

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

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### Original Research Article

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### 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<sub>50</sub> value of CPF for *Labeo rohita* was found to be 0.01 mg/L. During chronic exposure fish were subjected to 1/3<sup>rd</sup>, 1/5<sup>th</sup>, 1/7<sup>th</sup> and 1/9<sup>th</sup> of LC<sub>50</sub> 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

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### 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 organophosphate 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

(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<sub>50</sub> 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<sub>50</sub> 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<sub>1</sub> (control group), T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> (experimental groups). LC<sub>50</sub> value was recorded as 0.01mg/L. Each aquarium contained 40 L water and 20 fish individual were transferred. 1/3<sup>rd</sup>, 1/5<sup>th</sup>, 1/7<sup>th</sup> and 1/9<sup>th</sup> of LC<sub>50</sub> 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<sup>3</sup> 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<sub>1</sub> (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<sub>5</sub> 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 propria 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<sub>1</sub> (control group) exhibits normal architecture of renal tubules and showing Renal Corpuscles (showing glomerulus & Bowman'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<sub>2</sub> 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<sub>3</sub> treated with 1.4µl of CPF and showed shrinkage and vacuolar degeneration of glomeruli and Vacuole formation (Fig. 2C). T<sub>4</sub> 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<sub>5</sub> 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).

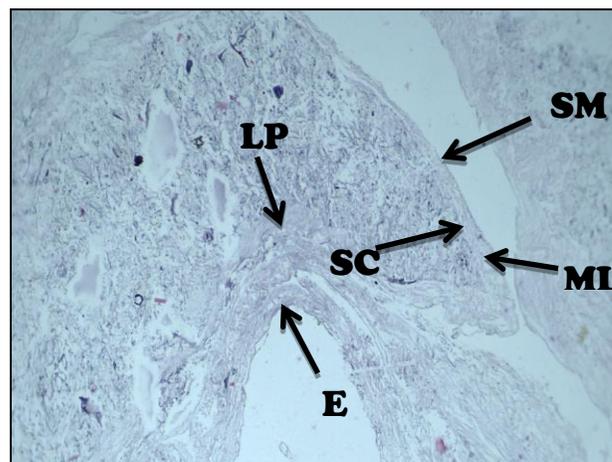
### Histological changes in liver

The liver tissues (Hepatic cell and Granular cytoplasm) of fish in T<sub>1</sub> (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<sub>3</sub> 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 melanomacrophages 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

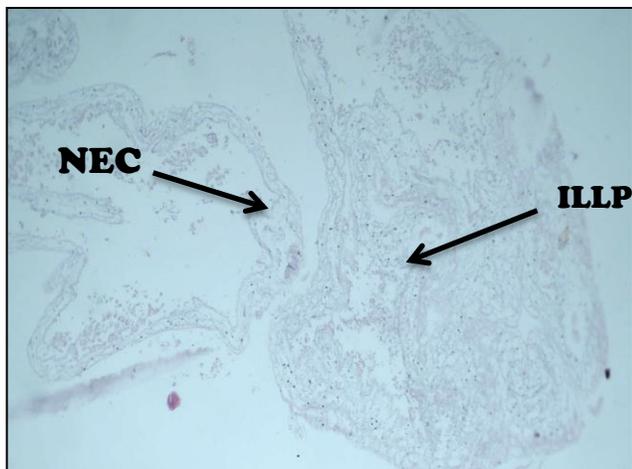
reported by Zodrow *et al.* (2004). Oropesa *et al.*, (2009) found necrotic foci and lipid droplets in liver of *Cyprinus carpio*, whereas histological analysis of Silver catfish (*Rhamdia quelen*) has showed vacuolation in the liver after exposure to the herbicide, clomazone (Crestani *et al.*, 2007). 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

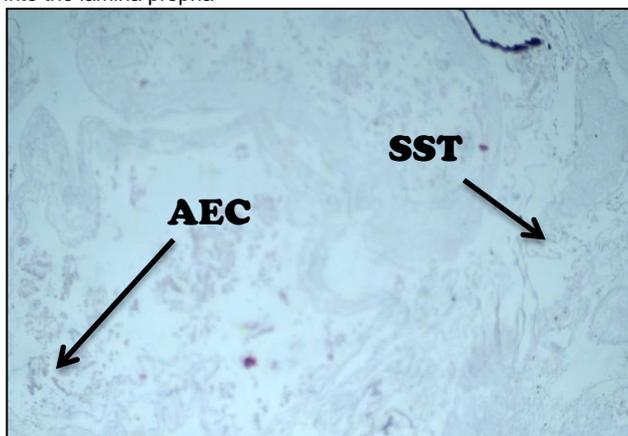
We conclude that CPF is highly toxic to *Labeo rohita* and its chronic exposure resulted in significant alterations in histology, which can ultimately affect the nutritional quality of *L. rohita*. After chronic study of the present study shows that, CPF adversely affects the intestine of fish. Higher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



