# Evaluation of histopathological alterations in kidney and liver of *Cirrhinus mrigala* and *Labeo rohita* following acute exposure to chlorpyrifos

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**Abstract**

Chlorpyrifos (CPF) is one of the most common insecticides found in freshwater ecosystem, and has been detected in agriculture and fish products worldwide. Pesticides affects the aquatic organism both directly and indirectly. Agricultural runoff has a direct entry path, whereas food chain consumption has an indirect entry path. The present study was conducted to assess the histopathological alterations in the kidney and liver of *Cirrhinus mrigala* and *Labeo rohita* treated with various concentration of chlorpyrifos. Fishes were divided into fourgroups for experiment. Group I taken as control group. Group II, III and IV were exposed to 4.0 mg/L, 4.5 mg/L and 5.0 mg/L respectively solution of chlorpyrifos for short term experiment (96 h). The histopathological changes in kidney and liver was observed after exposure of chlorpyrifos at 24, 48, 72 and 96 hours. During the exposure trial several behavioral changes including loss of equilibrium, increased opercular movement, rapid gulping of water and changes in skin colouration were observed in the fish.

**Key words:** Fishes, *Cirrhinus mrigala*, *Labeo rohita,* Kidney, Liver, Chlorpyrifos, Histopathology

## INTRODUCTION

The use of chemicals increasing day by day in industry and agricultural that contaminate the aquatic ecosystem. In agriculture crops, pesticides are widely used to control the pest. Pesticides are discharged into the environment and pollute it through a variety of processes. Organophosphate, organochlorine and carbamates are three major pesticide groups that constitute a significant threat [1]. Due to wide usage of pesticide in agricultural activities, harmful substances are added in environment [2]. These pesticides in environment may harm non- target organisms such as fish that are economically and nutritionally significant [3].

Three different ways insecticides enter the fish body, such as skin incorporation, gills uptake and by drinking or feeding. Acute and chronic toxicity can be caused by haematological, histopathological changes and antioxidant activities in fish [4]. Previously, it was stated that there are lot of fish mortality instances in the water due to chlorpyrifos exposure in water [5].

Pesticides degradation in aquatic ecosystem is a severe concern, and fish can be usually exposed to these pollutants, which can enter the body through gills, skin and polluted food. Therefore, study the effects of hazardous insecticides like chlorpyrifos on fish can help to determine whether or not pesticides are harmful to human health [6]. Chlorpyrifos is well-known crop pesticide that is commonly used in the Agriculture. As freshwater supplies are limited, drainage water can be recovered and utilized in fish farms. As a result, a number of pollutants and undesirable remains can be accumulating in the body of fish and result in severe direct or indirect effect [7]. Through

extreme lipid peroxidation, apoptosis, and neuro behavioral improvement, chlorpyrifos exposure inhibits the immunological and antioxidative activities in aquatic organism. Poisoning with chlorpyrifos resulted in physiological and antioxidative response in Caspian brown trout (*Salmo trutta caspius*) *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Cyprinus carpio* [8].

Many pesticides are widely used in agricultural production. These pesticides have a variety of physiological impacts on the pests, including inhibiting growth, food intake, metabolism, enzyme function and overall development, as well as negative impact on aquatic organism [9]. The use of histopathological testing to measure the effects ofenvironmental contaminants on aquatic animals is becoming increasingly popular [10].

The effects of chlorpyrifos on the histopathology of various fish species are well-known [11]. The liver is connected with detoxification and biotransformation, and it is also one of the organs most susceptible to harm caused by variety of toxins [12]. Kidney is an essential organ of excretion, osmoregulation and also maintaining the homeostasis. It is also accountable for specific reabsorbtion, that helps to maintain the volume and body fluid and erythropoiesis [13]. Fishes plays significant role in the food chain and measured as a major source of high quality animal protein as they have huge quantity of crucial amino acids [14].

## MATERIALS AND METHODS

### Study design

For this experiment fingerlings of *Cirrhinus mrigala* and *Labeo rohita* 48 each, were procured from fish seed hatchery Manawa Lahore. The fish weight and length ranges from 25 to 70 g and 13 to 19 cm, respectively. The fish was initially acclimatized under laboratory conditions for one week in glass aquaria. During acclimatization period, protein based diet was given to fish twice a day.

### Preparation for stock solution of chlorpyrifos

A stock solution of chlorpyrifos was made by dissolving 1 mg of chlorpyrifos in 100 mL of acetone. The fish were divided into four different groups and placed in different glass aquariums. Twelve fish were introduced in each aquarium filled with 35 litter of water. Group I was maintain in pesticide-free water to serve as control. Group II, III and IV were exposed to different concentration of chlorpyrifos stock solution, 4.0 $mgL^{-1}$, 4.5 $mgL^{-1}$ and 5.0 mg $L^{-1}$ respectively. The fish were exposed to these concentration for 96 h [15].

## Chemical

Chlorpyrifos is a broad-spectrum, chlorinated organophosphate insecticides. Common Name: Chlorpyrifos, Chemical Name: O,O-diethyl-O-(3,5,6-trichlor-2-pyridyl)-phosphorothioate, Empirical Formula: $C_9H_{11}Cl_3NO_3PS$

## Acute toxicity evaluation

Clean water was added to glass aquarium up to 70% of their water holding capacity and fish was introduced to gradually increasing test concentrations of chlorpyrifos with three replications of each doses. During the whole acute toxicity trial duration (96 hrs). Test aquaria was examined at periodic intervals of 24, 48, 72 and 96 hours.

## Histopathological evaluation

Fish were collected at different time intervals 24, 48, 72 and 96 hours and dissected tocollect organs i.e. kidney and liver. Histopathological examination of kidney and liver was done by preserving it in 70% formalin. These tissues were dehydrated by passing through various alcoholic grades, washed with clearing solution and embedded in paraffin wax (molten). Fine sectioning of embedded tissue (by using a microtome), hemotoxylin and eosin stain were used and observe under microscope for histopathological findings. The histopathological observations were classified into 4 categories ranging from no changes (-), mild changes (+, < 10%), moderate changes (++, 10-50%) and severe changes (+++, >50%). Throughout the experiment various behavioural changes following exposure to chlorpyrifos were observed that include rapid gulping of water, increased opercular movement and changes in skin colouration [16].

