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Research Article

HISTOPATHOLOGICAL CHANGES IN SELECTED TISSUES OF THE FISH *CYPRINUS CARPIO* DURING SUB – LETHAL EXPOSURE TO KARANJIN BASED BIOPESTICIDE – DERISOM

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ABSTRACT

Histopathology serves as a very good marker of environmental toxicology and also useful in monitoring health status of aquatic vertebrates. Histological alterations in organs i.e. gill, liver, kidney and muscle of *Cyprinus carpio* were observed during sub-lethal exposure of biopesticide Derisom for a period of 21 days. Fishes of exposed group showed alterations in gill like vacuolation, curling of secondary gill lamellae, infiltration of inflammatory cells, etc. The liver of exposed fishes showed alterations such as vacuolation, hydropic degeneration, necrosis, cytoplasmic degeneration, etc. The kidney of the exposed fishes showed changes such as epithelial rupture, degenerative changes, vacuolation, etc. The muscle of the exposed fishes showed changes such as splitting of muscle fibres, increased inter muscular space, etc.

Keywords: Histopathology, Biopesticide, Derisom, gill, liver, kidney and muscle

1. INTRODUCTION

Pesticides are one of the largest groups of agricultural pollutants that are used to control various insect pests [1]. Accumulation of pesticides in water bodies causes harmful effects on all members of food chain mainly affecting the health of fishes which constitute important food source for humans [2]. Exposure of aquatic organisms mainly fishes to low concentrations of pesticides may result in some histopathological alterations and lesions in different tissues of fish [3-5].

In view of the harmful effects caused by the synthetic pesticides to the environment, more research and importance is being given for the manufacture of biopesticides in the form of botanical pesticides which are one of the promising tools today to combat the hazards caused by the synthetic pesticides. The biopesticide which we have used in the present study is a plant based botanical pesticide named "Derisom". The biopesticide is present as a liquid formulation and is brown in color. It is effective against wide range of pests and used in variety of crop fields. The active ingredient is an alkaloid "**Karanjin**" and karanj oil which are extracted from the seeds of Karanja plant (*Pongamia pinnata*). This plant is a boon for mankind like neem tree. Not only the plant gives us a wonderful botanical pesticide but at the same time it is being used in manufacturing of "Bio-Diesel" along with Jatropha plant.

Tissue changes in test organisms exposed to a low concentration of toxicants provide information on the nature of the toxicant [6]. Histopathological studies of various organs like gill, liver, kidney and muscle provide us an understanding of extent of damage caused by the toxicants [7]. Histological alterations in organs serve as sensitive biomarkers for xenobiotic effects. Therefore, histopathological alterations in various fish tissues have been widely used as a key instrument in aquatic toxicology [8-12].

Cyprinus carpio is a commonly cultured and edible fish throughout India. The fish is used as a very good model in toxicology research which easily adapts to the laboratory conditions. It breeds twice in a year hence available throughout the year for research work.

2. MATERIALS AND METHODS

Juveniles of fish common carp (*Cyprinus carpio*) were brought from Kaikaluru village of Andhra Pradesh State and were acclimated in 500 Litre indoor tanks filled with dechlorinated tap water for a period of one month prior to the experiment. They were fed twice daily with commercially available fish feed. During the acclimatization period constant aeration was supplied to the tanks. Throughout the acclimatization period any fish found dead was removed immediately. The water in the tank was changed every day with fresh dechlorinated tap water.

Healthy fishes weighing 35±1.99 gm and measuring in length 14.03±1.217 cm were used for sub-lethal toxicity testing of biopesticide – Derisom. The 96 hrs LC50 value has already been calculated as 2.8ppm. 1/10th of 96hrs LC50 value i.e. 0.28ppm was taken as the sub-lethal value and the fishes were exposed to the sub-lethal value for a period of 21 days. Throughout the sub-lethal exposure period the pesticide concentration was renewed after every 24hrs. The experiment was carried out in 6 replicates. After the completion of 24hrs, 7 days, 14 days and 21 days the fishes from both control and exposed group were dissected, gill, liver, kidney and muscle tissues were carefully removed, washed in saline and fixed in 10% neutral formalin. Fixed tissues were dehydrated thoroughly in graded series of ethanol. Then the tissues were cleared with xylene and embedded in paraffin wax. In order to specify the thickness of serial section for histological purpose, generally sections were cut at 4-5 µm thickness and stained with haematoxylin and eosin for light microscopic examination, and sections were examined for investigation of histopathological lesions. The slides were observed using Olympus light microscope and the images were captured using Olympus camera attached to the microscope. The histological structures were clearly observed and studied under 40X and 100X oil immersion magnifications.

3. RESULTS

3.1. GILL

The structure of the gill of normal control *Cyprinus carpio* fish is composed of primary gill lamellae (PGL) and secondary gill lamellae (SGL) with well-marked inter lamellar spaces (ILS). Primary gill lamellae consisted of cartilaginous skeletal structure filament, multi-layered epithelium (EP) and vascular system. Numerous secondary lamellae were lined up along both sides of primary lamella. Secondary gill lamella was constituted of epithelial cells supported by pillar cells (PC). The gills of the control fish were healthy in appearance and red in colour. The opercular movement was very normal **(Fig: 1.1 and 1.6)**.

