, 1991). CPF is a crystalline organo-phosphate pesticide. Several billion fishes are died due to the chlorpyrifos according to a recent report (AbdelHalim *et al.,* 2006). In less alkaline soil, CPF has two months and in an average soil, CPF has half-life of 30 days and indoors, CPF can persist for weeks and months (Arcury *et al.,* 2007). CPF enters into water via air drift or surface runoff and then deposited in different aquatic organisms, particularly fish (Varo *et al.,* 2002). CPF has lethal and sub lethal levels of toxicities in aquatic environment. Lethal levels cause mass mortalities in fish and sub lethal toxicities induce morphological, neurobehavioural, oxidative, biochemical, histopathological, haematological and developmental alterations (Sunanda *et al.,* 2016). CPF also disturbs steroid hormone production and has harmful effect on reproductive system of fish (decreased serum estrogen and testosterone levels), developmental stages and neurobehaviour

Author's Contribution: A.S., Did experimental work; **D.H**., Provided the facility for experimental set up and helped for the determination of acute and chronic exposure; **U.R.,** Helped in the histological studies of fish; **S.M.,** Supervised research, helped in formulating the work, experimental design and wrote up of manuscript

(Levin *et al.,* 2004). Several studies have proven that by inhibiting brain acetylcholinesterase (AChE) CPF is noxious to living organisms including fish (Kwong*.,* 2002; Singh & Singh 2008; Xing *et al.,* 2012; Mishra & Devi 2014). Tissue histology is extensively used to study the effect of contamination and toxicity in organisms (Cengiz & Unlu, 2003). Histopathological changes can also be used as biomarkers to check the contamination in fishes both in laboratories and field studies (Thophon *et al.,* 2003; Schwaiger *et al.,* 1997). The coverage of sub lethal concentration of CPF led to the reduction in the level of total protein and glycogen, concentrations of pesticide also raise the glucose level which to lethargy (Kadam & Patil, 2013; Majumder & Kaviraj, 2017). CPF also disturbs chemical composition of fish which leads to cell damages and is responsible for mortality of fish (Khan, 2017). Dursban and Lorsban insecticides are the active form of CPF (Kienle *et al.,* 2009). Presently, varieties of organophosphate having chemical, physical and biological properties are used for agricultural purposes (Kumar, 2012). Due to direct contact with environment (water) fish gills are primary site of noxious action of many waterborne pollutants (Olson, 2002). Acute toxicity test is the best way to check the toxicity of organisms and the ecosystem as a whole. These tests are helpful in creating knowledge about potential destructive effects of such industrial discharges to the environment (Adedeji *et al.,* 2008; Onyedineke *et al.,* 2010). Fish as a source of food has been documented all over the world (Tacon & Metian, 2013). Proteins have a key role in human diet for appropriate growth and other essential activities. Fish is regarded as an excellent source of protein for human diet (WHO, 2007). In developing countries like Pakistan, fish manufacture sector is very significant not only as a major source of animal protein to guarantee food security but also to recover the value of food and raise protein supply in the food chain (Sheikh & Sheikh 2004; Bacha *et al.,* 2011). Thus, the present study was designed to evaluate the effects of CPF under acute and chronic exposure on the *Labeo rohita* and its effects on liver, kidney and intestinal tissues.

MATERIALS AND METHODS

Experimental fish

Healthy fingerlings of *Labeo rohita* were purchased from Manawa Fish Hatchery, Lahore and brought to the fish experiment room, Animal House in Department of Zoology at Government College University, Lahore. Prior to the start of the experiment, fish were acclimatized to laboratory conditions in round tanks for 15 days. Fish were fed with commercial pelleted diet at 5% body weight, twice daily.

Determination of LC_{50} for *Labeo rohita*

Labeo rohita were starved for 24hrs before start of the experiment. Eleven different concentrations of CPF (0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L) were prepared in ten equal sized aquaria, in addition to that one test aquarium kept for the control. Each aquaria contained 10 L water with 15-fishes/ aquarium. The fishes were exposed to the prepared test solutions for 96 hrs. Dead fish were watched and removed per day till the end of the fourth day. By the end of the fourth day, the mortality percentage was calculated according to the profit - analysis method. The experiment was repeated three times and the average of LC_{50} value for CPF was recorded as 0.01mg/L for 96hrs.

Chronic exposure of Chlorpyrifos for *Labeo rohita*

To determine the chronic toxicity fishes were collected and randomly divided into five groups. The first group represented the control and other four groups were experimental labeled as T_1 (control group), T_2, T_3, T_4 and T_5 (experimental groups). LC_{50} value was recorded as 0.01mg/L. Each aquarium contained 40 L water and 20 fish individual were transferred. $1/3^{rd}$, $1/5^{th}$, $1/7^{th}$ and $1/9^{th}$ of LC_{50} were considered for chronic study. Fish were fed with commercial food at least once a day during the study period (30 days) and fecal matter was removed daily from aquaria. After 30 days samples were collected for the histological studies of liver, kidney and intestine.

Histology of liver, kidney and intestine

For the study of histopathological affects, tissue specimens from three fish per treatment were removed by dissecting and preserved the tissues of kidney, liver and intestine in formalin solution (10% distilled water, 5% ethanol and 10% formaldehyde). For histology slide preparation fixed the tissues in 10% buffered formaline for 24hrs and ratio of formalin and tissue were 10:1 (10ml of formaline per $1cm^3$ tissue). After fixation tissues, specimens were trimmed by using a scalpel to enable them to fit into an appropriate labeled tissue cassette. The tissue cassettes were stored in formalin until

processing begins. Tissue processing began under three different steps, dehydration, clearing and embedding of specimens. After tissue processing tissue specimens were cut into sections and placed on the glass slides. Most cells were transparent. Histochemical stains (Haematoxylin and Eosin) were used to stain the tissue. A cover slip was mounted over the tissue specimen on the slide by using optical grade glues to protect the specimen.

