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Histopathological responses of kidney in the common carp *Cyprinus carpio* exposed to sublethal concentrations of phorate

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Abstract

Cyprinus carpio (*C. carpio*) fish were exposed to chronic sublethal toxicity (one-tenth of the LC_{50}/96 hours - 0.071 ppm/l) of phorate (CSTP) for one day, 7, 15 and 30 days and the effect of CSTP on kidney histopathology of the fish was investigated in the present study. On exposure for a period of 1 day to CSTP, glomerular Shrinkage and expansion of space inside the Bowman's capsule were observed in the kidney of the fish. On exposure of the fish for a period of 7 days, destruction in the renal tubules, vacuolization in cytoplasm and loss of architecture were observed in the kidney of the fish. Further on exposure of the fish *C. carpio* for a period of 15 days to CSTP, degeneration of glomerulus and increase in periglomerular space and degeneration in Bowman's capsules were observed. After the exposure period of 30 days, indistinct capillary structure, shrunken glomerulus, tubular destruction, vacuolation in the cytoplasm, a clear increase in periglomerular space, loss of cell architecture and degeneration of cell nuclei were observed in the kidney of the exposed fish. The findings of the present study demonstrate that the frequency of pathological changes in the kidney of the fish *C. carpio*, increase with the increasing exposure time to CSTP.

Keywords: Phorate, *Cyprinus carpio*, Histopathology, Bowman's capsule, Glomerulus, Periglomerular space.

1. Introduction

Nowadays farmers are using a number of pesticides in the operation of agricultural and other commercial crops for more production, protection and to eradicate or control the pests. The heavy dependence of modern agriculture on agrochemicals like pesticides is emerging as a threat to the ecological balance of aquatic ecosystems. Synthetic pesticides used for controlling pests in agriculture are one of the major causes of aquatic pollution. The residues of these pesticides mostly reach into aquatic ecosystems through surface run off and affect the health of non-target organisms like fish. Agricultural runoff, effluents from pesticide manufacturing industries or accidental spillage pose a serious threat to the aquatic environment, affecting aquatic lives including fish [1, 2].

Among synthetic pesticides organophosphates (OPs) are widely used in agriculture and in health and hygiene programmes due to their high effectiveness as insecticides and less persistence in the environment. Organophosphate (OP) compounds are a diverse group of chemicals used in both domestic and industrial settings. They are favored over organochlorines (OCs), which have long persistence and consequently easily bioaccumulate in food chain. The shift from OCs to OPs has resulted into increased occurrence of OPs in the water bodies causing acute and chronic toxicity to fish fauna [3-5]. The health effects associated with OP poisoning are a result of excess acetylcholine (ACh) present at different nerves and receptors in the body because acetylcholinesterase is blocked.

The knowledge of the histology is useful to distinguish normal cells from abnormal or diseased ones, which helps in the diagnosis of many diseases [6]. The physiological investigations when coupled with cytoarchitectural studies, the toxicological studies seem to be complete so as to give a picture of the extent of pesticidal effect. The cytoarchitectural dynamics of a tissue is very essential for maintaining the tissue integrity and for effective physiological, biochemical and metabolic functions. The cellular and sub-cellular constituents of tissue in terms of size, shape, number and position play an important role in the physiological and metabolic functions. Therefore, the histological structure of a tissue in an animal has a profound influence on its function. Hence the present investigation was aimed to assess the impact of CSTP, which is widely used in the local area to combat pests, on kidney histopathology in the fish *C. carpio*, a representative of the aquatic environment.

2. Materials and Methods

2.1. Test Species

The Indian major carp *C. carpio* (Linnaeus, 1758) has been selected as test species for the present investigation. It is an economically important edible fish, having great commercial value.

2.2. Test Chemical

Pesticide selected for this study is phorate (O, O-diethyl S-ethylthiomethyl phosphorodithioate) an OP insecticide which is widely used throughout the world and also in India as a broad spectrum insecticide on numerous crops including paddy and groundnut. Commercial names of phorate are thimet, rampart, granutox, agrimet etc and its molecular formula is $C_7 H_{17} O_2 PS_3$.