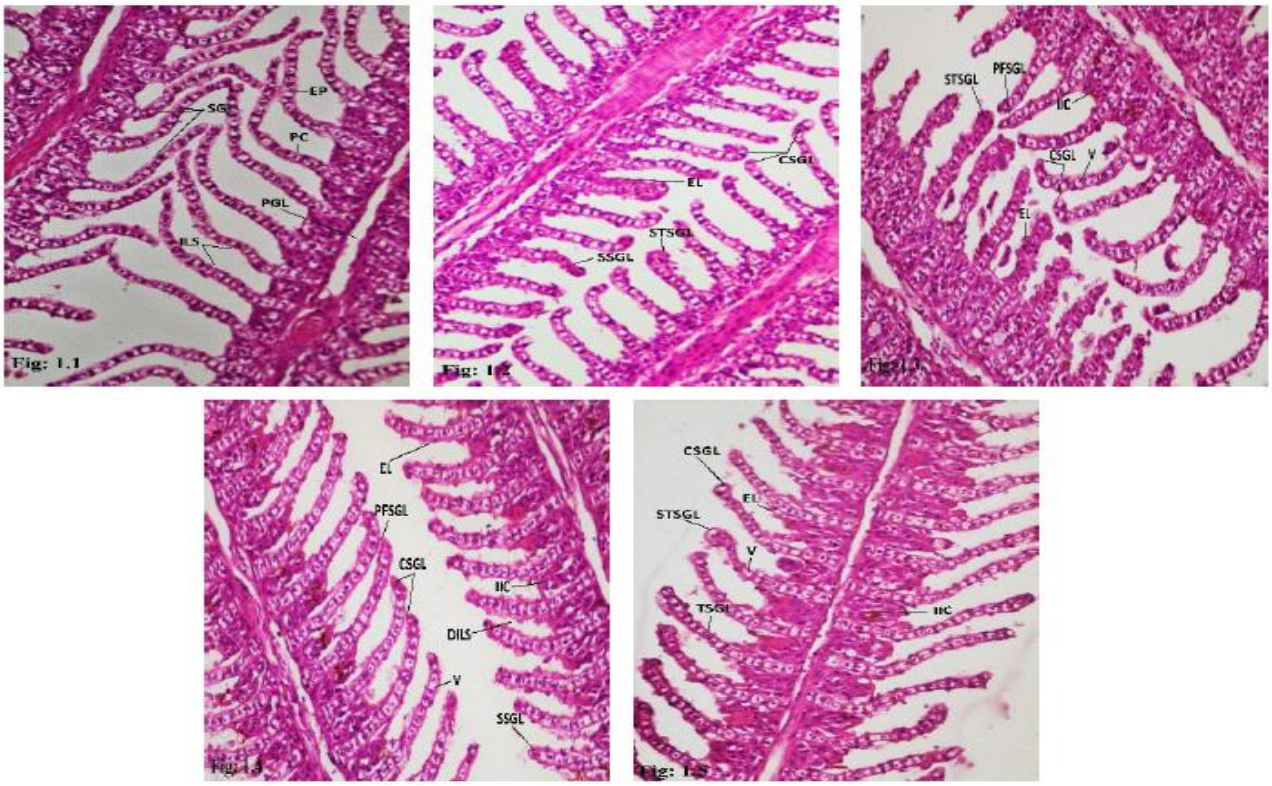

Fig. 1.1 contol gill of *C. carpio* at 40x, H&E
Fig. 1.2 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 40x, H&E
Fig. 1.3 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 40x, H&E
Fig. 1.4 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 40x, H&E
Fig. 1.5 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 40x, H&E

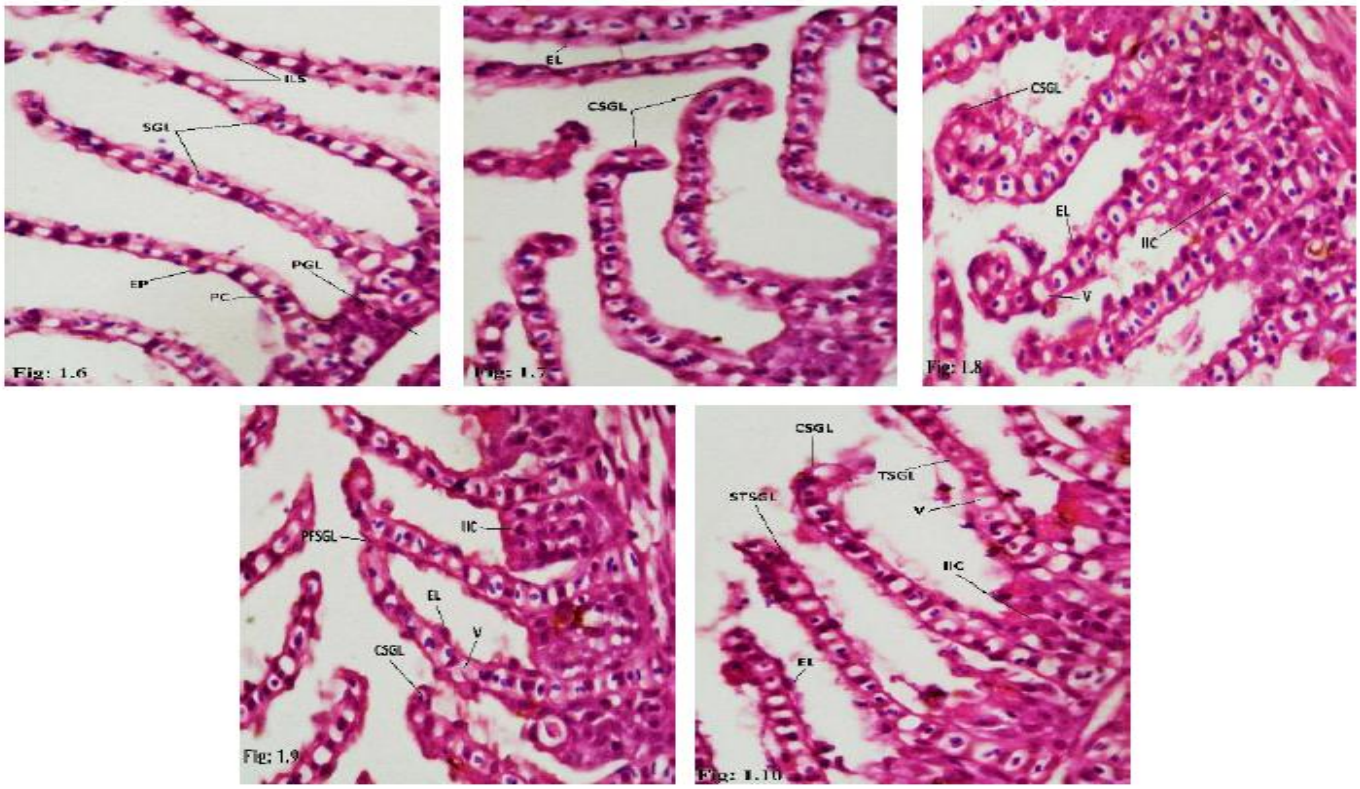

Fig. 1.6 contol gill of *C. carpio* at 100x, H&E
Fig. 1.7 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 100x, H&E
Fig. 1.8 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 100x, H&E
Fig. 1.9 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 100x, H&E
Fig. 1.10 gill of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 100x, H&E

PGL – primary gill lamellae, SGL – secondary gill lamellae, EP – epithelium, PC – pillar cell, ILS – inter lamellar space, CSGL – curling of secondary gill lamellae, EL – epithelial lifting, SSGL – shortening of secondary gill lamellae, STSGL – swollen tips of secondary gill lamellae, V – vacuolation, IIC – infiltration of inflammatory cells, PFSGL – partial fusion of secondary gill lamellae, DILS – decreased inter lamellar space, TSGL – thinning of secondary gill lamellae

Gills are the first organ to get exposed to any kind of toxicant and they are the primary organs to be affected. The gills of the fishes during sub-lethal exposure showed both morphological and histological alterations, as the days of exposure increased these changes were also increasing in their degree. The gills were little pale as compared to the control fish gills and the opercular movement was slow than the normal as a result of avoidance of contact behaviour. After completion of 24 hrs of sub-lethal exposure following changes were seen in the gills – curling of secondary gill lamellae (CSGL), epithelial lifting (EL), swollen tips of secondary gill lamellae (STSGL) and shortening of secondary gill lamellae (SSGL) **(Fig: 1.2 & 1.7)**. After the completion of 7 days of sub-lethal exposure more changes were observed in the gills – vacuolation (V), epithelial lifting (EL), infiltration of inflammatory cells (IIC), curling of secondary gill lamellae (CSGL), shortening of secondary gill lamellae (SSGL) and partial fusion of secondary gill lamellae (PFSGL) **(Fig: 1.3 & 1.8)**. The gills showed still high levels of structural and histopathological alterations after the completion of 14 days of sub-lethal exposure – epithelial lifting (EL), partial fusion of secondary gill lamellae (PFSGL), curling of secondary gill lamellae (CSGL), infiltration of inflammatory cells (IIC), vacuolation (V), decreased interlamellar spaces (DILS) and shortening of secondary gill lamellae (SSGL) **(Fig: 1.4 & 1.9).** After the completion of 21 days of sub-lethal exposure the gills showed severe morphological and pathological changes such as curling of secondary gill lamellae (CSGL), epithelial lifting (EL), vacuolation (V), swollen tips of secondary gill lamellae (STSGL), thinning of secondary gill lamellae (TSGL) and infiltration of inflammatory cells (IIC) **(Fig: 1.5 & 1.10).**

