Indian J. Applied & Pure Bio. Vol. 40(1), 112-120 (2025). **ISSN: 0970-2091**
A Web of Science Journal

Evaluation of histopathological changes in the kidney of common carp arising from exposure to Deltamethrin

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Abstract

These days, water contamination is a global problem. Hazardous chemical contamination from agricultural runoff, household and industrial wastewater is the primary cause of water pollution. Pesticides are one of the main compounds found in agricultural runoff, and they are crucial in boosting agricultural output by managing pests. However, these pesticides cause serious harm to non-target creatures in both terrestrial and aquatic ecosystems. It is therefore essential to thoroughly research the histology of fish and other aquatic species. Histological examination seems to be an extremely sensitive measure and is essential for identifying potential cellular alterations in vital organs. The current research investigated the toxic effects of deltamethrin at $1/20^{th}$ and $1/10^{th}$ of 96hrs LC_{50} value for a period of 28 days on kidney of common carp (*Cyprinus carpio*). Various alterations were observed after the exposure of pesticide in treated fish in comparison to control. Histological changes observed after the exposure of deltamethrin are damaged glomerulus, degenerated renal tubules, melanomacrophage centers, degeneration of epithelial cells, narrow lumen of tubules, pyknotic nuclei, dilation of Bowman's space, infiltration of blood cells and necrosis in the epithelial lining of various structures.

Key words : pesticides, synthetic pyrethroids, deltamethrin, common carp, kidney, histology.

A family of synthetic compounds known as pesticides was created to protect crops and control diseases and pests that may endanger them. Pesticides belonging to several categories, such as herbicides, insecticides, bactericides and fungicides, are widely utilized globally[1]. In addition, there are other groups of pesticides, according to the species they target or the makeup of their compounds. Pesticide residues usually enter aquatic systems through spray drift, direct application, or agricultural and urban runoff. Consequently,

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pesticide-related contamination is a serious environmental issue that requires attention and resolution[7]. It is thought that aquatic systems serve as the ultimate destination for all of these pollutants. Aquatic organisms, especially fish have become the silent victims of these contaminations. The toxicity of pesticides and their effects on aquatic non-target species, such as fish, are taken into consideration while evaluating the eco-toxicological dangers[17]. For example, understanding the detrimental effects of pesticide pollution on fish is essential since pesticides directly cause harm to important organs of fish and they also directly connect to the food chain and can contaminate water bodies[12].

The use of synthetic pyrethroids is chosen over other pesticide classes due to their high efficacy, minimal toxicity to mammals, high pest specificity, and quick environmental breakdown. Moreover, synthetic pyrethroids have controlled the global pesticide market significantly since the development of third-generation insecticides[4]. One of the most widely used pesticides in the pyrethroid family is deltamethrin (DM)[8]. It is a non-systemic pesticide that controls a wide range of sucking and chewing insects by contact and ingestion mechanism. Unfortunately, seepage through the soil, aerial transmission, and surface runoff from agricultural areas are how the majority of synthetic pyrethroids, including DM, end up in aquatic environments. Fish fauna and other non-target aquatic creatures are found to be seriously threatened by this. Numerous studies have examined the harmful effects of DM on various fish species[6,8,22].

The selection of bio-indicator species is the first step towards effective monitoring and risk-assessment programmes. Common carp is one of the most widely grown freshwater fish species globally because of its widespread distribution, high commercial value and growth qualities[30]. Because it is highly resistant to water contamination, including pesticides, it is also a great test organism for eco-toxicological investigations[9]. These properties are very important in selection of bio-indicators for both laboratory and field studies. Some recent studies have proven that common carp is an excellent mode organism to evaluate the toxic effects of different pesticides[21,25,29].