2.3. Procurement and maintenance of fish

Fingerlings of *C. carpio* fish were brought from the department of fisheries, Anantapur, Andhra Pradesh, released into large cement tanks with sufficient dechlorinated tap water and allowed to acclimatize for 15 days. Then the fish were separated into the batch of having the size of 10 ± 2gm and were maintained in static water without any flow [7]. As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of animals, size, sex etc [8], precautions were taken throughout this investigation to control all these factors as far as possible.

2.4. Chronic toxicity procedures

Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the probit method of Finney [9]. One-tenth of the LC_{50}/96 hours (0.071 ppm/l) concentration of phorate was taken as the sublethal concentration for chronic toxicity study.

2.5. Experimental Design

100 fishes were divided into 5 groups comprising of 20 fishes each. The group I was considered as normal control, group II, III, IV and V were experimental groups. The fishes of group II were exposed to CSTP (exposed to sub lethal concentration = 1/10th of LC_{50} - 0.071 ppm/l) for 1 day, group III for 7 days, group IV for 15 days and group V for 30 days. Then the fish were sacrificed and kidney tissues were isolated under laboratory conditions for histopathological studies after the completion of stipulated exposure period.

2.6. Histopathology

The histological sections of the kidney of the control and experimental fish were taken by adopting the procedure as described by Humason [10]. The kidney tissues were isolated from the control and phorate treated fish and rinsed with physiological saline solution (0.9% NaCl) to remove blood, mucus and debris adhering to the tissues. They were fixed in Bouin's fluid for 24 hours and the fixative was removed by washing through running tap water overnight. The tissues were processed for dehydration using ethyl alcohol as the dehydrating agent and were passed through a graded series of alcohols, cleaned in methyl benzoate and embedded in paraffin wax. Sections were cut at 5µ thickness and stained with hematoxylin [11] and counter stained with eosin (dissolved in 95% alcohol). Then the sections were mounted in Canada balsam after dehydration and cleaning and photomicrographs were taken using the magnus photomicrography equipment.

3. Results and Discussion

3.1. Results

The structure of the kidney of normal control *C. carpio* fish consists of a clear Bowman's capsule, glomerulus and renal tubules. The glomerulus, a cluster of capillaries is surrounded by the Bowman's capsule in the kidney (Fig 1).

3.1.1. Histopathological study in kidney

On exposure for a period of 1 day to CSTP, glomerular Shrinkage and expansion of space inside the Bowman's capsule were observed in the kidney of the fish *C. carpio*. Nuclear hypertrophy and cloudy swelling were observed in the epithelial cells of renal tubules. Cytoplasmic vacuolization with cellular degeneration was also noticed (Fig 2a). On exposure of the fish for a period of 7 days, destruction in the renal tubules, vacuolization in cytoplasm and loss of architecture were observed in the kidney of the fish. Necrosis in tubular epithelium and degeneration in Bowman's capsule were also seen (Fig 2b). Further on exposure of the fish *C. carpio* for a period of 15 days to CSTP, degeneration of glomerulus and increase in periglomerular space and degeneration in Bowman's capsules were observed. Loss of cell architecture with increased vacuolation in the cytoplasm of the kidney was noticed (Fig 2c). After the exposure period of 30 days, reorganized dilated Bowman's capsule, indistinct capillary structure, shrunken glomerulus, tubular destruction, vacuolation in the cytoplasm, a clear increase in periglomerular space, loss of cell architecture and degeneration of cell nuclei were observed (Fig 2d).