3.2. Liver

The structure of the normal liver of the *Cyprinus carpio* control fish consists of continuous mass of cells called parenchyma (P) in which are present hexagonal cells arranged in the form of groups called hepatocytes (H). The hepatocytes form a rather cord-like pattern and these cords are arranged around tributaries of the hepatic vein. In between the hepatocytes are the sinusoids (S) in which there is present the bile juice, all the sinusoids join to form a large bile duct. The liver cells are large in size, polygonal in shape with homogenous granular cytoplasm (GC) and either eccentric or centrally located distinct nuclei (N). Each cord of the liver was separated by the thick wall of the peripheral cells **(Fig: 2.1 and 2.6).**

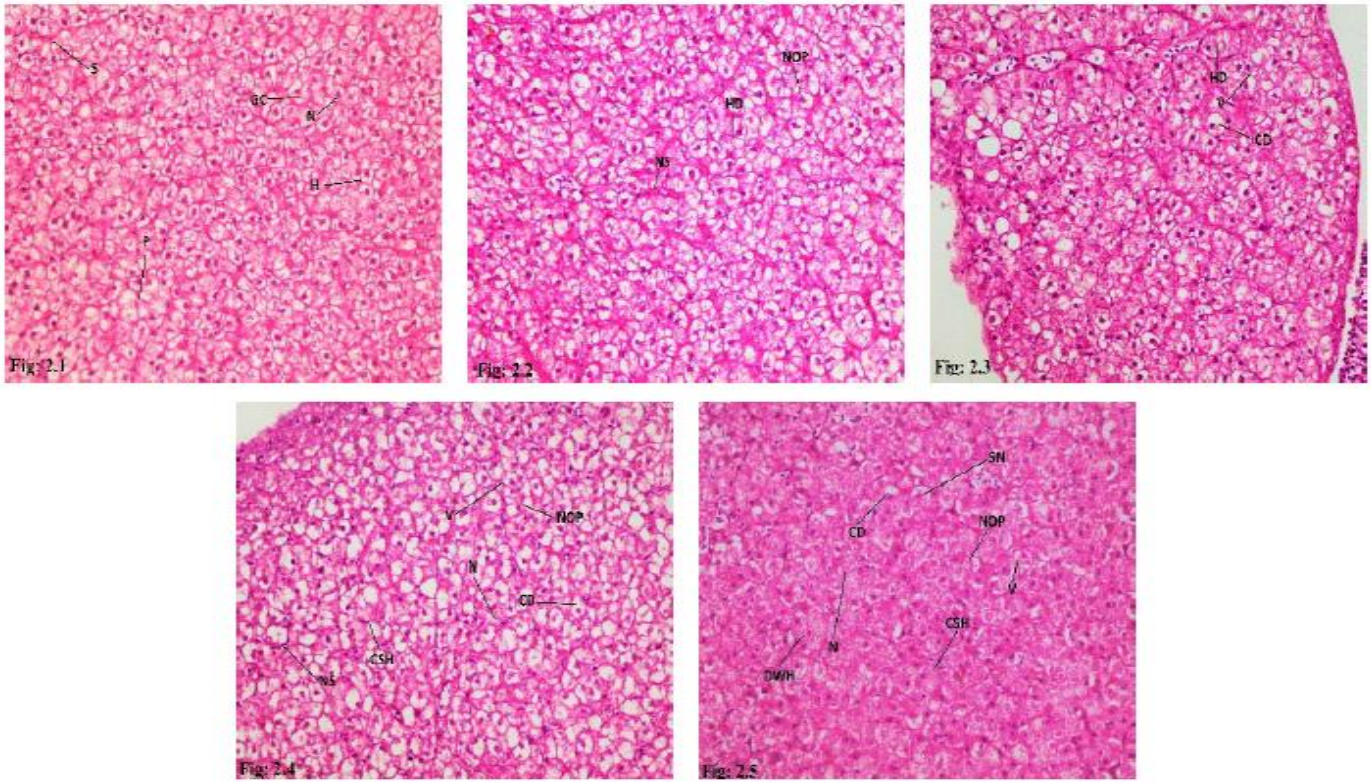

Fig. 2.1 contol liver of *C. carpio* at 40x, H&E
Fig. 2.2 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 40x, H&E
Fig. 2.3 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 40x, H&E
Fig. 2.4 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 40x, H&E
Fig. 2.5 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 40x, H&E

Liver is the foremost organ to counter and detoxify any kind of toxicant. After the completion of 24 hrs of sub-lethal exposure to biopesticide Derisom the liver showed changes such as narrowing of sinusoids (NS), hydropic degeneration (HD) and nucleus having an outlying position (NOP) **(Fig: 2.2 & 2.7).** After the completion of 7 days of exposure there were seen more histological changes such as vacuolation (V), hydropic degeneration (HD) and cytoplasmic degeneration (CD) **(Fig: 2.3 & 2.8).** As the days of exposure increased more severe changes were seen in the liver. There was size reduction of liver, i.e., as the fishes were not eating the feed properly it reflected on the size of liver and also the colour of the liver was slightly pale as compared to the liver of control fish. After the completion of 14 days of sub-lethal exposure the following changes were observed – vacuolation (V), necrosis (N), narrowing of sinusoids (NS), cloudy swelling of hepatocytes (CSH), cytoplasmic degeneration (CD) and nucleus having an outlying position (NOP) **(Fig: 2.4 & 2.9).** The liver showed maximum alterations in liver after the completion of 21 days sub-lethal exposure such as shrunken nucleus (SN), cytoplasmic degeneration (CD), necrosis (N), vacuolation (V), cloudy swelling of hepatocytes (CSH), disintegrating walls of hepatocytes (DWH) and nucleus having an outlying position (NOP) **(Fig: 2.5 & 2.10).**

