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# Histopathological Changes in Spleen and Kidney of Silver carp (*Hypophthalmicthys molitrix*) after Acute Exposure to Deltamethrin

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## ABSTRACT

The pyrethroid group of pesticides, which include deltamethrin, is preferably used for pest control programs as a replacement for organochlorines and organophosphates group of pesticides, as it has comparatively low toxicity and has low persistence in the environment. Deltamethrin is highly toxic to aquatic organisms including fishes as compared to terrestrial animals. The present study was carried out to explore the lethal effects of deltamethrin on spleen and kidney of silver carp. Numerous histological changes were observed in kidney and spleen of fish after acute exposure to pesticide. Doses were 25%, 50% and 75% of LC50. Histopathological changes like damage in tissues of spleen and kidney became severe with increasing dose concentration. Most pronounced histopathological changes in kidney included degeneration of renal tubules, dilatation of glomerular capillaries, degeneration of glomeruli, narrowing of the tubular lumen, and atrophy and lyses of glomerular tufts. Spleen of treated fish showed necrosis and significant changes in number of melano-macrophage centres as compared to control.

**Key Words:** *Deltamethrin, Silver carp, Degenerative changes, Kidney, Spleen*

## INTRODUCTION

Pesticides are substances or mixture of substances used to kill and destroy different pests. Pesticides contribute significantly to human welfare and at the same time they also have significant undesirable effects on non-target organisms (John., 2007; Hazarik & Das., 1998). There is a direct way of entry of Pesticides into the water when applied incorrectly, or by running off from surrounding treated products in an indirect way (Svobodova *et al.,* 2003). Pesticides which have tendency to persist in the environment for a long time, produce a risk to fish and its consumers. The increase and persistence of insecticides in the aquatic environment add up to threat biological life, as acute and chronic poisoning in many aquatic organisms including fishes have been observed (Soderlund *et al.,* 2002).

The pyrethroid group of pesticides, including deltamethrin, is considered as a preferable replacement for organochlorines and organophosphates as former group has low toxicity and less persistence in the environment (Kamal & Khalid, 2012). It is a natural compound pyrethrum characterized as non persistent sodium channel modulator, and is much less acutely toxic than organophosphate and carbamate usually applied against household pests (Anderson, 1989; Class *et al.,* 2012). The pyrethroids are extensively used as pesticides because they degrade shortly after their application, and lack the accumulation tendency in organisms (Laskowski., 2002). The major advantages of pyrethroids over organophosphrus pesticides are that these are very effective even in low concentration and stable in light and above all they disintegrate very easily. These pesticides have very low toxicity for birds and mammals (Koprucu & Aydin., 2004). Water is polluted by pesticides mainly due to rigorous agriculture along with surface run off and surface seepage after its application (Banaee *et al.,* 2011). Contamination of surface waters in agriculture by pesticides is a global issue (Hill, 1985; Sibley & Kaushik, 1991). Pesticides pose a threat to human life and aquatic animals when their degraded parts are found in drinking water. Keeping these facts in view pesticides are well documented globally and form major issues at local, regional, national and global levels (Cerejeira *et al.,* 2003; Spalding *et al.,* 2003). Being aquatic vertebrates fishes are mainly susceptible to environmental

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contamination of water by pesticides. When fishes are exposed to pollutants such as insecticides, their physiological and chemical processes are greatly disturbed (Banaee *et al.,* 2011). Furthermore, the histopathological changes and alteration in biochemical parameters of fish blood are clear indications of toxic effects of pesticides (Borges *et al.,* 2007; Sudova *et al.,* 2009). Acute exposure of pesticides to fish is a valid way to estimate the concentrations that cause direct, irreversible damage to experimental animals (Parrish, 1995).

The current study was performed to evaluate the sub-lethal effects of deltamethrin on histopathological alterations in kidney and spleen of silver carp (*Hypophthalmicthys molitrix*).