## RESULTS

The experiment was conducted for a short duration of 96 hours to find out the acute exposure of chlorpyrifos (CPF) on histopathology of kidney and liver of *Cirrhinus mrigala* and *Labeo rohita.*

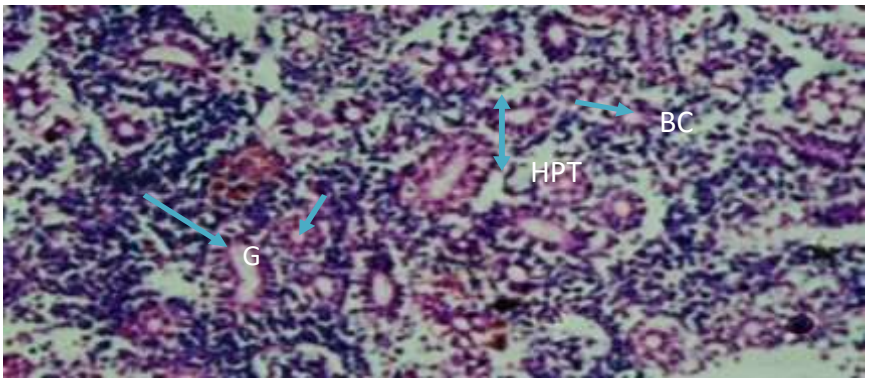


**Fig 1: Histopathology of *C. mrigala* kidney in group I (control)**

Bowmans capsule (BC), Glomerulus (G), Hematopoietic tissues(HPT).

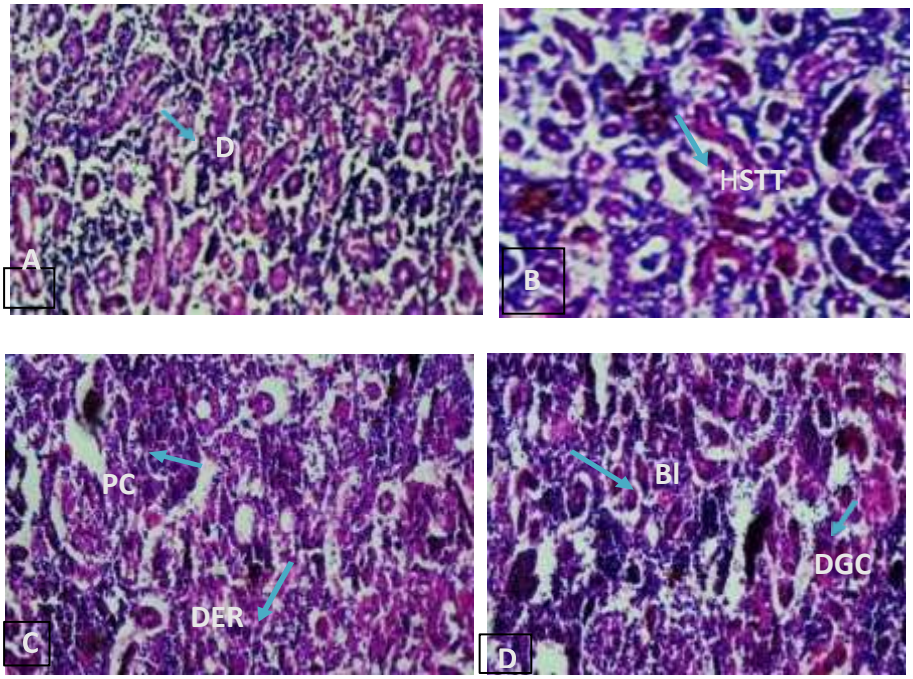

**Fig 2: Histopathology of *C. mrigala* kidney after exposure to chlorpyrifos 4.0 mg/L (GroupII)**

(A) 24 hrs: Degeneration (D). (B) 48 hrs: The hydropic swelling of tubules (HST). (C) 72 hrs: pycnotic cell (PC), degeneration in epithelial cells of renel tubules (DER). (D) 96 hrs: Bleeding (Bl), dilation of glomerulus capillaries (DGC).

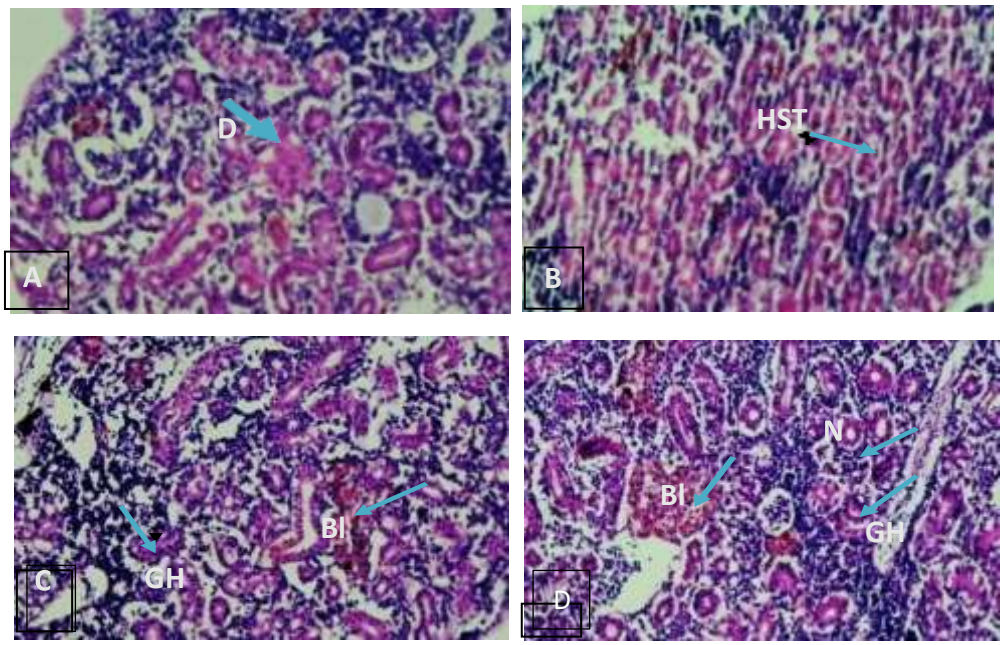


**Fig 3: Histopathology of *C. mrigala* kidney after exposure to chlorpyrifos 4.5 mg/L(Group III)**

(A): showing degeneration (D) after 24 hrs. (B): hydropic swelling of tubules (HST) after 48 hrs. (C): showing bleeding (Bl), glomerular hypertrophy (GH) after 72 hrs.(D): showing necrosis (N), glomerular hypertrophy (GH) and bleeding (Bl) after 96 hrs.