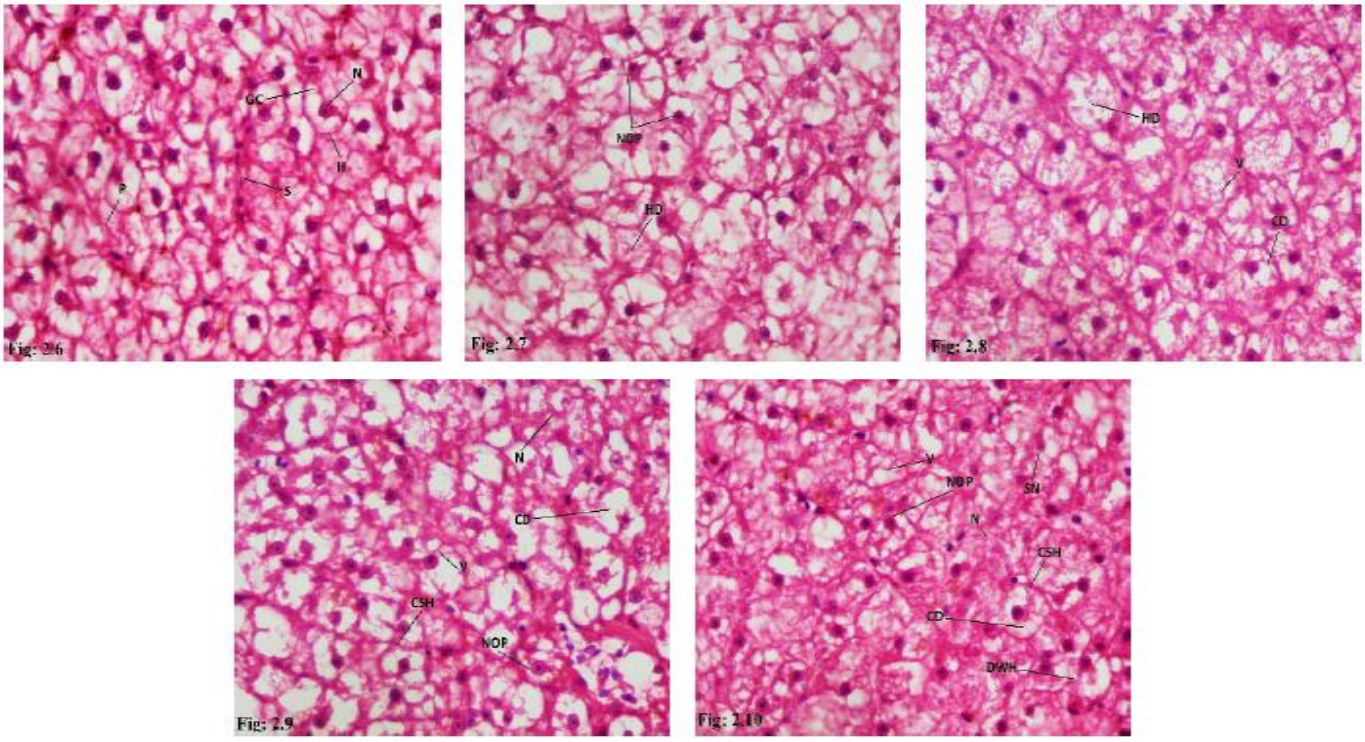

Fig. 2.6 contol liver of *C. carpio* at 100x, H&E
Fig. 2.7 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 100x, H&E
Fig. 2.8 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 100x, H&E
Fig. 2.9 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 100x, H&E
Fig. 2.10 liver of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 100x, H&E
P - parenchyma, H – hepatocytes, N – nucleus, S – sinusoids, GC – granular cytoplasm, NS – narrowing of sinusoids, HD – hydropic degeneration, NOP – nuclei having an outlying position, V – vacuolation, CD – cytoplasmic degeneration, CSH – cloudy swelling of hepatocytes, DWH – disintegrating walls of hepatocytes, N – necrosis.

3.3. Kidney

The kidney of the control group *Cyprinus carpio* showed the normal histology with structures such as a renal corpuscle containing the glomerulus (G) constituted by glomerular capillaries (GC) and the Bowman's capsule (BC). Around the renal corpuscle are also found the hematopoietic tissue with basophilic cells, proximal convoluted tubules (PCT) and distal convoluted tubules (DTC), each tubule had an epithelial layer (ERT), nucleus of cells of renal epithelial cells (NRT) and the lumen on tubules (LT) through which the blood passed and urine was filtered **(Fig: 3.1 and 3.6).**

Kidneys of fish are very sensitive and are greatly affected by pollutants and toxicants. After the completion of 24 hrs of sub-lethal exposure of Derisom the following pathological changes were seen at the initiation stage in the kidney such as vacuolation (V), infiltration of inflammatory cells (IIC), shrunken nucleus (SN), epithelial rupture (ER), occlusion of tubular lumen and some slight degenerative changes. **(Fig: 3.2 & 3.7).** As the days of exposure increased the pathological alterations also became more severe. After the completion of 7 days and showed alterations such as epithelial rupture (ER), vacuolation (V), glomerular shrinkage (GS), infiltration of inflammatory cells (IIC), occlusion of tubular lumen (OTL), initiation of degeneration of distal convoluted tubule (DDCT) and initiation of degeneration of proximal convoluted tubule (DPCT) **(Fig: 3.3 & 3.8).**

The pathological alterations were more precise and the intensity of alterations were also more after the completion of 14 days of exposure and showed following changes such as vacuolation (V), infiltration of inflammatory cells (IIC), glomerular shrinkage (GS), epithelial rupture (ER), occlusion of tubular lumen (OTL), degeneration of proximal convoluted tubule