RESULTS AND DISCUSSION

Histological changes in intestine

Intestinal sections of fish in the T_1 (control group) exhibited normal structure of intestine tissue with long and tapering villi with tightly packed sub mucosal tissues and epithelium, serous membrane, muscularis layers, stratum compactum, and lamina propria (Fig. 1a). In contrast, slight changes in villi, sub-mucosal tissue, necrosis of epithelial cells, infiltration of lymphocytes into the lamina propria of fish exposed to 1.1µl CPF were observed (Fig. 1b). Intestine of fish exposed to 1.4µl of CPF showed shrinkage of sub-mucosal tissue and the villi enlarged towards the tip, atrophy of epithelial cells and shrinkage of sub-mucosal tissues (Fig. 1c). Ruthless mucosal secretion has occurred due to suffering which enable the fish to deal with ecological stress (Samanta *et al.,* 2016). When exposed to 2.0µl of CPF, there was broadening and flattening of the villi towards their tips observed, some sign of deterioration were also visible and mucosal epithelium collapsed too (Fig. 1d). In the last group T_5 exposed to the 3.3µl of CPF, the intestine showed severe structural damage and intestine completely degenerated (Fig. 1e). Intestine is one of the most important part of a fish digestive system, performing main role in digestion and absorption of food materials. It is extremely sensitive to any toxic material and can be used as a significant biomarker organ for measurement of ecotoxicology (Kroon *et al.,* 2017). In this study, changes in intestinal tissues of *L. rohita* were primarily necrosis, hemorrhages, over production of goblet cells in villi, fusion, detachment and shortening of villi. When treated with deltamethrin, leukocytes infiltration, necrosis in gut tissues of Mosquito fish, *Gambusia affinis* has been reported by Cengiz & Unlu, 2006; Exposed to lambda-cyhalothrin for 60 days, *Cirrhinus mrigala* showed intestinal lesions, eosinophils invasion into the lamina properia and epithelial cells atrophy were observed which showed reduction of villi with inflammation, rupture of cells, disintegration changes in tips of villi, curved villi, hemorrhage, necrosis, numerous vacuoles, dilation in the blood vessels, completely damaged villi and loss of architecture in a number of fish species (Cengiz and Unlu, 2006; Velmurugan *et al.,* 2007; Vidhya and Nair, 2016).

Histological changes in kidney

Renal sections of fish were exposed to T_1 (control group) exhibits normal architecture of renal tubules and showing Renal Corpuscles (showing glomerulus & Bowmen's space) Proximal tubule and distal tubule (Fig. 2a). Cloudy swelling of epithelial cells of renal tubules, renal tubules with dilated lumens and occlusion lumens, fragmentation of glomeruli and renal corpuscles (showing glomerular expansion & absence of Bowman's space) and nuclear hypertrophy were observed, T_2 exposed to 1.1µl of CPF (Fig. 2b). Decreases in the tubular lumen may be due to the cloudy swelling of the epithelial cells of the renal tubules, which could be a reversible change. Also, the dilation in the tubules lumen may be due to the marked decrease in the length of the epithelial cells as a result of epithelial tubules degeneration (Issa *et al.,* 2011) whereas in the present study, the recognized homogenous eosinophilic deposits within the tubular lumens could be attributed to the protein leakage into the filtrate due to the glomerular disease as described by Roberts (2001). T_3 treated with 1.4µl of CPF and showed shrinkage and vacuolar degeneration of glumeruli and Vacuole formation (Fig. 2C). T_4 exposed to 2.0µl of CPF and showed cloudy swelling of epithelial cell of renal of renal tubules with narrowing lumens, renal tubules with degenerated epithelia and occlusion lumens (Fig. 2D). T_5 subjected to exposure of 3.3µl of CPF and showed renal tubules with degenerated epithelial cells and dilated lumens, complete destruction of tubule architectures, hyaline droplets degeneration and vacuole formation (Fig. 2e). These results partially agrees with (Hossain et al., 2002), where in more pathologies were found in *B. gonionotus*. This may be due to the use of

pesticides at sub-lethal concentrations, compared to the doses used in the present study. Oropesa *et al.* (2009) documented more or less similar findings for *Cyprinus carpio*. Although kidneys do not possess high levels of xenobiotic metabolizing enzymes as does the liver, many of the enzymatic reactions occurring in the liver have been shown to occur in the kidney (Mohssen, 2001). And kidney receives the bulk of the post branchial blood flow therefore its tissue play an important function in the detoxification and elimination of aquatic contaminants in fish (Durmaz *et al.,* 2006).