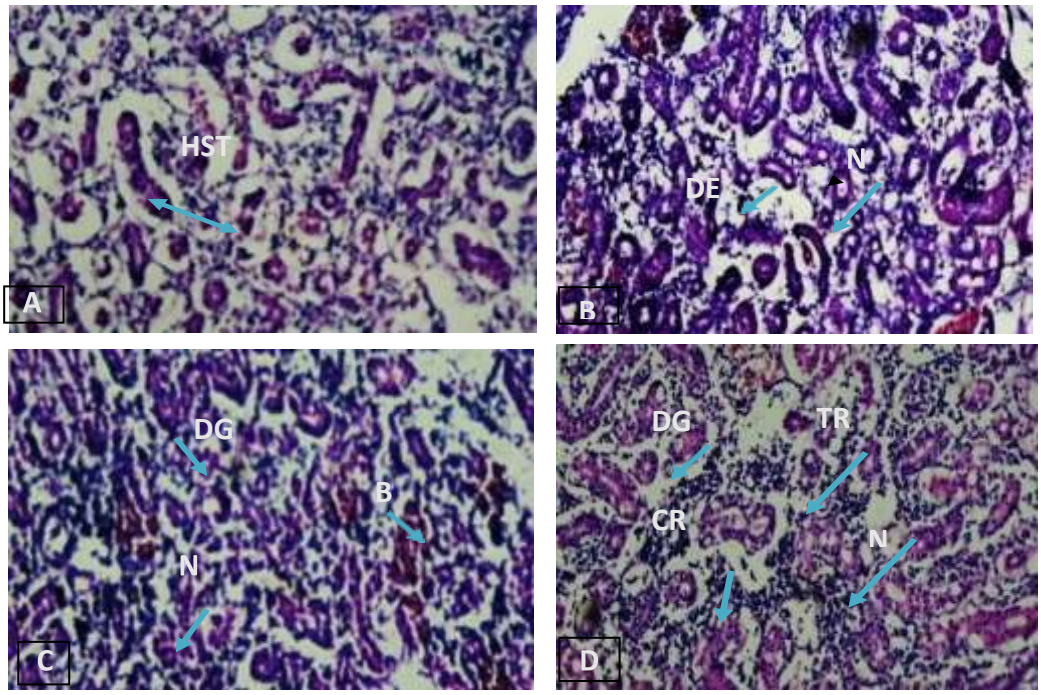


**Fig 4: Histopathology of *C. mrigala* kidney after exposure to chlorpyrifos 5.0 mg/L(Group IV)**

**(A):** shows hydropic swelling of tubules (HST) after 24 h. **(B):** Degeneration in epithelial cell (DES) and necrosis (N) after 24 hrs exposure. **(C):** Degeneration in glomerulus (DG), necrosis (N), bleeding (Bl) after 72 hrs exposure. **(D):** Necrosis (N) bleeding (Bl), degeneration in glomerulus (DG), tubular regeneration (TG) and cellular repture (CR) after 96 hrs.

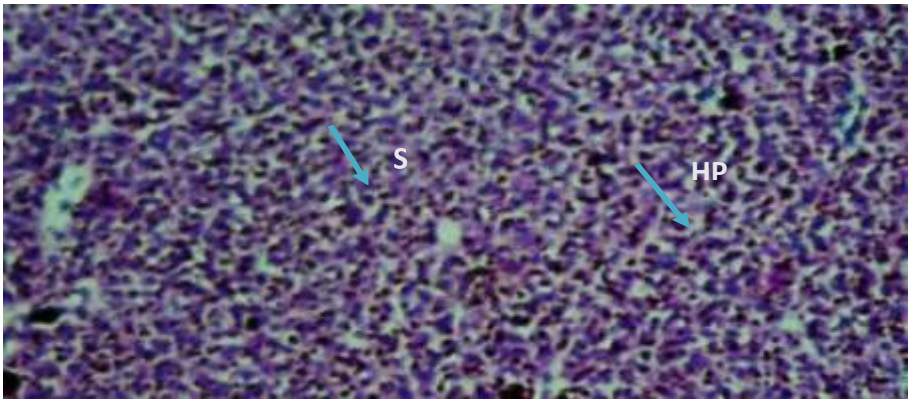


**Fig 5: Histopathology of *C. mrigala* liver in group I (control), Hepatic port (HP), sinusoids (S).**

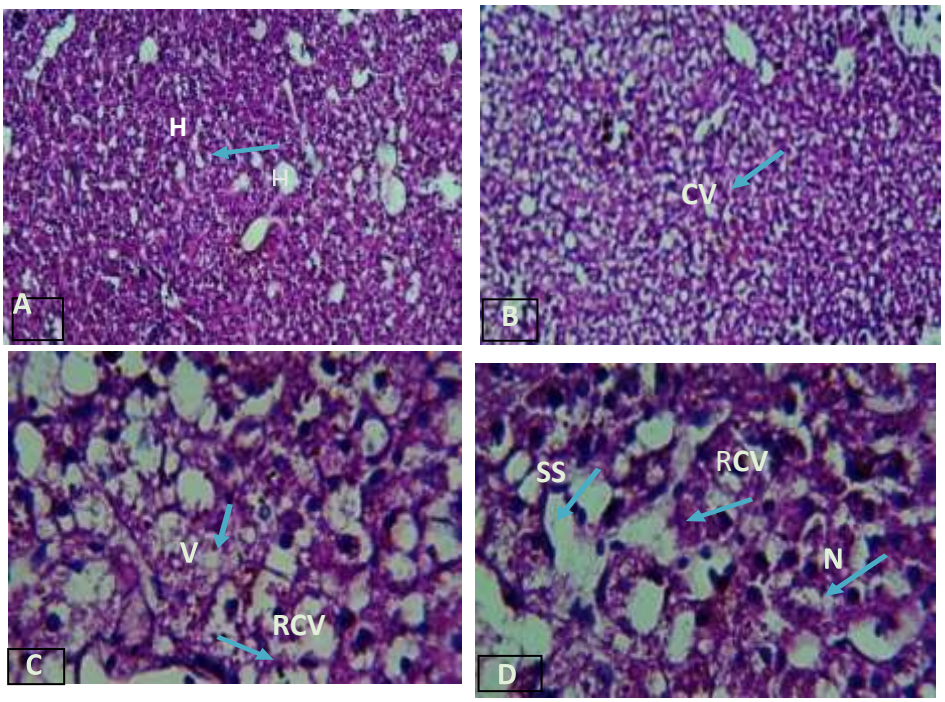


**Fig 6: Histopathology of *C. mrigala* liver after exposure to chlorpyrifos 4.0 mg/L (GroupII)**

(A): shows swelling in hepatic cells (H) after 24 hrs. (B): shows cytoplasmic vocalization (CV) after 48 hrs. (C): shows vocalization (V), reptured central veins (RCV) after 72 hrs. (D): shows vocalization (V), necrosis (N), repture in the central veins (RCV) and sinusoid space (SS) after 96 hrs.