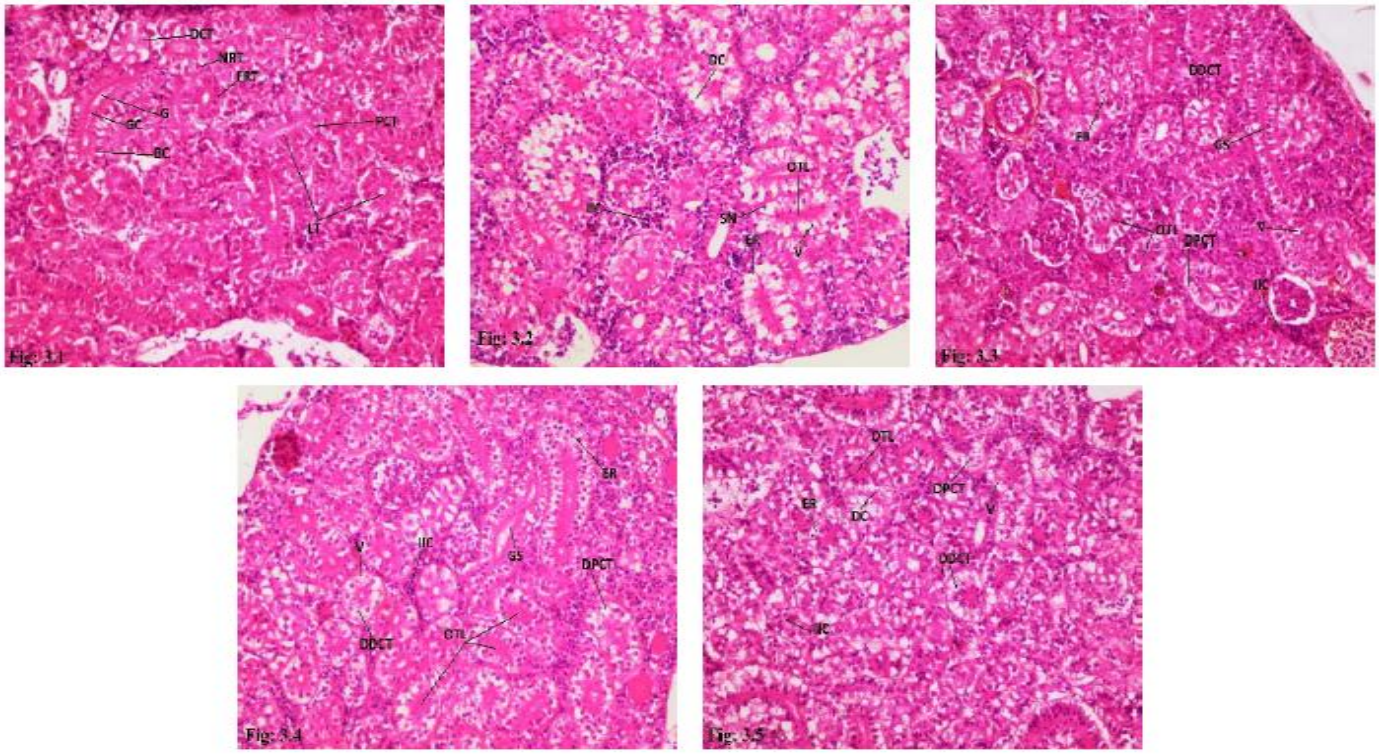

Fig. 3.1 contol kidney of *C. carpio* at 40x, H&E
Fig. 3.2 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 40x, H&E
Fig. 3.3 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 40x, H&E
Fig. 3.4 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 40x, H&E
Fig. 3.5 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 40x, H&E

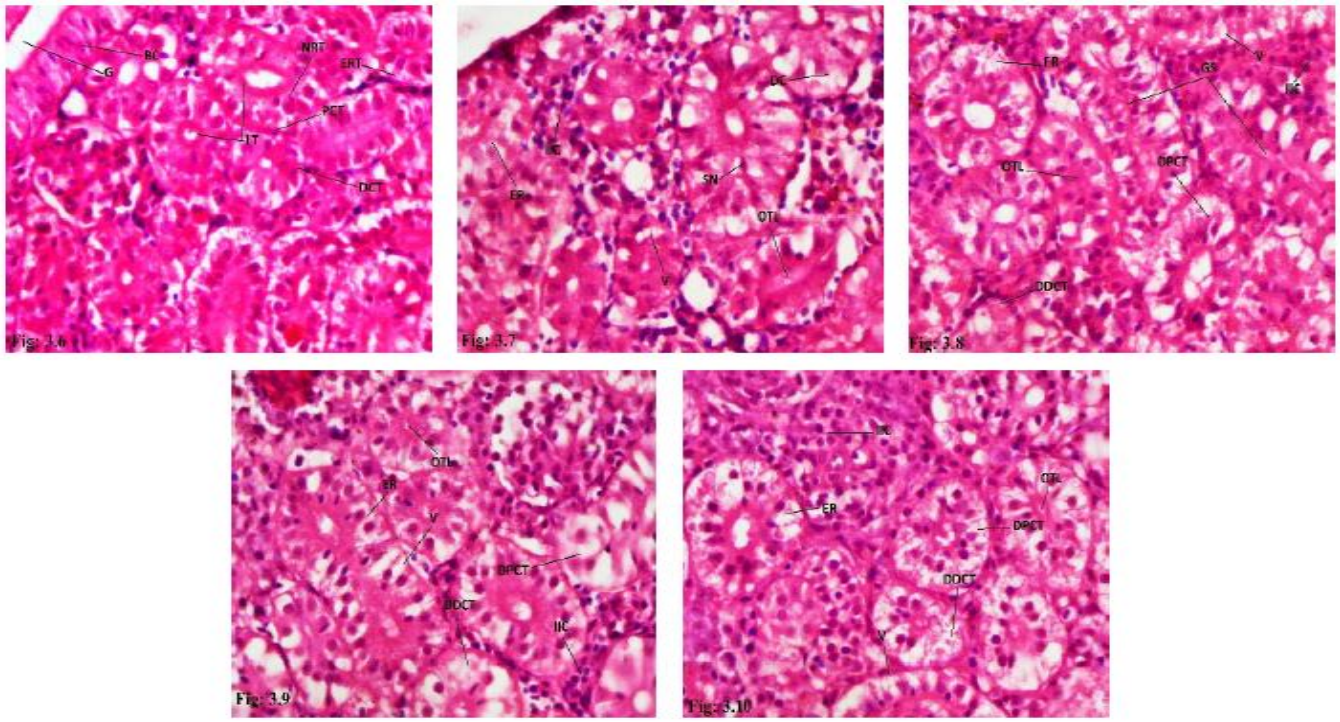

Fig. 3.6 contol kidney of *C. carpio* at 100x, H&E
Fig. 3.7 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 100x, H&E
Fig. 3.8 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 100x, H&E
Fig. 3.9 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 100x, H&E
Fig. 3.10 kidney of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 100x, H&E

G – glomerulus, BC – bowman's capsule, PCT – proximal convoluted tubule, DCT – distal convoluted tubule, LT – lumen of tubule, ERT – epithelium of renal tubule, NRT – nuclei of renal tubule, IIC – infiltration of inflammatory cells, V – vacuolation, ER – epithelial rupture, SN – shrunken nucleus, DC – degenerative changes, OTL – occlusion of tubular lumen, GS – glomerular shrinkage, DPCT – degeneration of proximal convoluted tubule, DDCT – degeneration of distal convoluted tubule.

(DPCT) and degeneration of distal convoluted tubule (DDCT) **(Fig: 3.4 & 3.9).** Kidney showed maximum and most severe pathological alterations after the completion of 21 days of sub-lethal exposure such as epithelial rupture (ER), degenerative changes (DC), infiltration of inflammatory cells (IIC), vacuolation (V), occlusion of tubular lumen (OTL), degeneration of proximal convoluted tubule (DPCT) and degeneration of distal convoluted tubule (DDCT) **(Fig: 3.5 & 3.10).**

3.4. Muscle

The structure of the muscle of the control fish consisted of compactly packed muscle bundle (MB), with definite intermuscular spaces (IMS). Each muscle bundle consisted of compactly arranged muscle fibres (CPMF). The intermuscular spaces appeared to be filled with viscous fluid. Round to spindle shaped nuclei (SSN) were found distributing all over the bundle length with occasional hyper chromacia (**Fig: 4.1 and 4.6).**