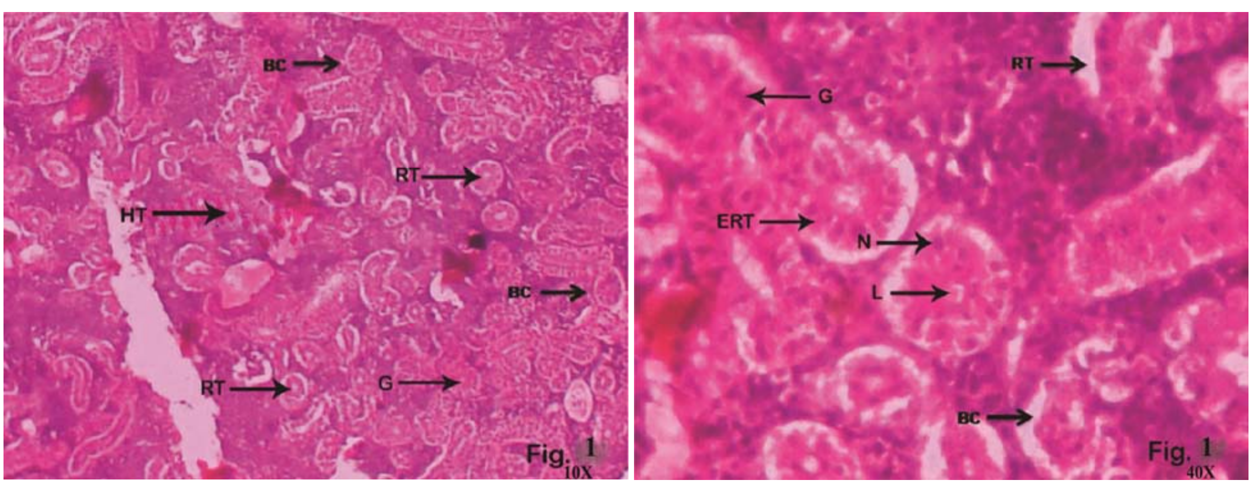

Fig 1: The normal architecture of the control fish kidney showing a clear Bowman's capsule (BC), glomerulus (G), renal tubules (RT), lumen (L), nuclei (N) and epithelial cells of renal tubules (ERT) and hematopoietic tissue (HT) with lower (10X) and higher magnification (40X).

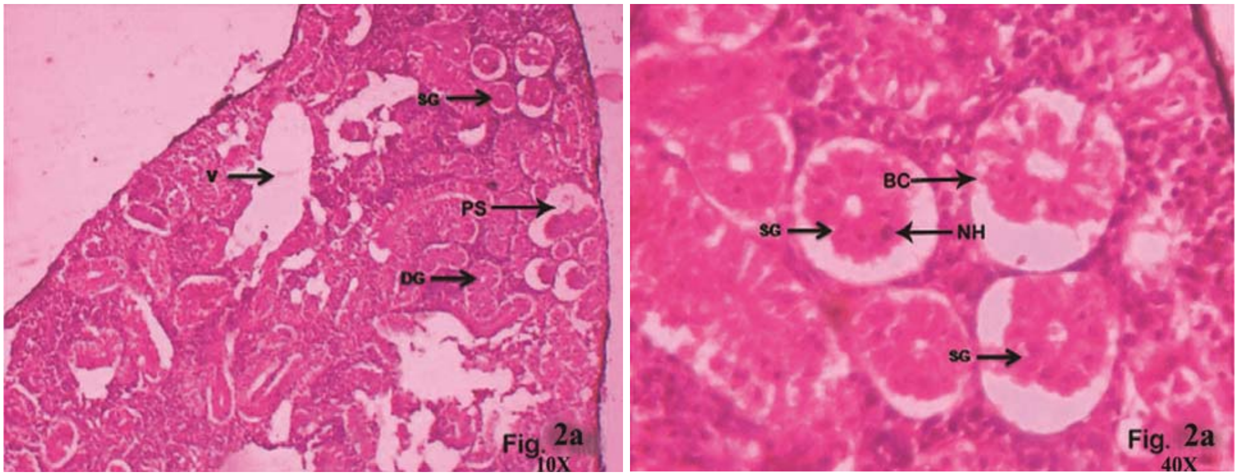

Fig 2a: The kidney of the fish exposed to CSTP for 1 day showing initiation of degeneration of glomerulus (DG) and Bowman's capsule (BC), shrunken glomerulus (SG), increase in periglomerular space (PS), nuclear hypertrophy in tubular epithelial cell (NH) and vacuolization (V) with lower (10X) and higher magnification (40X).