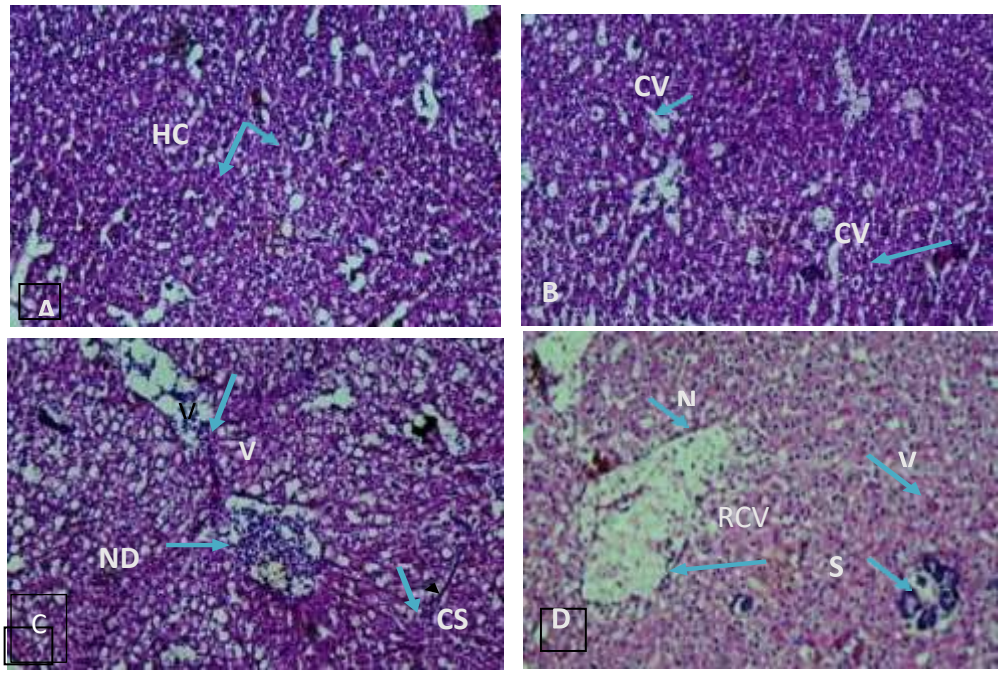


**Fig 7: Histopathology of *C. mrigala* liver after exposure to chlorpyrifos 4.5 mg/L(GroupIII)**

(A): liver shows hepatic cells (HC) after 24 hrs. (B): liver shows cytoplasmic vocuolation (CV) after 48 hrs. (C): liver shows vocuolation (V), congestion of sinusoid (CS), nulear degeneration (ND) after 72 hrs. (D): liver shows vocuolation (V), shrinkage of pancreatic cell (S), necrosis (N), ruptured central vein (RCV) after 9r hrs.

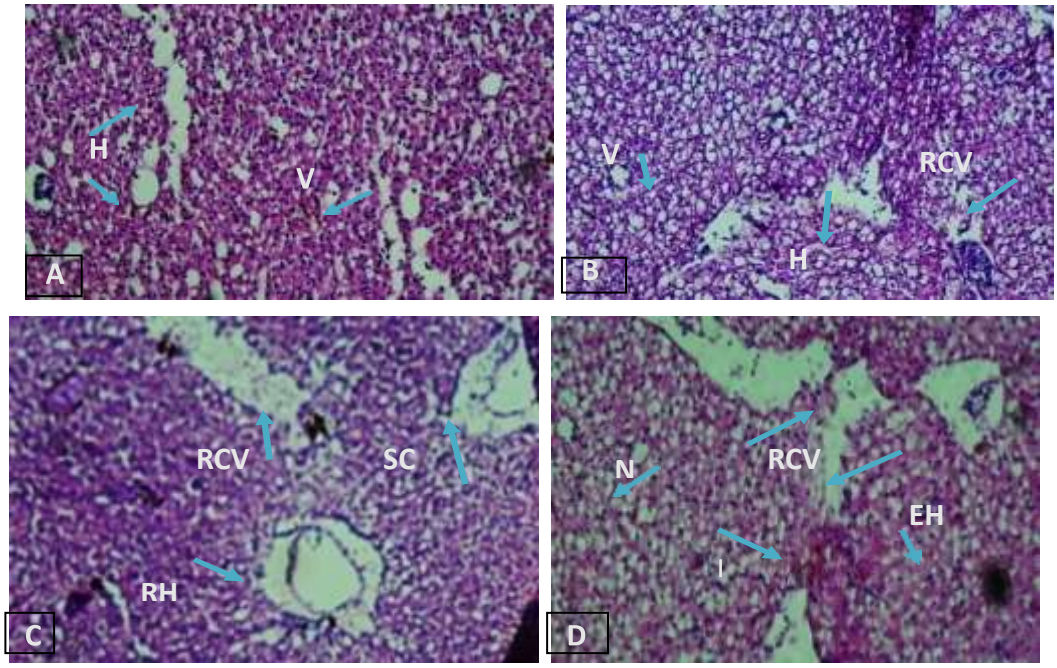


**Fig 8: Histopathology of *C. mrigala* liver after exposure to chlorpyrifos 5.0 mg/L (Group IV)**

After 24 hrs (A): shows vacuolization (V) and hemolysis (H). After 48 hrs (B): shows ruptured central vein (RCV), hemolysis (H). After 72 hrs (C): shows ruptured hepatocytes (RH), sinusoid congestion (SC). After 96 hrs (D): shows necrosis (N), inflammation (I), edema of hepatocytes (EH).

**Effect of chlorpyrifos on the behaviour of *Cirrhinus mrigala***

Several abnormal behaviours like rapid gulping of water, high opercular movement, loss of balance and changes in skin colouration with higher doses at the end of 96 hrs of exposure time (Table.1).