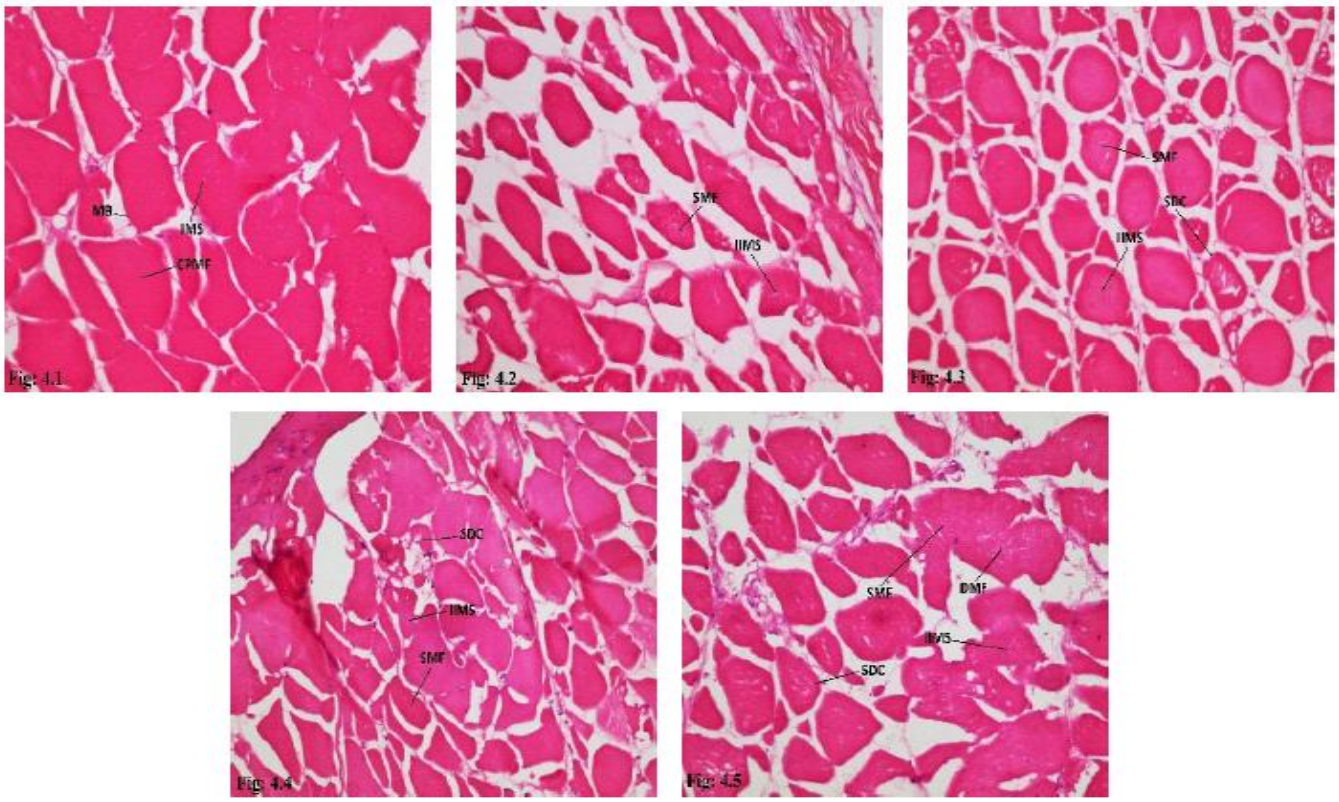

Fig. 4.1 contol muscle of *C. carpio* at 40x, H&E
Fig. 4.2 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 40x, H&E
Fig. 4.3 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 40x, H&E
Fig. 4.4 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 40x, H&E
Fig. 4.5 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 40x, H&E

The muscle is the organ to show least histological alterations after the sub-lethal exposure. After the completion of 24hrs the muscle showed very slight changes such as initiation of splitting of muscle fibres (SMF) and increased intermuscular space (IIMS) **(Fig: 4.2 & 4.7).** After the completion of 7 days of sub-lethal exposure the changes seen in muscle were splitting of muscle fibres (SMF), increased inter muscular space (IIMS) and structural degenerative changes (SDC) **(Fig: 4.3 & 4.8).** As the days of exposure increased the changes in muscle tissue also increased but very slowly unlike gill, liver and kidney. After the completion of 14 days there was seen splitting of muscle fibres (SMF), increase in the structural degenerative changes (SDC)

and increased inter muscular spaces (IIMS) **(Fig: 4.4 & 4.9).** The muscle showed maximum and high intensity of changes after the completion of 21 days of sub-lethal exposure, the changes seen were splitting of muscle fibres (SMF), increased intermuscular spaces (IIMS), structural degenerative changes (SDC) and degeneration of muscle fibres (DMF) **(Fig: 4.5 & 4.10).**

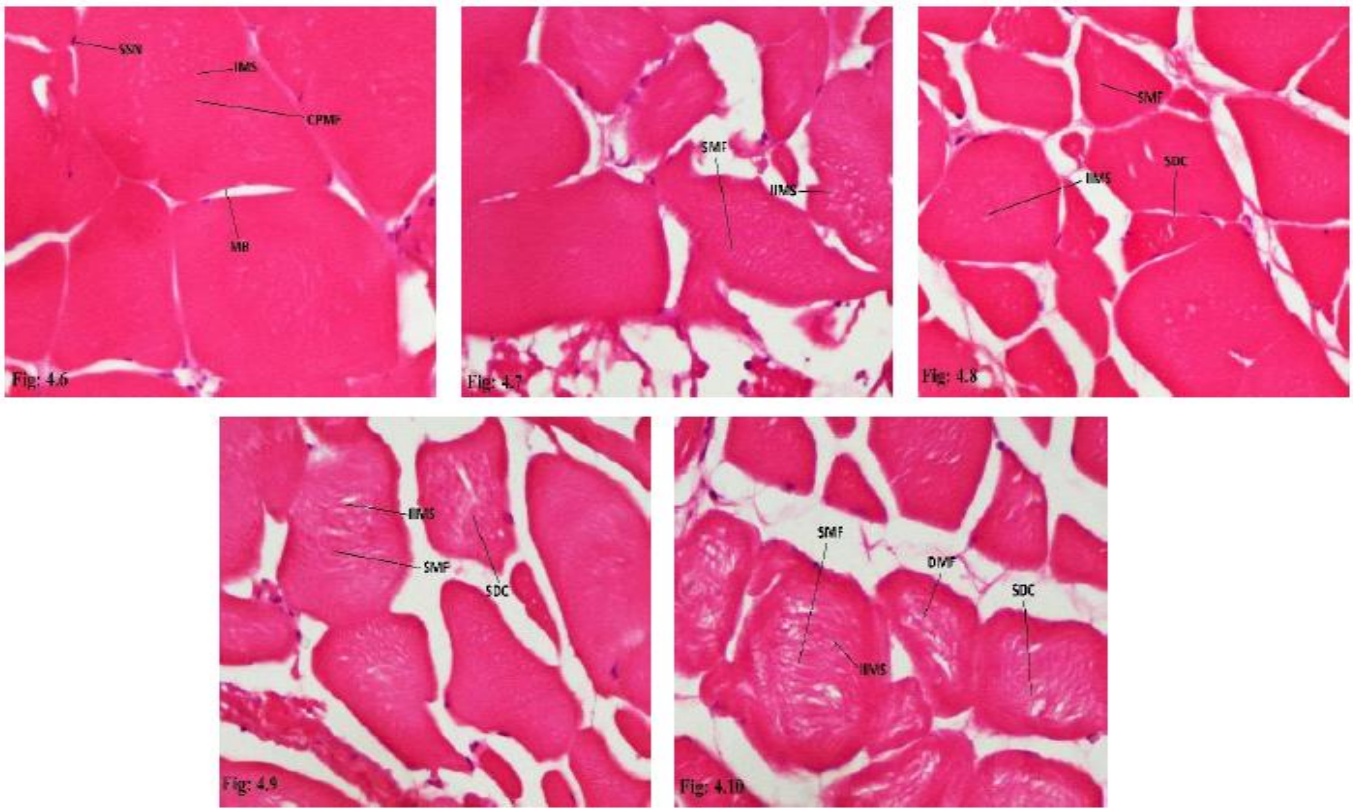

Fig. 4.6 contol muscle of *C. carpio* at 100x, H&E
Fig. 4.7 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 24hrs, 100x, H&E
Fig. 4.8 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 7 days, 100x, H&E
Fig. 4.9 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 14 days, 100x, H&E
Fig. 4.10 muscle of *C. carpio* exposed to sub-lethal concentration of Karanjin at 21 days, 100x, H&E
MB – muscle bundle, CPMF – compactly packed muscle fibres, IMS – inter muscular space, SSN – spindle shaped nucleus, SMF – splitting of muscle fibres, IIMS – increased inter muscular space, SDC – structural degenerative changes, DMF – degeneration of muscle fibres.