Histological changes in liver

The liver tissues (Hepatic cell and Granular cytoplasm) of fish in T_1 (control group) showed normal morphology because they were not exposed to any CPF intoxication (Fig. 3a). The fish exposed to 1.1µl of CPF, showed almost normal pattern in liver cells with very slight degenerative changes in cell arrangements and nuclear hypertrophy, bile stagnation and vacuole formation (Fig. 3b). This group T_3 represents 1.4 µl exposure of CPF. The liver tissue showed initial stage of cirrhosis and vacuolization of cytoplasm and eosinophilic granules and irregular shaped cells (Fig. 3c). The fish exposed to 2.0µl of CPF showed degeneration in the liver parenchymal cells, which was pronounced with severe damage and drastic karyolysis and necrosis were observed in some regions (Fig. 3d). Fish exposed to 3.3µl CPF has showed pronounced degeneration of liver tissues, vacuolization was severe, cirrhosis was remarkable, necrosis and karyolysis were highly pronounced and nuclear degeneration, cytoplasmic degeneration and melanomacrophases aggregate (Fig. 3e). In the present study common liver abnormalities were observed: loss of parenchymal architecture, fatty degeneration, vacuolar degeneration, atrophy and necrosis of hepatic and pancreatic cells with leucocytic infiltration. These results are in harmony with the previous studies of Tilak *et al.,* (2005) and Kunjamma *et al.,* (2008). Histological changes in the liver could be attributed to the fact that, the liver is the major site of detoxification (Nagai *et al.,* 2002), it is expected that the toxicant insecticide would reach there in abundance for detoxification and disposal (Mushigeri & David, 2005). Appearance of lipidosis and hepatocyte hypertrophy in zebrafish was reported by Zodrow *et al.* (2004). Oropesa *et al.,* (2009) found necrotic foci and lipid droplets in liver of *Cyprinus carpio*, whereas histological analysis of Silver catfish (*Rhamdia quelen*) has showed vacuolation in the liver after exposure to the herbicide, clomazone (Crestani *et al.,* 2007). Tissue histology is a helpful means to identify the level of pollution and it is considered as an indicator of exposure to pollutants for sublethal and chronic effects of fish (Cengiz and Unlu, 2003). Histopathological changes found in the present study accorded with the previous studies for liver, kidney and intestine of fish (*Labeo rohita*) treated with CPF. Moreover, present results demonstrated that as the concentration of CPF increased more distribution were observed in fish organs.

CONCLUSION

We conclude that CPF is highly toxic to *Labeo rohita* and its chronic exposure resulted in significant alterations in histology, which can ultimately affect the nutritional quality of *L. rohita*. After chronic study of the present study shows that, CPF adversely affects the intestine of fish. Higher value of chronic study, i.e., 3.3µl has the degenerated effects on intestine of fish.

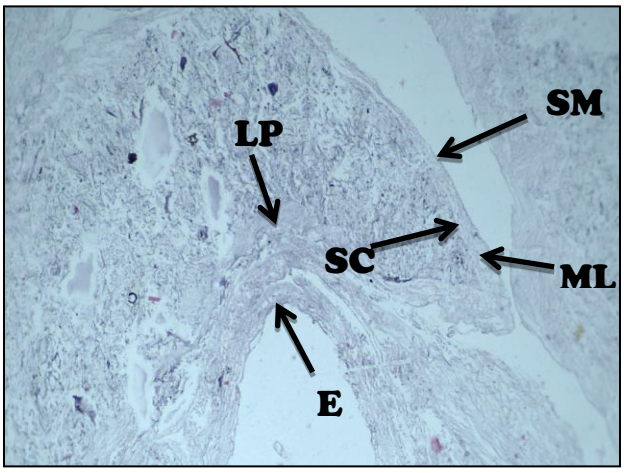

Fig. 1a: Micrograph showing Intestinal villi of *Labeo rohita* fed control diet (T_1). Cross section of intestine without any exposure to CPF showing normal structure of Intestine E: epithelium; LP: lamina propria; SC: stratum compactum; ML: muscularis layers; SM: serous membrane

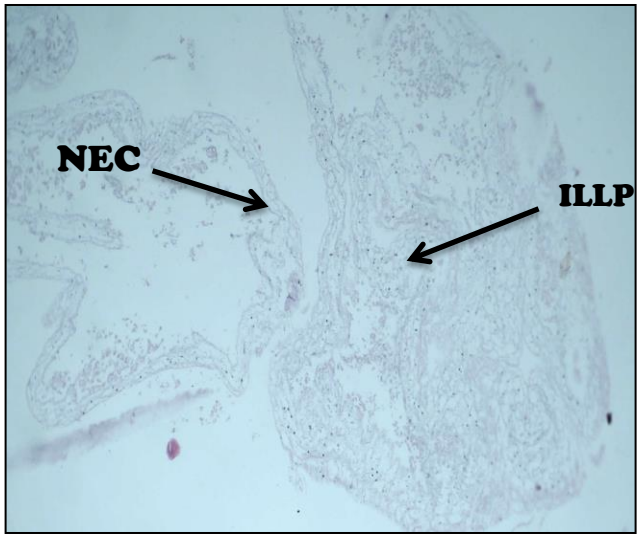

Fig. 1b: Micrograph showing intestinal villi of *Labeo rohita* in experimental group T_2. Slight changes occur in Intestinal villi
NEC: necrosis of epithelial cells; ILLP: infiltration of lymphocytes into the lamina propria