## MATERIALS AND METHODS

### Experimental animal

Live specimens of the silver carp (*H. molitrix)* were purchased from Himalayan Fish Hatchery Muredke, Sheikhupura. Average weight of fish was 159.33±5.38 g. Average length of fish was 9.95±0.138 cm. Fish were kept in Zoology Laboratory of Government College of Science, Wahdat Road, Lahore. Fish were acclimatized for two weeks in laboratory conditions with continuous aeration. Dead fish were removed immediately during this period.

### Toxicant and its doses

Pyrethroid pesticide deltamethrin was used as toxicant. Three doses of sub-lethal concentrations of deltamethrin were prepared according to LC 50 value. The doses of Group B, C and D were 0.4µg/l, 0.8µg/l, and 1.2µg/l**.** These doses were 25%, 50% and 75% of LC **50** value of the pesticide. Histopathological effects of pesticides were observed on spleen and kidney tissues.

### Experimental set up

Fish were divided into 4 groups. Group A was considered as control group while groups B, C & D were considered as experimental groups. Fishes in experimental groups were exposed to pesticide doses 0.4µg/l, 0.8µg/l, and 1.2µg/l respectively. Total number of fish used during experiment was 48 with 12 fishes in each group.

Fish were sacrificed after**,** 48 and 96 hours. Kidney and spleen of scarificed fish were removed and washed with saline. After this organs were preserved in 10% formalin.

### Histopathology

Small pieces from spleen and kidneys were fixed in formalin. Tissues were then dehydrated in different concentrations of alcohol and cleared in xylene. Infiltration was carried out by incubating the tissues in paraffin wax (100%) at 60°C overnight. After this, tissues were embedded in paraffin and blocks were formed which were mounted on microtome and tissues were sectioned at 5µm. Sections were stained with hematoxylin and eosin (H&E). Prepared slides were examined under trinocular microscope “Labomed”.

## RESULTS

Normal shape and structure of kidney tubules, Bowman’s capsules, glumeruli and blood vessels were observed in control fish. After 48 hours of pesticide exposure the histopathological alterations in the kidney of fish such as degeneration of renal tubule, shrinkage of glumeruli were observed (Fig., 1). Degeneration of blood vessels was also seen along with necrosis. Enlargement of renal tubule was also found. Spaces between glumerulus and Bowman capsule were seen which developed due to shrinkage of glomeruli (Fig., 2). Some epithelial cells became pyknotic due to breakdown of epithelial layer of tubules. After 96 hours of pesticide exposure more intensive degenerative changes were observed. Lumen of tubules increased noticeably (Fig., 3). More degenerative changes in epithelium of tubules were seen. Pyknotic nuclei were also visible in the epithelium of renal tubules. Nucleic degeneration and necrotic areas in tubules were visible. Atrophy in the glomeruli and dilation in renal blood vessels were also observed in 96 hours pesticide exposure (Fig., 3 & 4).