**Table 1: Effect of chlorpyrifos on the behaviou of *Cirrhinus mrigala***

| Behavior | Control | 4.0 mg/L | 4.5 mg/L | 5.0 mg/L |
|---|---|---|---|---|
| Rapid gulping of water | _ | + | ++ | +++ |
| High opercular movement | _ | + | ++ | +++ |
| Loss of balance | _ | + | ++ | +++ |
| Changes in skin Colouration | _ | + | ++ | +++ |

–, None; +, mild; ++, moderate; +++, severe

**Histopathological effect of chlorpyrifos on the kidney and liver of *Labeo rohita***

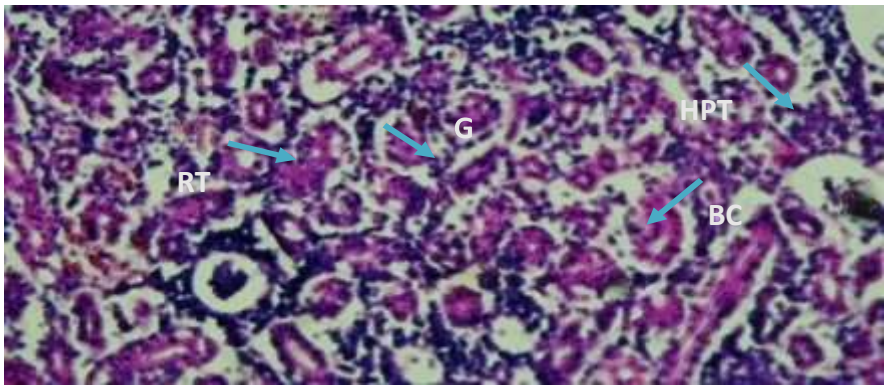


**Fig 9: Histopathology of *L. rohita* kidney group I (control)**

kidney structure of control group showing normal: renal tubules (RT), glomerulus (G),bowman capsule (BC), hematopoietic tissues (HPT).

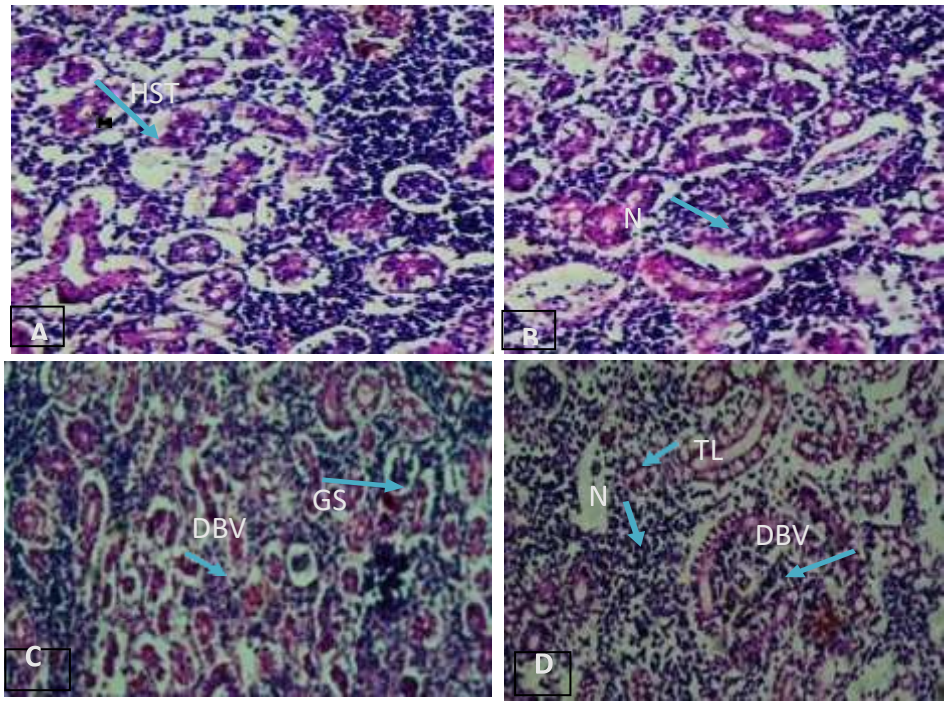


**Fig 10: Histopathology of *L. rohita* kidney after exposure to chlorpyrifos 4.0 mg/L (GroupII)**

After 24 hrs (A): exposure showing hydropic swelling of tubules (HST). After 48 hrs(B): necrosis (N). After 72 hrs (C): glomerular shrinkage (GS), damaged blood vessel (DBV). After 96 hrs (D): necrosis (N), increased tubular lumen (TL), damaged blood vessel (DBV).

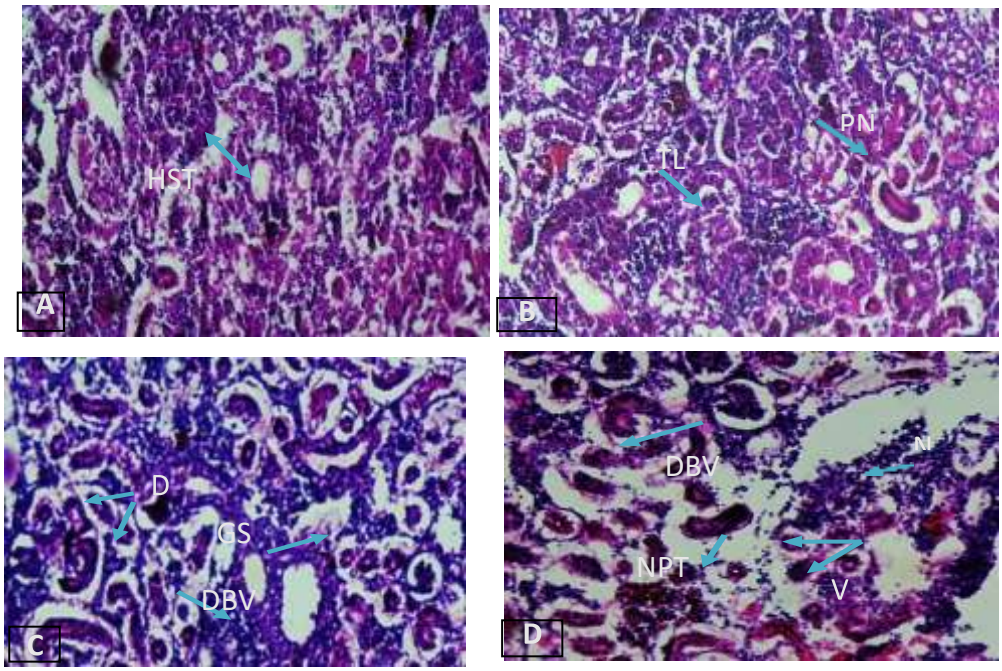


**Fig 11: Histopathology of *L. rohita* kidney after exposure to chlorpyrifos 4.5 mg/L (Group III)**

After 24 hrs (A): exposure showing hydropic swelling of tubules (HST). After 48 hrs (B): increased tubular lumen (TL), pycnotic nuclei (PN). After 72 hrs (C): degeneration of tubular epithelium (D), glomerular shrinkage (GS), damaged blood vessel (DBV). After 96 hrs (D): necrotic proximal tubules (NPT), necrosis (N), vacuolized(V), damaged blood vessel (DBV).