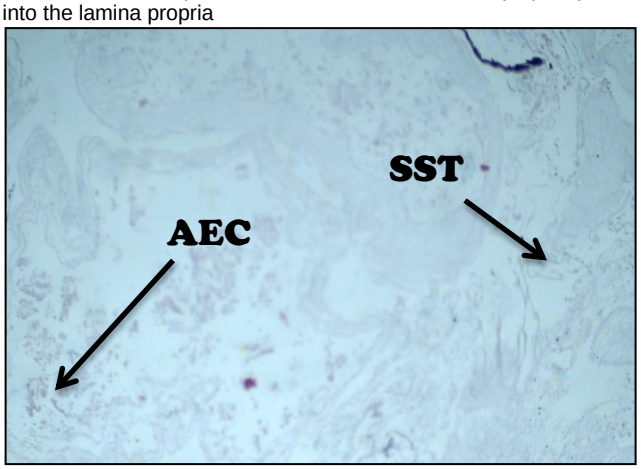

Fig. 1c: Micrograph showing intestinal villi of *Labeo rohita* in experimental group T_3. Shrinkage of sub-mucosal tissues is quite visible
AEC: atrophy of epithelial cells; SST: Shrinkage of sub-mucosal tissues

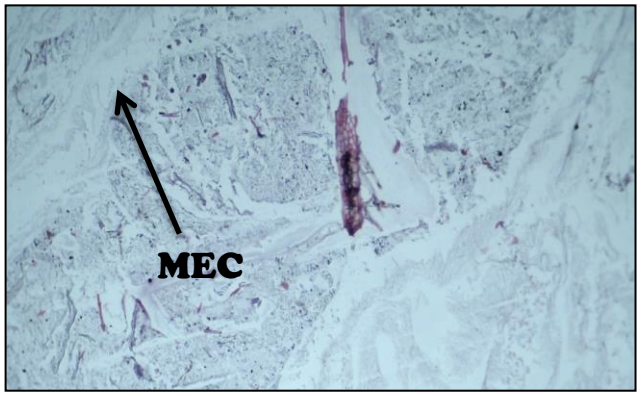

Fig 1d: Micrograph showing intestinal villi of *Labeo rohita* in experimental group T_4. Mucosal epithelium is collapsed.
MEC**:** Mucosal epithelium collapsed.

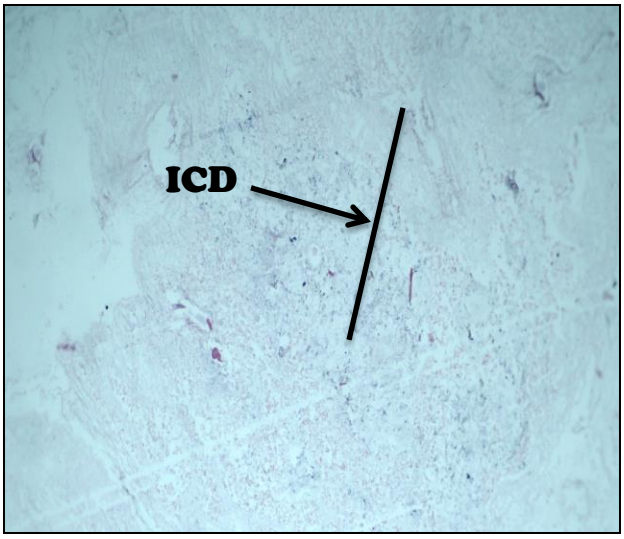

Fig. 1e: Micrograph showing intestinal villi of *Labeo rohita* in experimental group T_5. Intestine complete degeneration
ICD: Intestine complete degeneration.

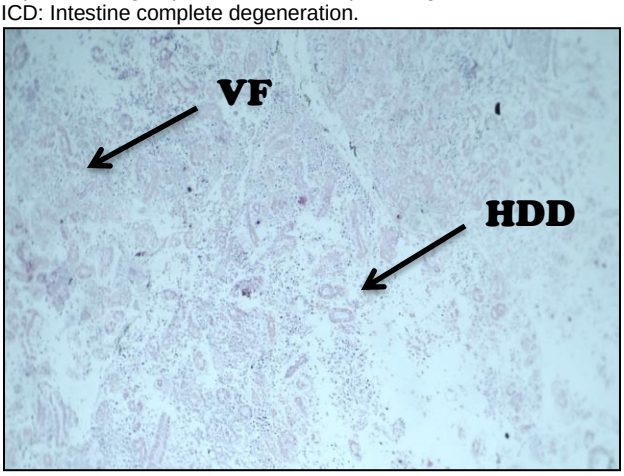

Fig. 1f: Micrograph showing internal structure of Kidney of *Labeo rohita* in experimental group T_5.Renal tubule with degenerated epithelial cells and dilated lumen, complete destruction of tubule architecture.
HDD: Hyaline Droplets Degeneration, VF: Vacuole Formation

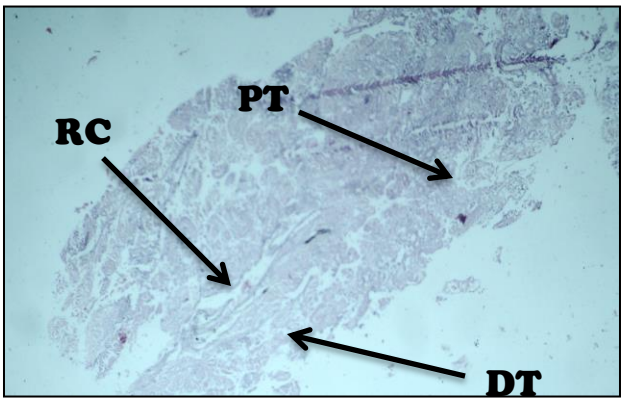

Fig. 2a: Micrograph showing internal structure of Kidney of *Labeo rohita* fed control diet (T_1) showing normal architecture, renal tubule.
RC: Renal Corpuscle (showing glomerulus & bowmen's space), PT: Proximal Tubule; DT; Distal Tubule