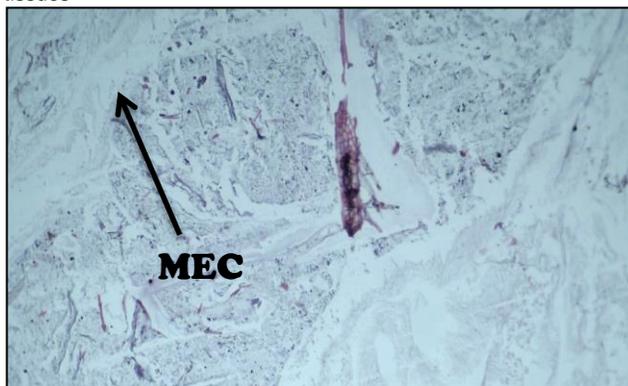
**Fig. 1a:** Micrograph showing Intestinal villi of *Labeo rohita* fed control diet (T<sub>1</sub>). Cross section of intestine without any exposure to CPF showing normal structure of Intestine  
E: epithelium; LP: lamina propria; SC: stratum compactum; ML: muscularis layers; SM: serous membrane



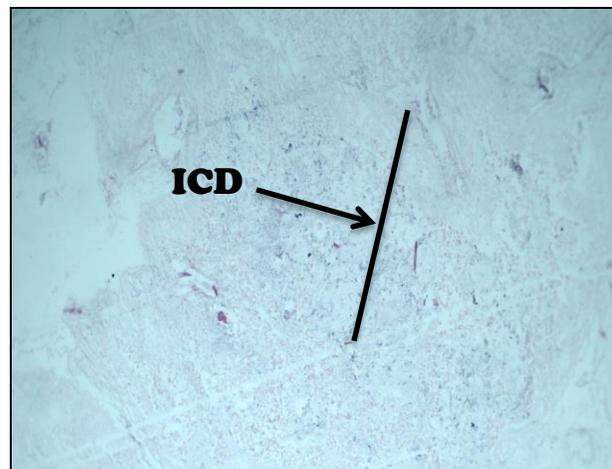
**Fig. 1b:** Micrograph showing intestinal villi of *Labeo rohita* in experimental group T<sub>2</sub>. Slight changes occur in Intestinal villi  
NEC: necrosis of epithelial cells; ILLP: infiltration of lymphocytes into the lamina propria



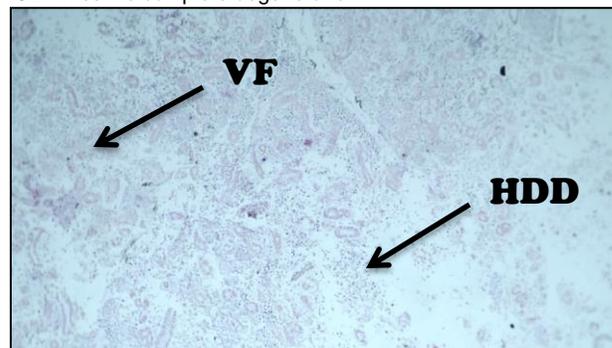
**Fig. 1c:** Micrograph showing intestinal villi of *Labeo rohita* in experimental group T<sub>3</sub>. Shrinkage of sub-mucosal tissues is quite visible  
AEC: atrophy of epithelial cells; SST: Shrinkage of sub-mucosal tissues



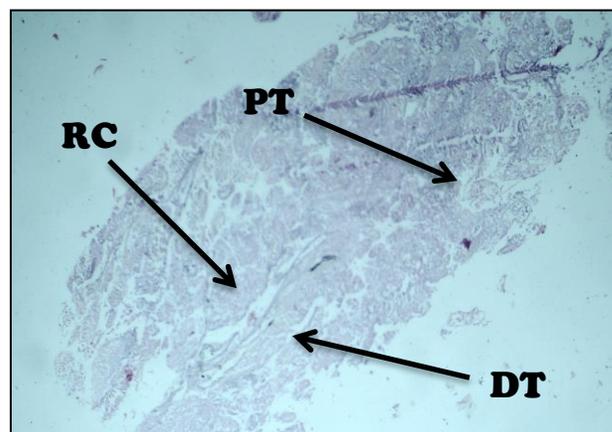
**Fig 1d:** Micrograph showing intestinal villi of *Labeo rohita* in experimental group T<sub>4</sub>. Mucosal epithelium is collapsed.  
MEC: Mucosal epithelium collapsed.