4. DISCUSSION

Studies relating to histopathological investigations in various organs of fish are a valuable tool for toxicological studies and also environmental monitoring. Histopathological observations provide us with useful information regarding the health of fish and the condition and functionality of organs. Injuries of various kinds to the organs and tissues of fishes due to pesticide exposure can result in reduced survival, growth and fitness, low reproductive success or increased susceptibility to infections. The intensity of leisions and frequency of leisions in tissues depend on two important factors, i.e., concentration of pesticide and exposure period.

Gills are the most important organs of fishes as they are concerned with important functions such as respiration, osmoregulation and excretion. Gills are in continuous contact with the external environment and are very sensitive to any kind of changes in the water quality, hence they are considered the primary target of any kind of contaminants [13-14]. In the present study, the most common histological changes seen in the gills were curling of secondary gill lamellae, epithelial lifting, shortening of secondary gill lamellae, swollen tips of secondary gill lamellae, vacuolation, infiltration of inflammatory cells, partial fusion of secondary gill lamellae, decreased inter lamellar space and thinning of secondary gill lamellae. Similar findings were also reported by various investigators in

different fish species on exposure to variety of pollutants. Histopathological alterations in gills such as the lifting of epithelial layer from gill lamellae, shorting of secondary lamellae, and club shaped lamellae in the gills of *Lepistes reticulatus* were observed after exposure to cyphenothrin [15]. Secondary lamellae showing fusion and lifting of epithelium have also been observed in *Gambusia affinis* [16]. The presence of sub epithelial edema and the disarrangement of the secondary lamellae was reported for *C. carpio* [17] and for *O. niloticus* exposed to GFT [18]. The appearance of irregular gill lamellae, increased vacuolation in epithelial cell, lamellar fusion and complete destruction of gill lamellae in *Poecilia reticulate* exposed to chlorpyrifos [19]. Changes such as epithelial ruptures, secondary gill lamellae fusion and hyperplasia of branchial epithelium in *Carassius auratus* after acute exposure to Malathion [20]. Epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, oedema, shortening of secondary lamellae and lamellar fusion in *Cirrhinus mrigala* exposed to dichlorvos [21]. Epithelial lifting, lamellar fusion, lamellar disorganization, rupture of the lamellar epithelium, rupture of pillar cells and necrosis in the gill of common carp, *Cyprinus carpio*, on exposure to sub-lethal concentrations of chlorpyrifos pesticide for a period of 14 days [22].

Liver is the organ associated with the detoxification and biotransformation process. Due to its function, position and blood supply, it is the organ to be affected most by any kind of contaminants in the water [13]. The present study showed alterations in liver such as narrowing of sinusoids, hydropic degeneration, nuclei having an outlying position, vacuolation, cytoplasmic degeneration, cloudy swelling of hepatocytes, disintegrating walls of hepatocytes and necrosis. These results are also in agreement with those observed by many authors who have studied the effects of different pollutants on fish liver [23-25]. Histological alterations in liver such as hepatic lesions, including degeneration, hypertrophy, sinusoids enlargement, haemorrhage, pycnosis position of nuclei, vacuolization of cell cytoplasm and infiltration of mononuclear lymphocyte in fish exposed to pollutants [26]. In another study, cloudy swelling, focal necrosis and vacuolization have been reported in the *C. paleatus* exposed to methyl parathion [25]. Hypertrophy of hepatocytes, increasing kuffer cells, circulatory disturbance, narrowing of sinusoids and focal necrosis in the liver of *G. affinis* exposed to deltamethrin [27]. Histopathological lesions such as, nuclear pycnosis, narrowing of sinusoids, congestion, hypertrophy of hepatocytes, vacuolar degeneration, necrosis and fatty degeneration were observed in the tissue of nile tilapia induced to cypermethrin [7]. In other study, hyperplasia, disintegration of hepatic mass, focal coagulative necrosis were seen in *Labeo rohita* exposed to cypermethrin [28]. The cellular nucleus displacement to the periphery and the hepatocytes hypertrophy was also observed for *O. niloticus* exposed to GFT [18]. The liver of *P. mesopotamicus* showed hepatocytes vacuolization and cells hypertrophy when exposed to 3.0 or 4.0 mg L^{-1} glyphosate as Roundup Ready® [29]. Cloudy swelling, focal necrosis, atropy and vacuolization was observed in *Corydoras paleatus* exposed to methyl parathion [25]. Histological alterations such as nuclear and cellular hypertrophy, cellular atrophy, irregular contour of cells and nucleus, cytoplasmic vacuolation, cytoplasmic and nuclear degeneration, cellular rupture, pyknotic nucleus, necrosis and melanomacrophages aggregations were observed in the liver of common carp, *Cyprinus carpio*, on exposure to sub-lethal concentrations of chlorpyrifos for a period of 14 days [22].

The kidney is a vital organ of body and proper kidney function is to maintain the homeostasis. It is not only responsible for selective reabsorption, which helps in maintaining volume and pH of blood and body fluids and erythropoiesis [30]. The kidney is one of the first organs to be affected by contaminants in the water [31]. The present study showed histological changes in kidney such as infiltration of inflammatory cells, vacuolation, epithelial rupture, shrunken nucleus, degenerative changes, occlusion of tubular lumen, glomerular shrinkage, degeneration of proximal convoluted tubule and degeneration of distal convoluted tubule. These results are also in agreement with those observed in *C. carpio* exposed to sewage [32], *P. lineatus* exposed trichlorfon [33] and *L. calcarifer* exposed to cadmium [31]. The disarrangement of the proximal tubules epithelium and the distal tubule epithelium was also observed on *O. mykiss* exposed to linuron herbicide [34] and on *O. niloticus* exposed to Roundup® [18]. The Bowman's capsule space increase and the disarrangement of the tubules cells was also observed on *C. gariepinus* exposed to GFT, which leads to the kidneys physiological functions loss [35]. Dilation of tubules, necrotic changes characterized by karyolysis of the affected cells were observed in the fish *Labeo rohita*

exposed to hexachlorocyclohexane [36]. Necrosis, cloudy swelling in the renal tubules, cellular hypertrophy, granular cytoplasm, vacuolization were observed in kidney tissues of *Ctenopharyngodon idellus* exposed to fenvalerate [37]. Degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen were observed in the kidney tissues of fish *Cyprinus carpio* exposed to deltamethrin [38]. Shrinkage of glomerulus, changes in tubular lumen, vacuolization, desquamation and hydropic swelling were observed in *Cyprinus carpio* after sub lethal exposure to dimethoate [39].