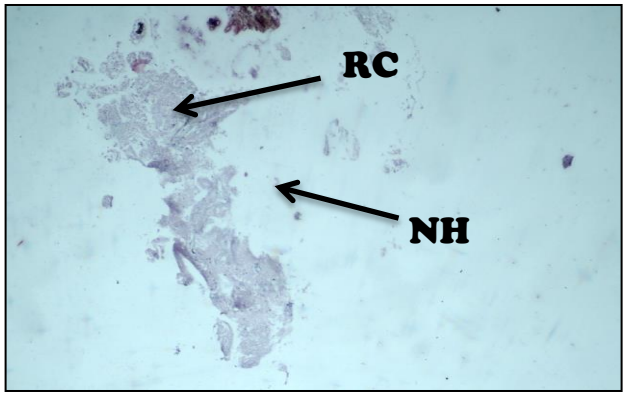

Fig 2b: Micrograph showing internal structure of kidney of *Labeo rohita* in experimental group T_2. Cloudy swelling of epithelial cells of renal tubule, renal tubule with dilated lumen and occlusion lumen and fragmentation of glomeruli.
RC: Renal corpuscle (showing glomerular expansion & absence of bowmans space) , NH : Nuclear Hypertrophy

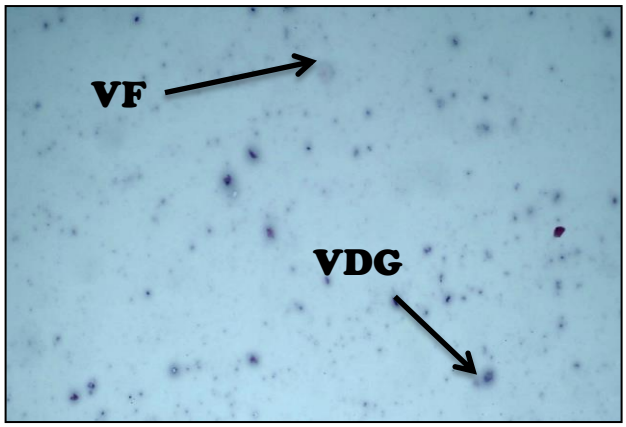

Fig. 2c: Micrograph showing internal structure of Kidney of *Labeo rohita* in experimental group T_3.Showing shrinkage and vacuolar degeneration of glomeruli.
VF : Vacuole Formation; VDG: Vacuolar degeneration of glomeruli

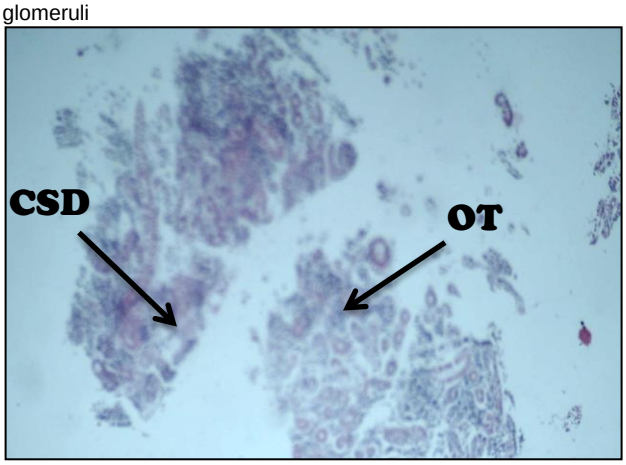

Fig. 2d: Micrograph showing internal structure of Kidney of *Labeo rohita* in experimental group T_4.Cloudyswelling of epithelial cell of renal tubule with narrowing lumen, renal tubule with degenerated epithelia and occlusion lumen.
OT : Occlusion of Tubular lumen , CSD :Cloudy Swelling Degeneration

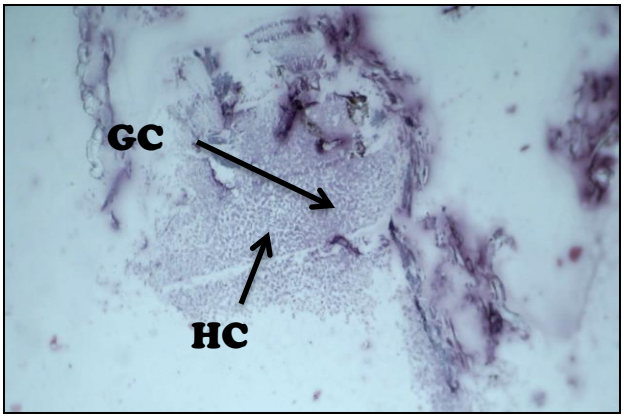

Fig. 3a: Micrograph showing Liver of *Labeo rohita* fed control diet (T_1) showed normal morphology of liver cells.
HC: Hepatic cell, GC: Granular cytoplasm