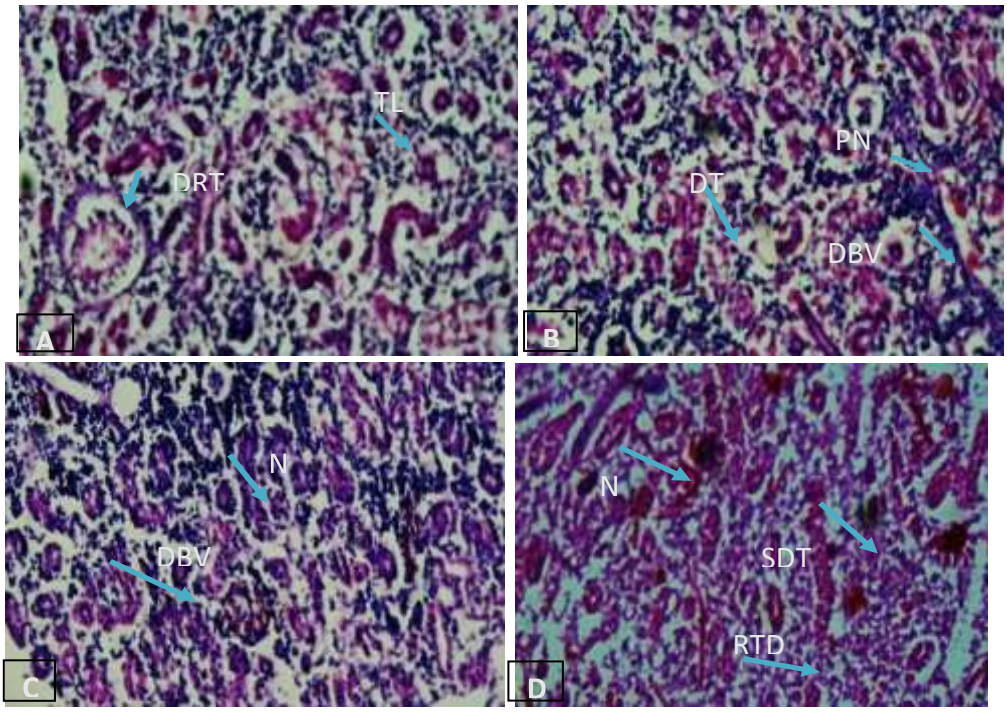

**Fig 12: Histopathology of *L. rohita* kidney after exposure to chlorpyrifos 5.0 mg/L (GroupIV)**

(A) 24 hrs: degeneration of renel tubules (DRT), tubuler lumen (NL). (B) 48 hrs: degeneration of blood vessels (DBV), necroris (N), pyknotic nuclei (PC). (C) 72 hrs: degeneration of blood vessels (DBV), necroris (N). (D) 96 hrs: renal tubules damges (RTD), necrotic (N) and severe tubules damges (STD).

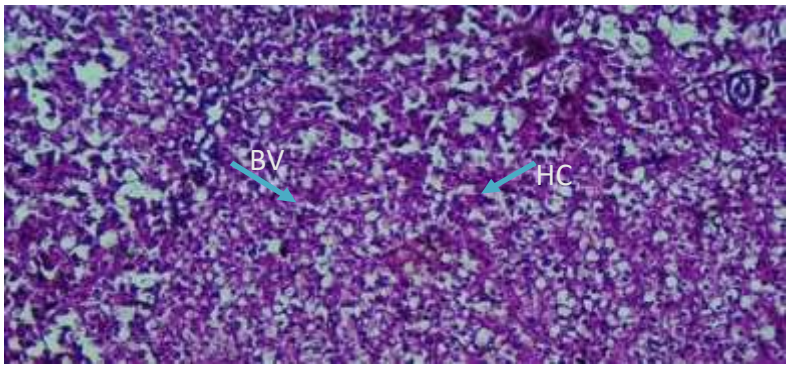


**Fig 13: Histopathology of *L. rohita* liver group I (control)**

Normal hepatic cells (HC), blood vessels (BV), no inflammation and hydropicdegeneration.

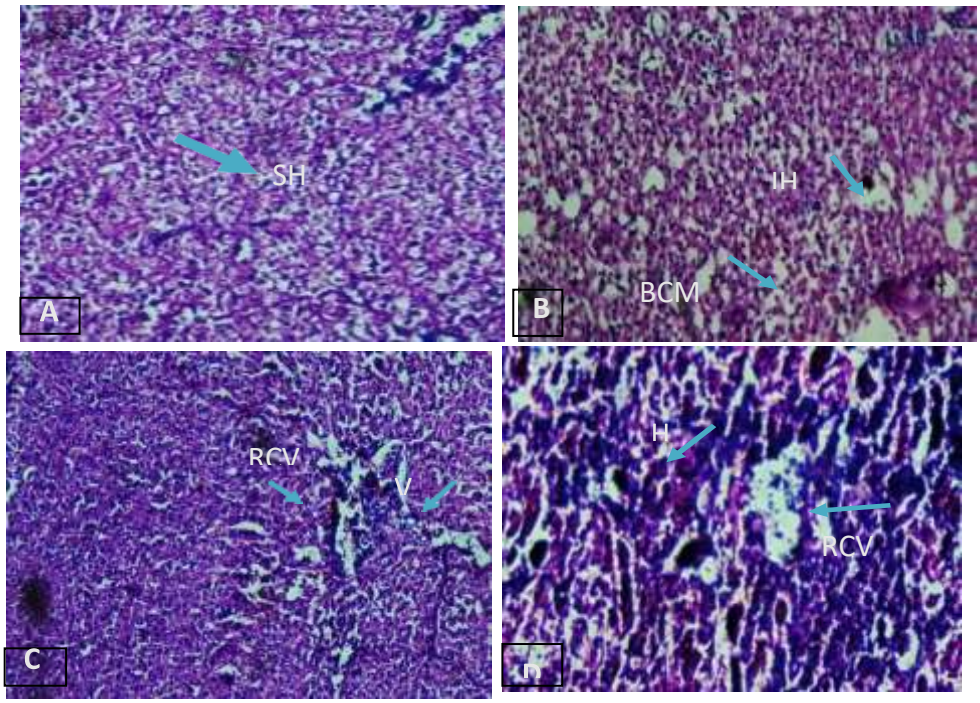


**Fig 14: Histopathology of *L. rohita* liver after exposure to chlorpyrifos 4.0 mg/L (GroupII)**

(A): swelling in hepatocytes (SH) after 24 hrs. (B): broken cell membrane (BCM), irregular structure of hepatocytes (IH) after 48 hrs. (C): showing accumulation ofvacuoles (V), ruptured central veins (RCV) after 72 hrs. (D): showing abnormal hepatocytes (H), ruptured central veins.