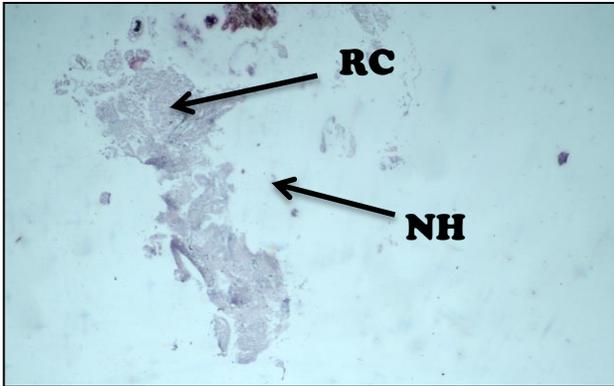
**Fig. 1e:** Micrograph showing intestinal villi of *Labeo rohita* in experimental group T<sub>5</sub>. Intestine complete degeneration  
ICD: Intestine complete degeneration.



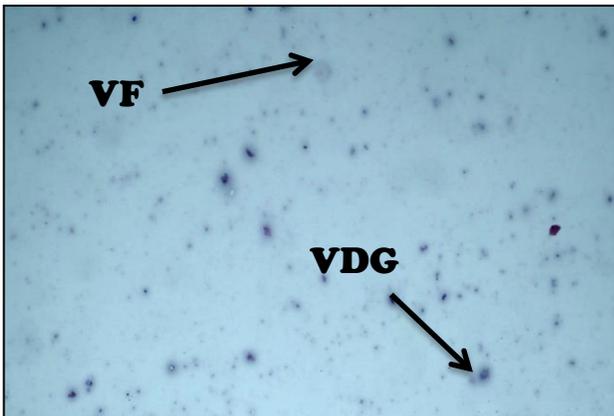
**Fig. 1f:** Micrograph showing internal structure of Kidney of *Labeo rohita* in experimental group T<sub>5</sub>. Renal tubule with degenerated epithelial cells and dilated lumen, complete destruction of tubule architecture.  
HDD: Hyaline Droplets Degeneration, VF: Vacuole Formation



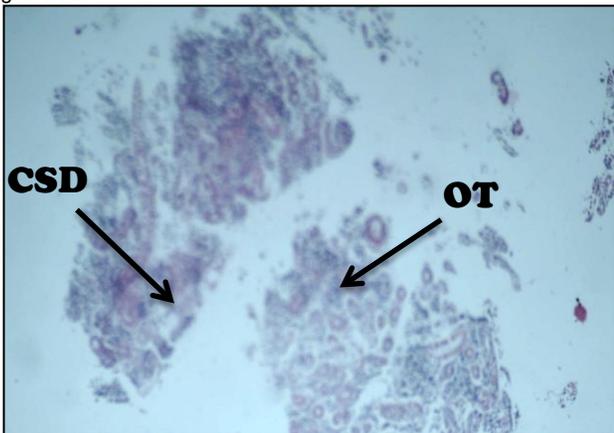
**Fig. 2a:** Micrograph showing internal structure of Kidney of *Labeo rohita* fed control diet (T<sub>1</sub>) showing normal architecture, renal tubule.  
RC: Renal Corpuscle (showing glomerulus & bowmen's space), PT: Proximal Tubule; DT; Distal Tubule



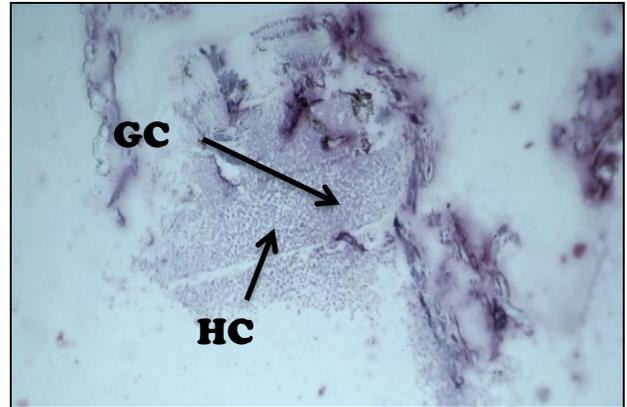
**Fig 2b:** Micrograph showing internal structure of kidney of *Labeo rohita* in experimental group T<sub>2</sub>. Cloudy swelling of epithelial cells of renal tubule, renal tubule with dilated lumen and occlusion lumen and fragmentation of glomeruli.  
RC: Renal corpuscle (showing glomerular expansion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