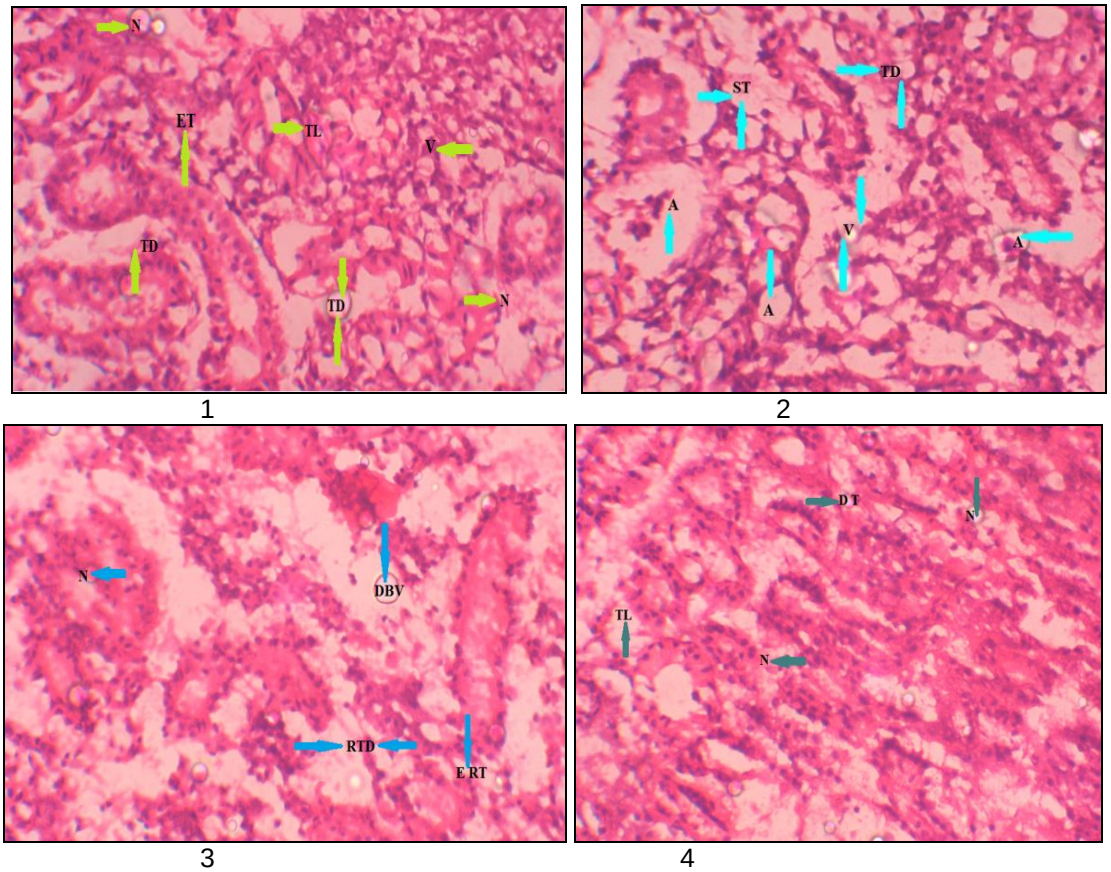


**Fig., 1-4:** Degenerative changes in kidney after48 and 72 hours showing Atrophy (A), Bowman's capsule(BC), blood vessels(BV), capillaries in glomerulus (C), Damaged blood vessel(DBV),Damaged tubule (DT), enlargement of tubule(ET), Enlargement of renal tubule (ERT), glomerulus (G), hematopoietic tissue(HPT), kidney tubules(KT), necrosis(N) , Renal tubular damage(RTD), Severe damaged tubule(ST), tubular degeneration (TD), tubular lumen(TL), vacuolization(V), widened tubular lumen(WTL)

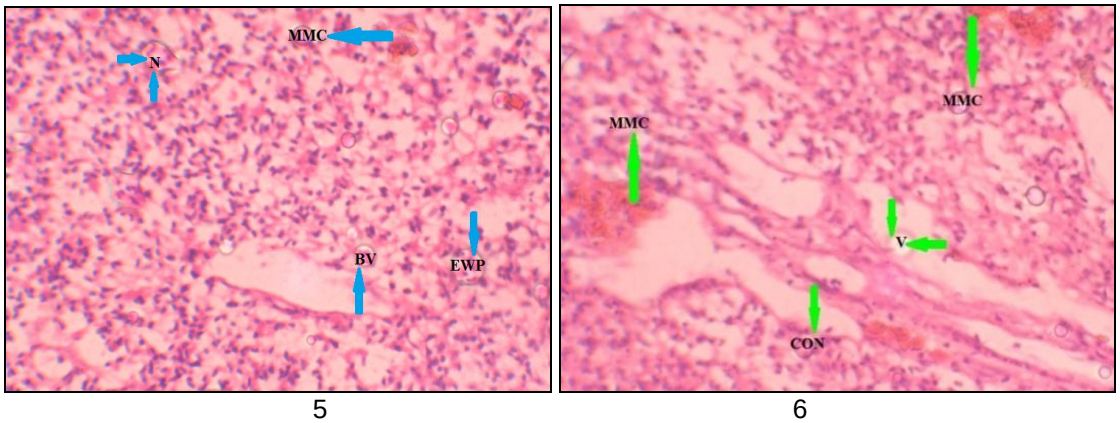


**Fig., 5 & 6:** Degenerative changes in spleen after 48 and 72 hours showing Melano-macrophage centers(MMC), Congestion (CON), Degeneration(D) vacuolization, expansion of blood vessel (EBV), Expansion of white pulp (EWP), Blood vessel (BV) , Necrosis (N), Red pulp (RP), White pulp(WP).