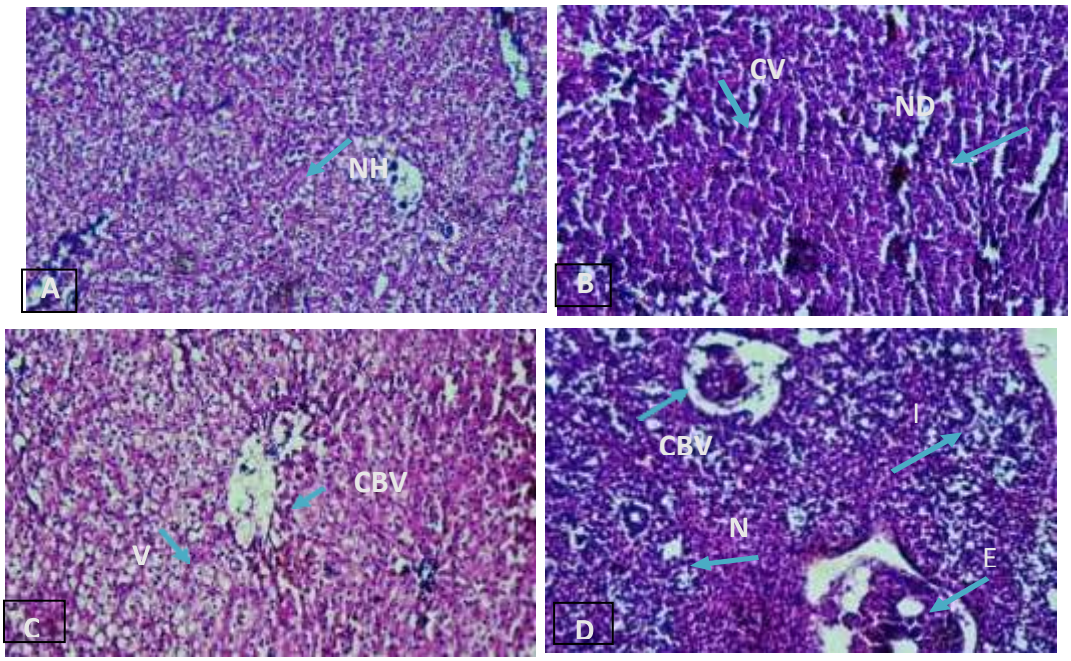


**Fig 15: Histopathology of *L. rohita* liver after exposure to chlorpyrifos 4.5 mg/L (GroupIII)**

The liver shows the nuclear hypertrophy (NH) after 24 h (A). Cytoplasmic vocuolation(CV), nuclear degeration (ND) after 48 h (B). Vaculation in hypatocytes(V), congested blood vessels (CBV) after 72 h (C). Necrosis (N), inflammation (I), edema (E) after 96h.

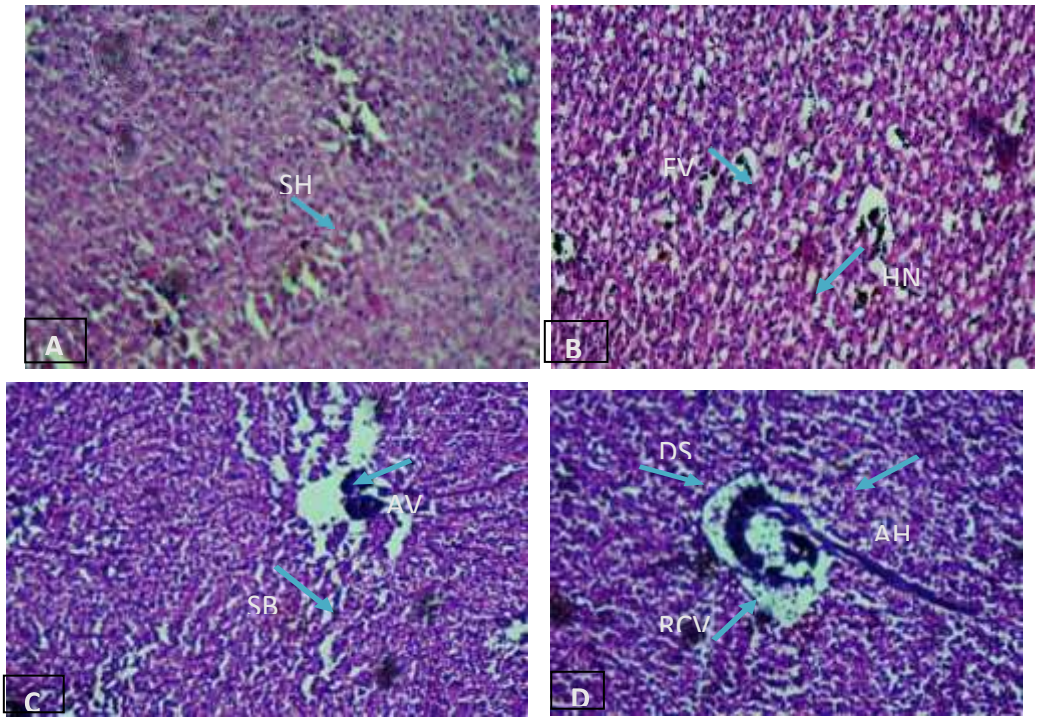


**Fig 16: Histopathology of *L. rohita* liver after exposure to chlorpyrifos 5.0 mg/L (GroupIV)**

(A): swelling in hepatocytes (SH) after 24 h. (B): fat vacuoles (FV), hepatocytesnecrosis (HN) after 48 h. (C): accumulation of vacuoles (AV), sinusoid blood congestion (SB) after 72 h. (D): abnormal hepatocytes (AH), dilation of sinusoid (DS), ruptured central veins (RCV) after 96 h.