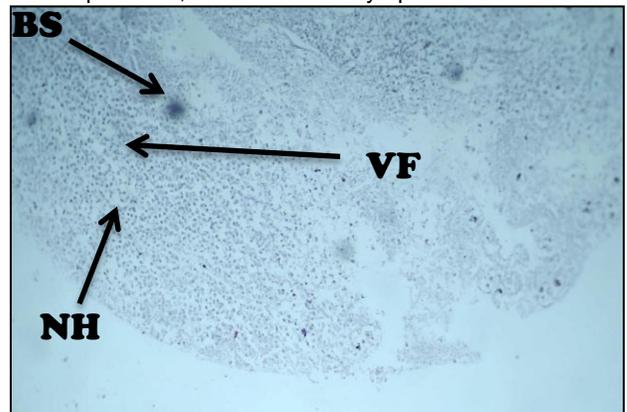
**Fig. 2c:** Micrograph showing internal structure of Kidney of *Labeo rohita* in experimental group T<sub>3</sub>. Showing shrinkage and vacuolar degeneration of glomeruli.  
VF : Vacuole Formation; VDG: Vacuolar degeneration of glomeruli



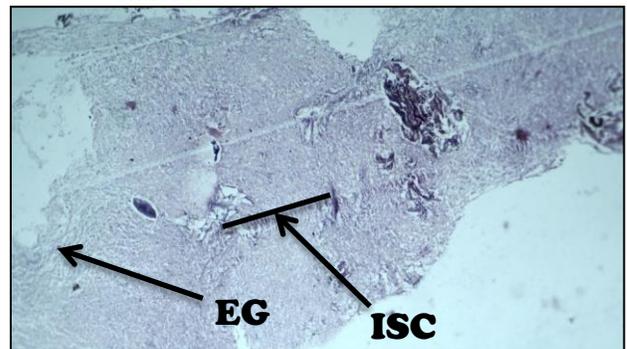
**Fig. 2d:** Micrograph showing internal structure of Kidney of *Labeo rohita* in experimental group T<sub>4</sub>. Cloudy swelling of epithelial cell of renal tubule with narrowing lumen, renal tubule with degenerated epithelia and occlusion lumen.  
OT : Occlusion of Tubular lumen , CSD :Cloudy Swelling Degeneration



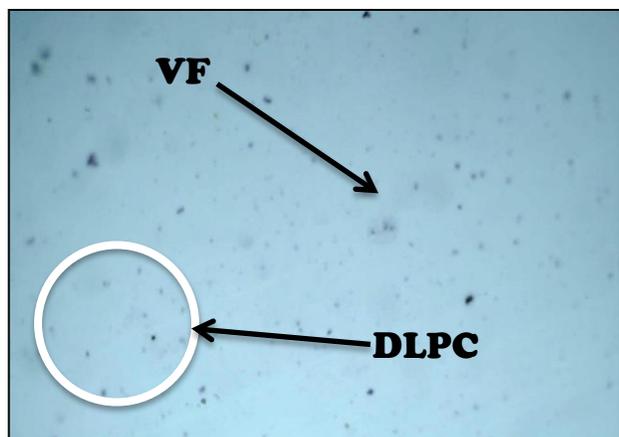
**Fig. 3a:** Micrograph showing Liver of *Labeo rohita* fed control diet (T<sub>1</sub>) showed normal morphology of liver cells.  
HC: Hepatic cell, GC: Granular cytoplasm



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

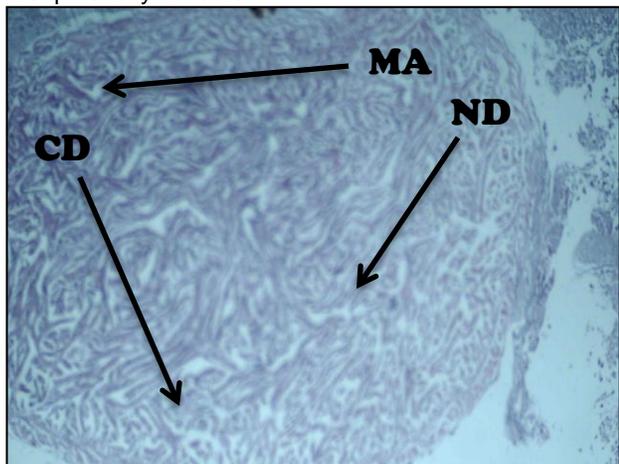


**Fig. 3c:** Micrograph showing internal structure of Liver of *Labeo rohita* in experimental group T<sub>3</sub>. The liver tissue showed initial stage of cirrhosis and vacuolization of cytoplasm.  
EG: Eosinophilic Granules, ISC: Irregular Shaped cells



**Fig. 3d:** Micrograph showing internal structure of Liver of *Labeo rohita* in experimental group T<sub>4</sub>. 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<sub>5</sub>. 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 : Melanomacrophages Aggregate

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

Stages					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
<b>Intestine</b>	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
<b>Kidney</b>	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
<b>Liver</b>	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