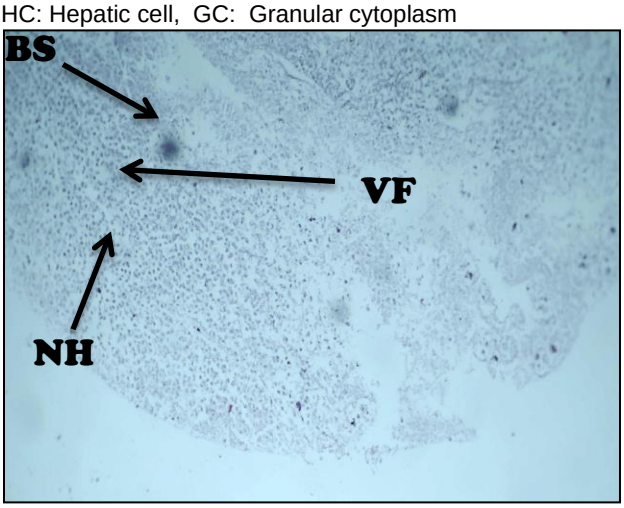

Fig 3b: Micrograph showing internal structure of Liver of *Labeo rohita* in experimental group T^2. Showed normal pattern in liver with very slight degenerative changes in cell arrangements
NH : Nuclear Hypertrophy , BS : Bile Stagnation, VF : Vacuole Formation

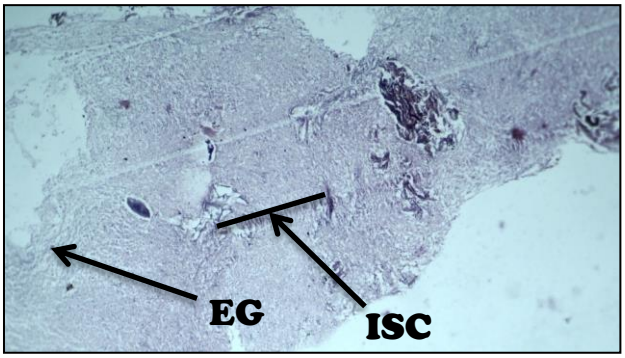

Fig. 3c: Micrograph showing internal structure of Liver of *Labeo rohita* in experimental group T_3. The liver tissue showed initial stage of cirrhosis and vacuolization of cytoplasm.
EG: Eosinophilic Granules, ISC: Irregular Shaped cells

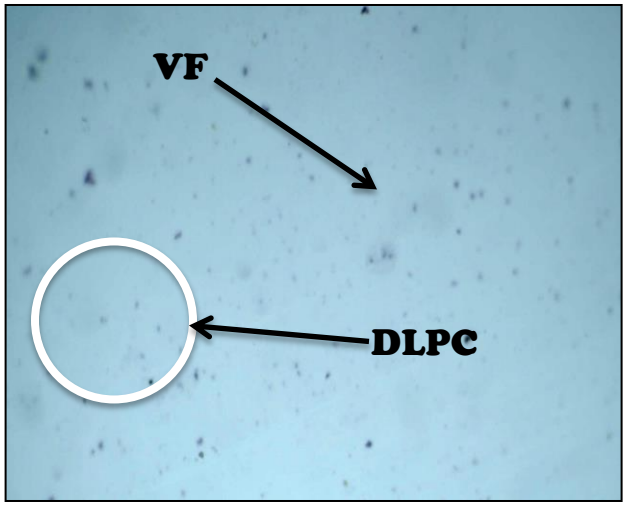

Fig. 3d: Micrograph showing internal structure of Liver of *Labeo rohita* in experimental group T_4. Degeneration in the liver parenchymal cells is pronounced with severe damage and drastic karyolysis and necrosis is observed in some regions.
VF : Vacuole formation, DLPC: Degeneration in the liver parenchymal cells

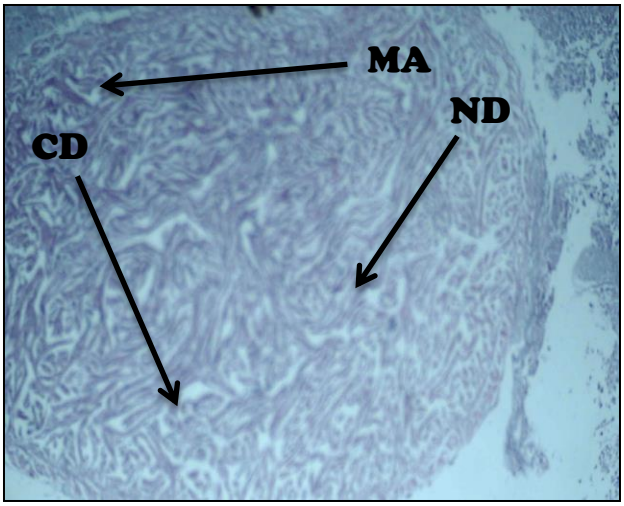

Fig. 3e: Micrograph showing internal structure of liver of *Labeo rohita* in experimental group T_5. Showed pronounced degeneration of liver tissues, vacuolization is severe, cirrhosis is remarkable, necrosis and karyolysis are highly pronounced.
ND: Nuclear Degeneration, CD: Cytoplasmic degeneration , MA : Melanomacrophases Aggregate

Table 1: Effects of different concentrations of CPF on intestine, kidney and liver of *Labeo rohita*

	Stages				
	T_1	T_2	T_3	T_4	T_5
Intestine	Epithelium Lamina Propria Stratum Compactum Muscularis Layer Serous Membrane	Necrosis of epithelial cells Infiltration of lymphocytes into lamina propria	Atrophy of epithelial cells Shrinkage of sub-mucosal tissues	Mucosal epithelium collapsed	Intestine complete degeneration
Kidney	Renal corpuscle (Showing glomerulus & bowmen, s Space) Proximal tubule Distal tubule	Renal corpuscle (showing glomerular expansion & absence of bowmn, s space) Nuclear Hypertrophy	Vacuole formation Vacuolar degeneration of glomeruli	Occlusion of tubular lumen Cloudy swelling degeneration	Hyaline droplets degeneration Vacuole formation
Liver	Hepatic cell degeneration Granular cytoplasm	Nuclear hypertrophy Bile stragnation Vacuole formation	Eosinophilic granules Irregular shaped cells	Vacuole formation Degeneration in the liver parenchymal cells	Nuclear degeneration Cytoplasmic degeneration Malenomacrophages aggregate

REFERENCES

AbdelHalim, K.Y., Salama, AK., Elkhateeb, E.N. and Barky, N.M., 2006. Organophosphorus pollutants (OPP) in aquatic environment at Damietta Governorate, Egypt: implications for monitoring and biomarker responses. *Chemosphere.,* 63: 1491–1498.