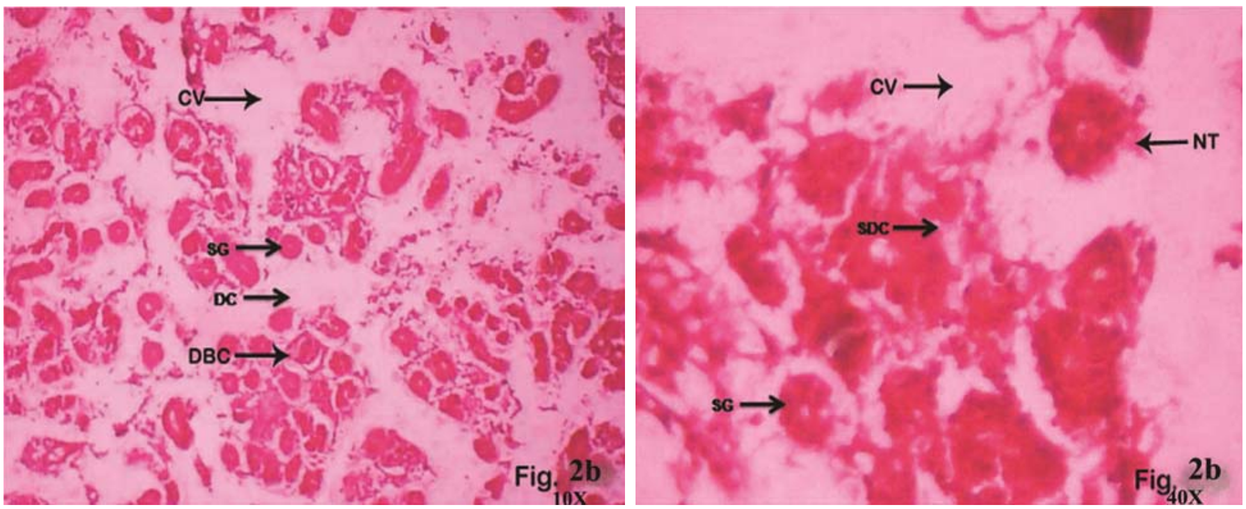

Fig 2b: The kidney of the fish exposed to CSTP for 7 days showing structural degenerative changes (SDC) such as shrunken glomerulus (SG), degeneration of Bowman's capsule (DBC), necrosis in tubular epithelium (NT), degenerative changes (DC) and cytoplasmic vacuolization (CV) with lower (10X) and higher magnification (40X).

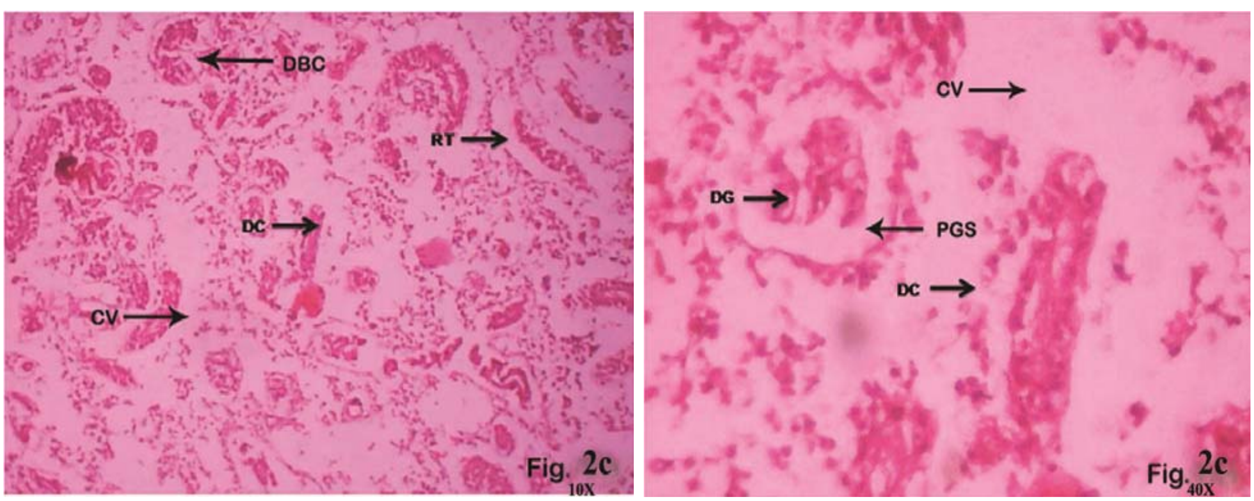

Fig 2c: The kidney of the fish exposed to CSTP for 15 days showing degenerative changes (DC) such as degeneration of glomerulus (DG), increase in periglomerular space (PGS),degeneration of Bowman's capsule (DBC) and renal tubule (RT) and increasing cytoplasmic vacuolization (CV) with lower (10X) and higher magnification (40X).