Certain lesions that develop in fish organs exposed to harmful pesticides in a laboratory conditions serve as useful indicators of toxicity. Because of this, histopathological analysis is acknowledged as a useful method for determining how environmental contaminants affect fish. The kidney of fish is among the first organs to be impacted by pollutants. In terms of the hydroelectrolytic balance (water and salt) and the excretion and metabolism of xenobiotics, this organ is crucial to maintaining a stable internal environment. Unlike mammals, where the primary function of the kidney is the elimination of nitrogenous waste, primary role of fish kidneys is the osmotic regulation of water and salts. Since the kidney absorbs the majority of post-branchial blood and is a primary pathway for the excretion of xenobiotic compounds, it is more prone to have histo-pathological changes in response to pesticide stress. Various researchers in recent years have studied the histopathological changes in kidney of various fish species after the exposure of different pesticides[15,25]. The current research was carried out in order to evaluate the histological damage to the kidney in common

carp (*C. carpio*), following sub-lethal exposure to deltamethrin.

Experimental animal :

Fish, common carp (*C. carpio*) was used as experimental animal which was obtained from Deoli fish farm, Ghagus, Bilaspur, H.P. and then transferred from fish farm to laboratory of Department of Biosciences, Himachal Pradesh University, Summerhill, Shimla. To avoid dermal contamination, fish was washed with a 0.1% solution of Potassium Permanganate ($KMnO_4$). Before beginning the experiment, the fish were kept for 15 days in glass aquaria so they could become acclimatized to the lab environment. Every day, about 40% of the aquarium's water is replaced in order to expel out waste and preserve water quality during the acclimatization period. Water was continually oxygenated by an aerator. During the acclimatization period, they were given commercial pellets at a rate of 3% body weight twice a day.

Chemical used :

Commercial grade Deltamethrin in 11% EC formulation was used for the current experiment which was purchased from local market, Shimla.

Experimental Design :

The physico-chemical characteristics of the water were noted before the start of the experiment. All the water parameters (pH = 7.23 ± 0.51, dissolved oxygen = 7.86±0.26 mg/L, water temperature = 25±2 °C) were within the recommended range. Fish were maintained in photoperiod of 10±2 (light): 14±2 (dark). 96hrs LC_{50} of test chemical, DM (11% EC) value was determined which was found to be 0.114 µl/L. Sub-lethal concentrations that were selected for the toxicological study are1/ 20^{th} (0.005 µl/L/ T1) and $1/10^{th}$ (0.011 µl/L/T2) of the 96 hrs LC_{50} concentration of DM. Experiment was conducted for a period of 28 days with 7 days sampling frequency. No mortality was observed during the experimental (sub-lethal exposure) period.

Fish was divided into three groups

1. Group I was designated as control
2. Group 2 received 0.005 µl/L concentration of DM for a period of 28 days
3. Group 3 received 0.011 µl/L concentration of DM for a period of 28 days

Histological analysis :

Kidney tissue of fish was excised immediately after sacrificing the fishes. Tissue was fixed in Bouin's fixative for 24 hours. After that tissue was washed in running tap water until the entire yellow color disappeared. Tissue was dehydrated serially in different grades of alcohol (30%, 50%, 70%, 90%, 100%) and cleared in xylene. Tissues were then embedded in paraffin wax (58-60°C). Sections of about 5-6µm thickness were cut on the rotary microtome and subjected to hematoxylin-eosin staining.

Hematoxylin-Eosin staining :

Ribbons of tissue (kidney) sections were cut and stretched on albuminized slides in warm water. These were subjected to de-waxing in xylene at 37°C overnight, followed by hydration and passing through descending grades of alcohol 100% to 30% (30 minutes each) and then finally transferred to distilled

water. Slides were then dehydrated in ascending grades of alcohol (30-90%) for 30 minutes each. Counterstaining was done in 1% alcoholic eosin for 2 minutes. Excessive stain was removed by dipping in 90% alcohol. Tissue sections were dehydrated completely in absolute alcohol and then subjected to xylene for clearance. The dehydrated and cleared sections were mounted directly in DPX. The permanent slides were dried, tissue sections examined and thereafter photographed.

Semi-quantitative analysis :

Semi-quantitative histological changes were noted, in accordance with **Mishra and Mohanty**[16]. From a total of fifty slides for organ of study (kidney), the mean of 10 randomly chosen slides was used to examine the histological alterations in the tissues. There were three categories based on the mean prevalence of each histopathological parameter: mild abnormalities (+, 10% of sections), moderate abnormalities (++, 10% to 50% of sections), and severe abnormalities (+++, > 50% of sections).