The muscle of the fish *Cyprinus carpio* on exposure to sub-lethal concentration of Derisom showed different pathological changes such as splitting of muscle fibres, increased inter muscular space, structural degenerative changes and degeneration of muscle fibres. There were very few changes observed in muscle as compared to the other three organs i.e., gill, liver and kidney, this proves that muscle is the last organ to be affected by any kind of toxicant. These changes ultimately affect the contractile ability of the muscle and results in disfunctioning of muscle fibres. The histopathological alterations observed in muscle in the present study are agreement with those observed by many investigators who have studied the effects of different pollutants on fish muscles [40-41, 36].

5. CONCLUSION

The present study showed that the karanjin based biopesticide–Derisom showed noticeable histological changes in *Cyprinus carpio*. It can be concluded that alterations in gill, liver, kidney and muscle may serve as a sensitive biomarker for sub-lethal toxicity of Derisom. The results indicates that the contamination of an aquatic ecosystem by Derisom may affect the aquatic life especially fish. Hence, a close monitoring of its usage in agricultural fields, rice – fish integrated farming practice and in aquatic ecosystems is essential.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. Begum G. *Aquatic Toxicology,* 2004; **66:**83-92.
2. Palaniappan PLRM, Sabhanayakam S, Krishnakumar N, Vadivelu M. *Catla catla, Food and Chemical Toxicology,* 2008; **46:**2440-2444.
3. Rao JV. *Chemosphere,* 2006a; **65(10):**1814-1820.
4. Velmurugan B, Selvanayagam M, Cengiz EI, Unlu E. *Environmental Toxicology and Pharmacology,* 2007; **24(3):**286-291.
5. Oliveira Ribeiro CA, Filipack F, Mela M, Silva PH, Randi MAF, Costa JRA, Pelletier E. *Environmental Research,* 2006; **101:**74-80.
6. Mathur S, Gupta AK. *J. Environ. Biol,* 2008; **29:**201-204.
7. Korkmaz N, Cengiz EI, Unlu E, Uysal E, Yanar M. *Environmantal Toxicology and Pharmacology,* 2009; **28:**198-205.
8. Greenfield BK, Teh SJ, Ross JRM, Hunt J, Zhang GH, Davis JA, et al. *Archives of Environmental Contamination and Toxicology,* 2008; **55(2):**270-281.
9. Olarinmoye O, Taiwo V, Clarke E, Kumolu-Johnson C, Aderinola O, Adekunbi F. *Applied Ecology and Environmental Research,* 2009; **7(3):**277-286.
10. Van Dyk JC, Marchand MJ, Smit NJ, Pieterse GM. *African Journal of Aquatic Sciences,* 2009; **34:**273-282.
11. Grund S, Keiter S, Bottcher M, Seitz N, Wurm K, Manz W, Hollert HTB. *Diseases of Aquatic Organisms,* 2010; **88:**235-248.
12. Rajeshkumar S, Munuswamy N. *Chemosphere,* 2011; **83(4):**415-421.
13. Camargo MM, Martinez CB. *Neotrop. Ichthyol,* 2007; **5:**327-336.
14. Fernandes MN, Mazon AF. Environmental pollution and fish gill morphology, *In: Val, A.L and B.G.Kapoor (Eds.). Fish adaptation. Enfield, Science Publishers,* 2003; 203-231.
15. Erkmen B, Caliskan M, Yerli SV. *Veterinary and Human Toxicology,* 2000; **42(1):**5-7.
16. Cengiz EI, Unlu E. *Journal of Environmental Science and Health B,* 2003; **38(5):**581-589.
17. Neskovic NK, Poleksic V, Elezovic I, Karan V, Budimir M. *Contam. Toxicol,* 1996; **56:**295-302.
18. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P. *Sci. Asia,* 2002; **28:**121-127.

19. De Silva PMCS, Samayawardhena LA. *Ecotoxicology and Environmental Safety,* 2002; **53:**248-254.
20. Cristina RT, Cosoroaba J, Trif A, Parvu D, Hadaruga N, Dumitrescu E, et al. *International Conference of cellular and tissue comparative pathology. July 3-5, 2008, Cluj Napoca. Bull USAMV-CN,* 2008; **65(1-2):**358-363.
21. Velmurugan B, Selvanayagam M, Cengiz EI, Unlu E. *Braz. Arch. Biol.Technol,* 2009; **52:**1291-1296.
22. Sandipan Pal, Emiko Kokushi Jiro Koyama, Seiichi Uno, Apurba R Ghosh. *Journal of Environmental Science and Health,Part B,* 2012; **47:**180-195.
23. Mohamed FA. *J. Egypt. Acad. Soc. Environ. Develop,* 2001; **2:**25-42.
24. Ptashynski M, Pedlar R, Evan R, Baron C, Klaver Kamp J. *Aquat. Toxicol,* 2002; **58:**229-247.
25. Fanta E, Rios FSA, Romao S, Vianna ACC, Freiberger S. *Ecotoxicology and Environmental Safety,* 2003; **54:**119-130.
26. Cengiz EI, Unlu E, Balci K. *Journal of Environmental Science and Health B, 2001;* **36(1):**75-85.
27. Cengiz EI, Unlu E. *Environ. Toxicol. Pharmacol,* 2006; **22:**200-204.
28. Sarkar B, Chatterjee A, Adhikari S, Ayyappan S. *Journal of Applied Ichthyology.,* 2005; **21(2):**131-135.
29. Shiogiri NS, Paulino MG, Carraschi SP, Baraldi FG, Cruz C, Fernandes MN. *Environ. Toxicol. Pharmacol,* 2012; **34:**388-396.
30. Iqbal F, Qureshi IZ, Ali M. *J. Res. Sci,* 2004; **15:**411-418.
31. Thophon S, Kruatranchuc M, Upathau E, Pokcthitiyook P, Sahaphong S, Jarikhuan S. *Environ. Pollut,* 2003; **121:**307-320.
32. Kakuta I, Murachi S. *Environ. Toxicol. Water Quality,* 1997; **12:**1-9.
33. Veiga M, Rodrigues E, Pacheco F, Ranzani-Paiva M. *Brazilian Arch. Biol. Technol,* 2002; **45:**171-175.
34. Oulmi Y, Negele RD, Braunbeck T. *Dis. Aquat. Org,* 1995; **21:**35-52.
35. Ayoola SO. *Am. Euras. J. Agric. Environ. Sci,* 2008; **4(3):** 362-367.
36. Das BK, Mukherjee SC. *Veterinarski Arhiv,* 2000; **70:**169-180.
37. Tilak KS, Veeraiah K, Yacobu K. *Pollut. Res,* 2001; **20:**387-393.
38. Cengiz EI. *Environmental Toxicology and Pharmacology,* 2006; **21:**246-253.
39. Ram Nayan Singh. *Ind J Sci. Res,* 2012; **3(1):**127-131.
40. Sakr S, Gabr S. *Proceed. Zool. Soc.A.R.E.,* 1991; **21:**1-14.
41. Abo Nour A, Amer A. *J. Egypt. Ger. Soc. Zool,* 1995; **18:**153-175.