**Table 2 Effect of different doses of chlorpyrifos on the behaviour of *Labeo rohita***

| Behaviour | Control | 4.0 mg/L | 4.5 mg/L | 5.0 mg/L |
|---|---|---|---|---|
| **Loss of equilibrium** | _ | + | ++ | +++ |
| **Motionlessness** | _ | + | ++ | +++ |
| **Erratic swimming** | _ | + | ++ | +++ |
| **Changes in skin Colouration** | _ | + | ++ | +++ |

–, None; +, mild; ++, moderate; +++, sever

## DISCUSSION

### Effect of chlorpyrifos on *Cirrhinus mrigala*

The kidneys are responsible for excreting xenobiotics and metabolites. Although, when exposed to harmful chemicals, the body may exhibit some histological changes. In the present study, no histopathological changes were observed in kidney of control group. The kidney of fish showed well developed glomeruli, renal tubules and hematopoietic tissues. The histopathological examination of chlorpyrifos treated *Cirrhinus mrigala* groups were showed that at low dose 4.0mg/L kidney tissue showed mild alteration in epithelial cells of the renal tubules, as compered to control group. The moderate alteration inthe epithelial cells of renal tubules were showed hydropic swelling of tubules in second dose 4.5 mg/L. At high dose 5.0 mg/L severe toxicity in kidney tissues were observed. The hydropic swelling of tubules, necrosis, bleeding, degeneration in glomerulus and cellular repture.

Similar histopathological changes were caused due to pesticidal exposure in kidney of *Prochilodus lineatus* and *Piaractusmesopo tamicus* exposed to trichlofon [17]. After exposure to different concentrations of chlorpyrifos, histopathological changes in kidney, degeneration in the endothelial cells of renal tubules, contraction of glomerulus, degradation of the glomerular capillary and narrowing of the renal tubules. Similarly, *Heteropneustes fossilis* treated to chlorpyrifosshowed dilation of the lamellae of the kidney tubules, destruction of tubules, shrinking of the glomerular tuft, and dilatation of blood vessels in the renal tuft [18].

The liver is the major metabolic site for xenobiotic detoxification, and it can frequently produce very toxic secondary products. As a result, the liver is exposed to a lot of toxic substances and accumulates them. In the present study the liver tissues of control group, revealed normal hepatic structure which show normal hepatic portal, sinusoids and hepatocytes. However in group II exposed to 4.0 mg/L dose of chlorpyrifos liver tissue show mild alteration in hepatic cells and sinusoids as compered to control group. Where are in group III exposed to 4.5 mg/L dose ofchlorpyrifos liver tissue show moderate alteration in hepatocytes, sinusoidal congestion. In addition, group IV exposed to 5.0 mg/L doseof chlorpyrifos show severe toxicity in liver tissue including hepatocytes, necrosis and ruptured central vein of liver.

Other studies have examined the impact of several insecticides on the liver of different fish species. In the liver tissues of the common carp, chlorpyrifos exhibited melanomacrophage accumulations, irregular structure of hepatocytes, blood congestion and ruptured central veins, hyperplasia, inflammation, necrosis and edema[19]. On the other hand normal behavior of fishes were observed in the control group. Similar behavioral changes reported by in the fishes exposed to different pesticides [20].

## Effect of chlorpyrifos on *Labeo rohita*

The principal haematological and osmoregulatory organs in fish are the kidneys.Because the majority of post-branchial blood flows to fish kidneys, altered fish histology is a good indicator of environmental pollution. In the present study kidney tissues show no clear changes in control group showing normal renal tubules, glomerulus, bowman capsule, hematopoietic tissues. The kidney of fish exposed to 4.0 mg/L chlorpyrifos stock solution, showing hydropic swelling of tubules, shrinkage of glomerulus and damaged blood vessels were observed. The kidney of fish exposed to 4.5 mg/L chlorpyrifos stock solution, showing hydropic swelling of tubules. The kidney of fish exposed to 5.0 mg/L chlorpyrifos stock solution, the histopathological changes in the kidney of fish such as degeneration of renel tubule and tubuler lumen were observed. Degeneration of blood vessels was also seen with necroris.

Many researchers have observed histological changes in the kidney at the glomerulus and tubular epithelium level in fish following exposure to harmful substances like insecticides. In *Labeo rohita* treated to hexachloro-cyclohexane, dilationof proximal tubule and inflammatory alterations characterised by karyorrhexix and karyolysis. After exposure to fenvalerate, *Ctenopharyngodon idella* kidney tissues showed necrosis, cloudy edoema in the renal tubules, glomerular shrinkage and vacuolization [21].

The liver is the principal organ for metabolism, xenobiotic detoxification, and hazardous chemical excretion. It has the potential to digest harmful chemicals, but highconcentrations of these compounds can exceed its regulatory mechanisms, which canlead to structural damage[22].

In the present study, liver tissue of control group fish revealed nomal hepatic and sinusoids architecture. The histological examination of chlorpyrifos treated *Labeo rohita* groups were showed that at 4.0 mg/L liver tissues showing abnormal hepatocytes, dilation of sinusoid as compared to control group, and 4.5 mg/Lliver shows the nuclear hypertrophy, cytoplasmic vocuolation and nuclear degeration and 5.0 mg /L the histopathological changes

observed in the liver tissues were swelling in hepatocytes. broken cell membrane, irregular structure of hepatocytes and necrosis.

Hepatocyte degeneration and accumulation of vacuoles, hepatocytes necrosiswere seen in Nile tilapia treated to deltamethrin. In *O. niloticus*, histopathological alterations were observed in hepatocytes where diazinon-exposed liver revealed bleeding or haemorrhage, fatty deterioration, and hepatocytes. Fish behavior changes are important indicators of pesticide exposure which cause possible harmful effects. In the present study, numerous abnormal behaviors such as loss of balance, motionlessness, erratic swimming and changes in the skin colouration were exposed to chlorpyrifos. They stated that due to the toxicity of diazinon, thefish equillibrium became paralysed in all cases, and eventually settled down to the bottom of the aquarium, where they remained until death [23].

## CONCLUSION

Chlorpyrifos is a commonly used organophasphate insecticides that cause toxicological effects in aquatic organism especially in fish. In the present study, it was concluded that exposure of chlorpyrifos caused histopathological and behavioural changes in *Cirrhinus mrigala* and *Labeo rohita* after 96 hours of trial. After exposure, histopathological study of organ (kidney, liver) of *Cirrhinus mrigala* and *Labeo rohita* showed prominent variations in these organs as compared to control group.

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