Damaged glomerulus, degenerated renal tubules, melanomacrophage centers, degeneration of epithelial cells, narrow lumen of tubules, dilation of Bowman's space, dilation of tubules and infiltration of blood cells are histopathological changes that were recorded for semi-quantitative analysis (Table-1).

Table-1. Semi-quantitative analysis of several histopathological changes after the treatment of sub-lethal concentrations of deltamethrin at 7th, 14th, 21st and 28th day of experimental period.

Histopathological alterations	Treated Groups	Exposure time (days)			
		7	14	21	28
Damaged glomerulus (DG)	T1	+	+	++	++
	T2	+	+	++	+++
Degenerated renal tubules (DRT)	T1	+	++	+++	+++
	T2	+	++	+++	+++
Melanomacrophage centers (MMC)	T1	+	+	++	++
	T2	+	++	++	+++
Degeneration of Epithelial cells (DEpC)	T1	+	++	++	+++
	T2	+	++	++	+++
Narrow lumen of tubules (NLT)	T1	+	+	++	++
	T2	+	+	++	+++
	T2	+	+	++	+++
Dilation of Bowman's space (DBS)	T1	+	+	++	++
	T2	+	++	++	+++
Dilation of tubules (DT)	T1	+	+	++	++
	T2	+	++	++	+++
Infiltration of Blood cells(IFBc)	T1	+	+	++	++
	T2	+	++	++	+++

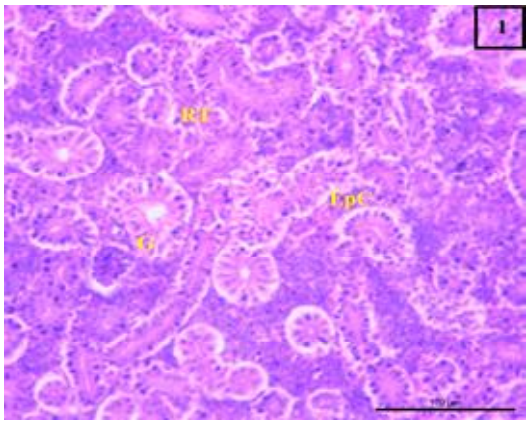

Fig. 1. Normal histo-architecture of kidney of common carp showing normal renal tubular (RT) structure, epithelial cells (EpC) and glomerulus (G).