Adedeji, O.B., Adedeji, A.O., Adeyemo, O.K. and Agbede, S.A., 2008. Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*). *Afr. J. Biotechnol.,* 7: 651-654.

Ali, Nagpure, D., Kumar, N.S., Kumar, S., Kushwaha, R.B. and Lakra., 2009. Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Food Chem. Toxicol.,* 47: 650-656.

Anita, B., Yadav, A.S. and Cheema, N., 2016. Genotoxic effects of chlorpyrifos in freshwater fish *Cirrhinus mrigala* using micronucleus assay. *Advan. Biol*. 1-6.

Arcury, T.A., Grzywacz, J.G., Barr, D.B., Tapia, J.C.H. and Quandt, S.A., 2007. Pesticide urinary metabolite levels of children in eastern North Carolina farm worker households. *Environ. Health Perspect*., 115: 1254–60.

Bacha, U., Nasir, M., Khalique, A. and Anjum, A.A., 2011. Comparative assessment of various agro-industrial wastes for *Saccharomyces cerevisae* biomass production and its quality evaluation as single cell protein. *J. Anim. Plant Sci.* 21(4): 844-849.

Banaee, M., Haghi, B.N. and Ibrahim, T.A., 2013. Sub-lethal toxicity of chlorpyrifos on common carp, *Cyprinus carpio* (Linnaeus, 1758): biochemical response. *Int. J. Aquat. Biol.,* 1(6): 281- 288.

Cengiz, E. and Unlu, E., 2003. Histopathology of gills in mosquito fish, *Gambusia affinis* after long-term exposure to sub-lethal concentrations of malathion. *J. Environ. Sci. Health.,* 38(5): 581-589.

Cengiz, E.I. and Unlu, E., 2006. Sublethal effects of 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.

Crestani, M., Menezes, C., Glusczak, L., Miron, D.S.D., Spanevello, R., Silveira, A. and Loro, VL., 2007. Effect of clomazone herbicide on biochemical and histological aspects of silver catfish (*Rhamdia quelen*) and recovery pattern. *Chemosphere*., 67(11): 2305-2311.

Durmaz, H., Sevgiler, Y. and Üner, N., 2006. Tissue-specific antioxidative and neurotoxic responses to diazinon in *Oreochromis niloticus*. *Pestic. Biochem. and Physiol,.* 84:215-226.

Hossain, Z., Rahman, M.Z. and Mollah, M.F.A., 2002. Effect of Dimecron 100 SCW on *Anabas testudineus, Channa punctatus and Barbobes gonionotus*. *Indian. J. Fish*., 49(4): 405-417.

Issa, A.M., Gawish, A.M. and Esmail, G.M., 2011. Histological Hazards of Chlorpyrifos Usage on Gills and Kidneys of *Tilapia nilotica* and the Role of Vitamin E Supplement in Egyp. *Life Sci. J.,* 4(8): 113-123.

Kadam, P. and Patil, R., 2013. Effect of Chlorpyrifos on Some Biochemical Constituents in Liver and Kidney of Fresh Water Fish, *Channa Gachua* (F.Hamilton). *IJSR.,* 5(4): 1975-1979.

Khan, S., 2017. Sublethal Effect of Chlorpyrifos on some Biochemical Constitution of Guppy Fish. WJPPS. ISSN 2278 -4357.

Kienle, C., Kohler, H.R. and Gerhardt, A., 2009. Behavioural and developmental toxicity of chlorpyrifos and nickel chloride to zebrafish (*Danio rerio)* embryos and larvae *Ecotoxicol. Environ. Saf.,* 72: 1740-1747.

Kroon, F., Streten, C., & Harries, C., 2017. A protocol for identifying suitable biomarkers to assess fish health: A systematic review. *PLoS One.* 12(4): e0174762.

Kumar, S.P., 2012. Micronucleus assay: a sensitive indicator for aquatic pollution. *IJRBS*., 1(2): 32-37.

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

Kwong, T.C., 2002. Organophosphate pesticides: biochemistry and clinical toxicology. *Ther. Drug. Monit*., 24: 144–149.

Levin, E.D., Swain, H.A., Donerly, S. and Linney, E., 2004. Developmental chlorpyrifos effects on hatchling zebrafish swimming behaviour. *Neurotoxicol. Teratol*., 26: 719–723.

Majumder, R., & Kaviraj, A., (2017) Cypermethrin induced stress and changes in growth of freshwater fish *Oreochromis niloticus. Int Aquat Res.* 9(2): 117-128.

Mishra, A. and Devi, Y., 2014. Histopathological alterations in the brain (optic tectum) of the freshwater teleost *Channa punctatus* in response to acute and sub chronic exposure to the pesticide chlorpyrifos. *Acta Histochem*., 116:176–181.