Spleen of control fish was clearly differentiated into two regions, white pulp and red pulp. White pulp was made up of lymphoid tissues and red pulp was composed of cells with haemopoietic activities.

Exposure to pesticide produced significant changes in melanomacrophage centres (MMCs). Deposition of hemosidrin in melanomacrophage centres was noted after pesticide exposure. After 48 hours of pesticide exposure MMCs were smaller in size and irregular in shape and appeared as aggregate clusters near the blood vessels (Fig., 4). After 96 hours of exposure the degree of congestion and number of MMCs increased in the spleen (Fig., 5). The spleen of deltamethrin exposed fish showed hyper activation of melanomacrophage centres (Fig., 5 & 6).

## DISCUSSION

Water pollution concentration assessment and its effects on aquatic fauna like fish can be measured by histopathological studies of different tissues of exposed fish. When fish is exposed to different concentrations of insecticides, nature of toxicant is indicated by intensity of histo-pathological changes in different tissues of organism. Histopathology provides information about the physical condition and functionality of organs (Banaee 2013). During present investigations acute deltamethrin exposure produced important alterations in the histology of kidney and spleen of silver carp. Fish were exposed to sub-lethal doses at the rate of 0.4µg/l, 0.8µg/l, and 1.2µg/l and sampling was done after 48 and 96 hours.

Morphology of the nephron of bony fishes includes glomerulous, tubules and collecting duct (Tripathi *et al*., 2011). Degeneration and necrosis in the renal tubular epithelium & glomerular cells were recorded in present experimental work, just as observed by Vinodini & Narayanan, (2009); Satyanarayan *et al.* (2012), in Atlantic salmon exposed to Endosulfan and Diazinon at doses 0.2 mg/l and 0.1 mg/l respectively. The changes in kidney tissues reported here after a pyrethroid pesticide deltamethrin exposure were also in accordance with the damages found in kidneys of *Cirrhinus mrigala* which was exposed to another pyrethroid pesticide fenvalerate (Velmurugan *et al.,* 2007). The changes observed in this case were necrosis of tubular epithelium, hypertrophied epithelial cells of renal tubules, narrowing of the tubular lumen, expansion of space inside the Bowman's capsules and shrinkage of the glomeruli in the kidney of fish. (Figs., 1-4).

Our findings also match with the findings of Banaee *et al*. (2013), who found disorientation in the glomerular structure, dilation in the inter space of urinary tubules and cloudy swelling in rainbow trout exposed to 0.1 mg/L diazinon.

The thymus, kidney (anterior and middle) and spleen are the largest lymphoid organs in teleosts (Zapata *et al*., 2006). The spleen is composed of a system of splenic ellipsoids, Melano-Macrophage Centres (MMCs) and lymphoid tissues. In most species, ellipsoids are clustered together and are organized around the other two components (Ferguson, 1989). During our experiment an increase in the size of spleen of experimental fish after 96 hours was observed, this is a typical macroscopic change and was in accordance with the change in fish exposed to diazinon toxicity along with the roughness of the spleen (Monteiro *et al*., 2006; Boran *et al*., 2012). Wolke *et al.* (1985) first suggested MMC as potential monitors of fish health. Our experimental fish Silver carp showed hemosiderosis and vascular congestion after pesticide exposure, this was in accordance with the findings of Montero *et al.* (1999). He observed that stressful situations related to aquaculture practices result in increased numbers of splenic and kidney MMCs, growth of red pulp with clogging of blood vessels and a considerable deposition of hemosiderin in MMCs. These changes were observed in fish exposed to different doses of diazinon. In another study when the Common goby was exposed to RT / 3, 4 dichloraniline, the important histopathological changes were Hyperactivation of Melano-Macrophage Centers (MMC), excessive hemosiderosis and congestion (Boran *et al.,* 2012) , similar changes were also recorded in our experimental work (Fig., 5 & 6).

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