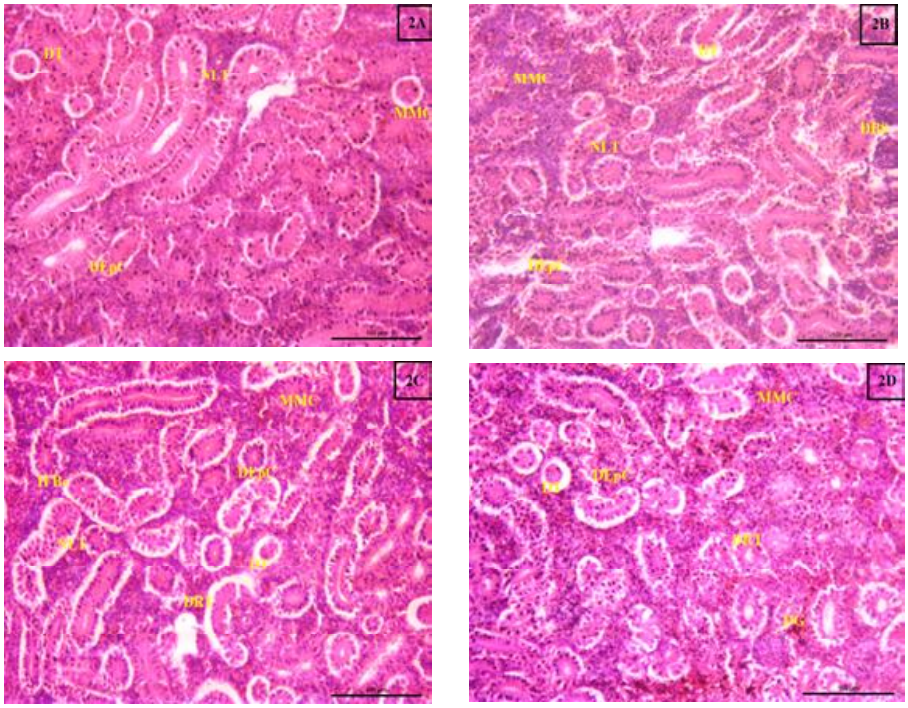

Fig. 2. Changes in normal histology of kidney after treatment of 0.005 µl/L/T1 of DM (11% EC). 2A, 2B, 2C and 2D showing histopathological alterations after 7th, 14th, 21st and 28th day of treatment of DM. Changes like degenerated renal tubules (DRT), melanomacrophage centres (MMC), infiltration of blood cells (IFBc), Dilation of Bowman's space (DBS), damaged glomerulus (DG), degenerated epithelial cells (DEpC), hyaline droplets (HD) and narrow lumen of tubules (NLT) were observed.

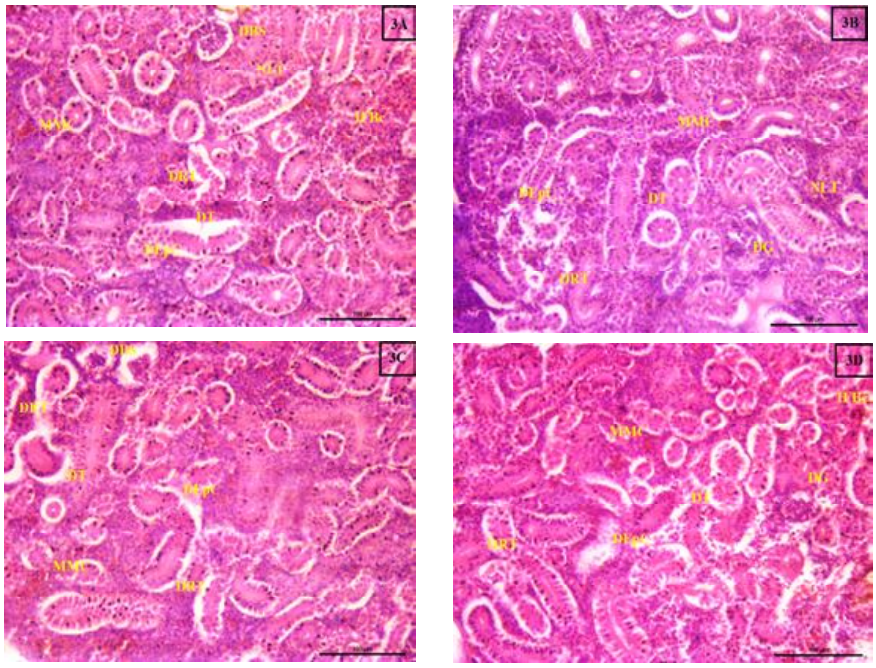

Fig. 3. Changes in normal histology of kidney after treatment of 0.011µl/L/T2 of DM (11% EC). 3A, 3B, 3C and 3D showing several histopathological alterations after 7th, 14th, 21st and 28th day of treatment of DM. Changes like degenerated renal tubule (DRT), melanomacrophage centres (MMC), infiltration of blood cells (IFBc), dilation of Bowman's space (DBS), damaged glomerulus (DG), degenerated epithelial cells (DEpC), and narrow lumen of tubules (NLT) were seen.

Histopathological analysis is considered as a sensitive bio-monitoring tool in toxicant effect assessment that may be used to determine how toxicants affect fish in aquatic ecosystems contaminated with pesticides[23]. When fish reside in polluted aquatic habitats, pesticides mostly accumulate in their tissues that are metabolically[18]. The kidney in fish is responsible of maintaining stable internal homeostasis and proper balance of electrolytes and water. It has been shown that histological examination of kidney is a valuable method for researching the toxic effects of harmful pesticides on non target organisms like fish as well as critical markers of stress brought on by different anthropogenic contaminants[24]. Before beginning an experiment, a physico-chemical analysis of the water is required since these properties of water affect the toxicity of pesticides. Water quality values from the tap water used in this study were within the normal range, indicating that pesticide usage was the only factor contributing to the pathological problems seen throughout the experiment.