Mohssen, M., 2001. Biochemical and Histopathological changes in serum creatinine and kidney induced by inhalation of thimet (phorate) in male swiss albino mouse, *Mus musculus*. *Eviron*. 87: 31-36.

Mushigeri, S.B. and David, M., 2005. Fenvalerate induced changes in the Ach and associated AChE activity in different tissues of fish *Cirrhinus mrigala* (Hamilton) under lethal and sub-lethal exposure period. *Environ. Toxicol. Pharmacol.,* 20:65-72.

Nagai, T., Yukimoto, T. and Suzuki, N., 2002. Glutathione peroxidase from the liver of Japanese sea bass *Laeolabrax japonicus*. *Z. Naturforscher.,* 57:172-176.

Olson, K.R., 2002. Vascular anatomy of the fish gill. *J Exper Zool.* 293: 214–231.

Onyedineke, N.E., Odukoya, A.O. and Ofoegbu, P.U., 2010. Acute toxicity tests of cassava and rubber effluents on the Ostracoda Strandesia prava Klie, 1935 (Crustacea, Ostracoda). *Res J Environ Sci.,* 4:166-172.

Oropesa, A.L., Cambero, J.P.G., Gomez, L., Roncero, V. and Soler, F., 2009. Effect of Long-Term Exposure to Simazine on Histopathology, Hematological, and Biochemical Parameters in *Cyprinus carpio*. *Environ Toxicol*. 24(2): 187-199.

Padmanabha, A., Reddy, H.R.V., Khavi, M., Prabhudeva, K.N., Rajanna, K.B. and Chethan, N., 2015. Acute effects of chlorpyrifos on oxygen consumption and food consumption of freshwater fish, *Oreochromis mossambicus* (Peters). *IJRSR.,* 6(4): 3380-3384.

Rao, J.V., Rani, C.H.S., Kavitha, P., Rao, R.N. and Madhavendra. S.S., 2003. Toxicity of chlorpyrifos to the fish, *Oreochromis mossambicus*. *BECT.,* 70: 985-992.

Roberts, R.J., 2001. Fish Pathology. 3rd (Ed.), W.B. Saunders, New York.

Samanta, P., Pal, S., Mukherjee, A.K., Kole, D. and Ghosh, A.R., 2016. Histopathological study in stomach and intestine of *Anabas testudineus* (Bloch, 1792) under Almix exposure. *Fish Aqua J.,* 7: 1–7.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W. and Triebskorn, R., 1997. The use of histopatological indicators to evaluate contaminant-related stress in fish. *J. Aquat. Ecosyst. Stress. Recovery.,* 6: 75-86.

Sheikh, B. A. and Sheikh, S.A., 2004. Aquaculture and integrated farming system. *Pakistan J. Agri. Agril. Engg. Vet. Sci*., 20: 52-58.

Singh, P.B. and Singh, V., 2008. Pesticide bioaccumulation and plasma sex steroids in fishes during breeding phase from north India. *Environ. Toxicol. Pharmacol*., 25: 342–350.

Sunanda, M.J., Rao, C.S., Neelima, P., Rao, K.G. and Simhachalam, G., 2016. Department of Zoology & Aquaculture. *Int. J. Pharm. Sci. Rev. Res.,* 39(1): 299-305.

Tacon, A.G.J. and Metian, M., 2013. Fish Matters: Importance of Aquatic Foods in Human Nutrition and Global Food Supply. *Rev. Fish. Sci.,* 21(1): 22–38.

Thophon, S., Kruatrachue, M., Upathan, E.S., Pokethitiyook, P., Sahaphong, S. and Jarikhuan, S., 2003. Histopathological alterations of white seabass, Lates calcarifer in acute and subchronic cadmium exposure. *Environ. Pollut.,* 121: 307-320.

Tilak, K., Rao, K. and Veeraiah, K., 2005. Effects of Chlorpyrifos on histopathology of the fish Catla catla. *J. Ecotoxicol. Environ. Monit.,* 15(2):127-140.

Tilak, K.S., Veeraiah, K. and Ramanakumari, G.V., 2001. Toxicity and effect of Chloropyriphos to the freshwater fish *Labeo rohita* (Hamilton). *Neurol. Res.,* 20: 438– 445.

Ufodike, E.B.C. and Omoregie, E., 1991. Growth of Nile Tilapia *Oreochromis niloticus* subjected to sublethal concentration of Gammalin 20 and Actellic 25EC in a continuous flow toxicant autodelivery system. *Aquat. Anim. Health.,* 3: 221-223.

Velmurugan, B., Selvanayagam, M., Cengiz, E.I. and Unlu, E., 2007. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. *Environ. Toxicol. Pharmacol.,* 24: 286–291.

Vidhya, V. and Nair, C.R., 2016. International Journal of Advanced Research in Biological Sciences. *Int. J. Adv. Res. Biol. Sci.,* 3: 43-47.

Varo, I., Serrano, R., Pitarch, E., Amat, F., Lopez, F.J. and Navarro, J.C., 2002. Bioaccumulation of chlorpyrifos through an experimental food chain: study of protein HSP70 as biomarker of sub-lethal stress in fish. *Arch. Environ. Contam. Toxicol.* 42: 229–235.

WHO., 2007. Proteins and Amino Acid Requirements in Human Nutrition. WHO Technical report series 935, World Health Organization, Geneva, Switzerland.

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.

Zodrow, J.M., Stegemanb, J.J. and Tanguay, R.L., 2004. Histological analysis of acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in zebrafish. *Aquat. Toxicol*., 66(1): 25-38.