The kidney tissue of control fish depicted an intact renal corpuscle with

glomeruli and Bowman's space, epithelial cells, and normal tubule network (Fig. 1). After the treatment of sub-lethal concentrations of DM, several alterations were seen (Fig. 2, 3 and Table-1).

Various researchers have studied the detrimental effects of DM on the histology of kidney of different fish species. Study by Cengiz[6] depicted several alterations in kidney of common carp after exposure to DM for short period of time. Changes in the histology of kidney of *Carassius auratus gibelio* after the treatment of deltamethrin were studied by Staicu *et al*.[26]. Aziz *et al*.[3] also conducted a research to study effects of deltamethrin (DM) concentrations of 0.5ug/L, (for one week), 0.02 and 0.01ug/L (for 4 weeks) on kidney of healthy male catfish (*Clarias lazera*). The results of these studies, which examined the effects of deltamethrin as a toxicant on the kidney histology of several fish species, align with what is being observed in present investigation. Different researchers observed similar changes in the histology of kidney of various species after using different synthetic pyrethroids as experimental toxicant. According to Akter *et al*.[2] fish (*Anabas testudineus*) treated with cypermethrin at different concentrations for a period of thirty days revealed several histopathological changes. Our results were in consistence with these findings. Comparable histological changes along with the appearance of degraded hyalin droplets and the display of nucleus and tubular epithelial cell hypertrophy were noted in *Oreochromis niloticus* treated with cypermethrin[14]. Cypermethrin caused significant histological alterations in the kidney of *Cirrhinus mrigala*, according to Prashanth[20]. Consistent with our findings, study on the kidney of *Cirrhinus mrigala* treated to monocrotophos displayed hypertrophied renal tubule epithelial cells, glomerulus constriction, pycnotic nuclei in tubular epithelium, and enlargement of space inside the Bowman's capsule[28]. Haque *et al*.[11] noted that the cypermethrin exposure caused histological alterations in the kidney tissues of *Mystus tengara*. Kenthao *et al*.,[13] studied the acute toxic effects cypermethrin on kidney of Nile tilapia. According to their observations cypermethrin exposure induced enlargement and degeneration of epithelial cells of renal tubules along with the damage of glomerulus (together with afferent and efferent arterioles). Results of current research are positively supported by all of the aforementioned findings.

Similar histopathological changes on kidney of several fish species were observed by various researchers after the exposure to different pesticides (other than synthetic pyrethroids). After 14 days of exposure to chlorpyrifos pesticide, common carp were similarly shown to have histological abnormalities in their kidney tissue[19]. Uikey[27] also discovered significant degenerative alterations in renal tissue of fish *Labeo rohita* when exposed to sub lethal concentration of malathion. Various histopathological lesions were observed by Bharti and Rasool[5] in kidney of *Channa punctatus* after the treatment of malathion. The severity of these lesions was increased with the increase in the days of exposure of malathion. Following exposure to Thiomethoxam (THM), a number of anomalies in the kidney tissues of Banded Gomorami (*Trichogaster fasciata*) were noted by Hasan *et al*.,[10]. The frequency of histological alterations rose as THM concentrations and exposure times

increased. Similar dose and time dependent increase in intensity of histopathological alterations was seen in current investigation. So we can say that our results are consistent with findings of different researchers mentioned above.

This investigation clearly demonstrated the adverse impacts of deltamethrin pesticide on histology of kidney of a freshwater fish, *C. carpio*. Kidney exhibited several changes after the treatment of sub-lethal concentrations of DM. On the basis of these results we can conclude that histopathological examination of different tissues provides a valuable insight of toxicity of pesticides, hence acts as crucial as well as important marker of toxicity evaluation.

One of the authors (Rajinder Kumar) is highly thankful to ICMR (Indian Council of Medical Research) for providing financial assistance to complete this research work. Authors express their gratitude to the chairperson of the Department of Biosciences, HPU, Shimla for providing lab facilities. The Himachal Pradesh Department of Fisheries is also appreciated by the authors.

Conflict of Interest

The authors affirm that there is no conflict of interest associated with this paper's publication.

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