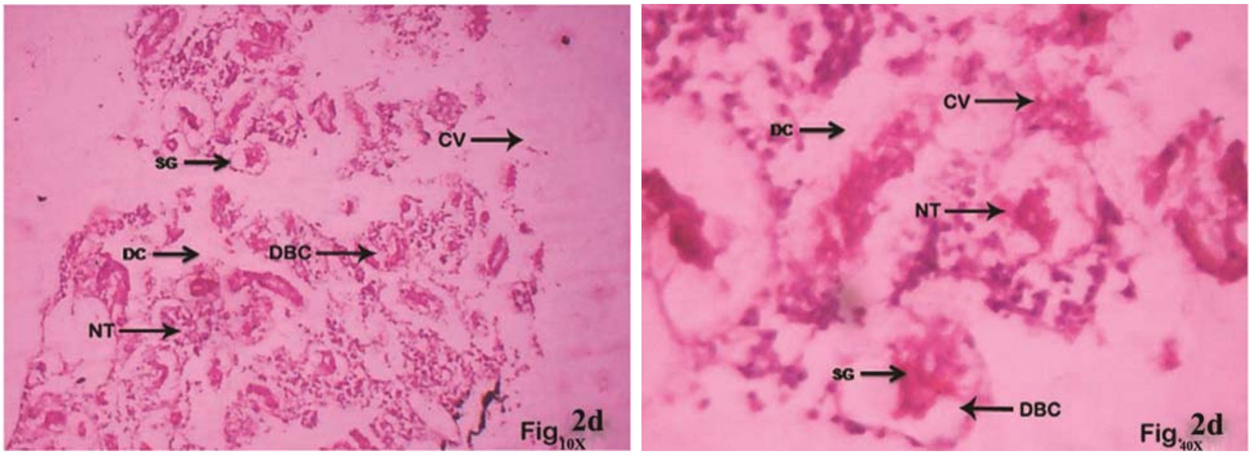

Fig 2d: The kidney of the fish exposed to CSTP for 30 days showing degenerative changes (DC) such as shrunken glomerulus (SG), degeneration of Bowman's capsule (DBC),necrosis in tubular epithelium (NT) and cytoplasmic vacuolization (CV) with lower (10X) and higher magnification (40X).

3.2. Discussion

Pathological lesions due to lethal and sublethal effects of xenobiotic compounds are very important for delineating fish health status and for future ecological impact [12]. Specific lesions occurring in the organs of fish exposed to toxic substances under laboratory conditions are helpful as biomarkers of exposure. As a result histopathological examination is increasingly being recognized as a valuable tool for assessing the impact of environmental pollutants like pesticides on fishes [13, 14]. In the present study, it is clearly indicated that the phorate has induced pronounced pathological changes in the kidney of the fish *C. carpio* exposed to CSTP (Fig 2a to 2d). The histopathological responses of the fish *C. carpio* exposed to CSTP in the present study reveal the degree of damage caused by this pesticide to the kidney tissues of the fish. The extent of damage caused by phorate to the kidney of the fish is progressive over the period of exposure to the CSTP suggest that the histopathological responses depend on the concentration of pesticides as well as the length of the fish exposure period to pesticides.

Kidney serves as a major route of excretion of metabolites of xenobiotics, receives the largest proportion of postbranchial blood and therefore it is more likely to undergo histopathological alterations under pesticide stress [15]. Hence the renal lesions might be expected to be good indicators of environmental pollution [15]. Several investigators have been found histological alterations earlier at the level of the tubular epithelium and glomerulus in the kidney of the fish [16-19]. Cengiz [18] observed 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 in the kidney of the fish *C. carpio* exposed to deltamethrin. Ram Nayan Singh [19] reported shrinkage of glomerulus, dilation of tubular lumen, vacuolization, desquamation, hydropic swelling and hyaline degeneration of tubular epithelium, cyst formation and hemorrhage in the kidney of common carp, *C. carpio* after sub lethal exposure to dimethoate and stated that the duration of exposure appears to have a profound effect on the kidney.

The histological changes that were taken place in the present study, at the initial period of exposure in the kidney of the fish on exposure to CSTP might be a part of defense mechanism. On prolonged exposure due to further accumulation of phorate in the kidney of the fish, it caused destruction in the organ structures. The slight structural reorganization of the kidney of the fish observed at day 30 of exposure to CSTP gives support to some extent that the ability of the fish to resist the sublethal stress and in repair of the damage caused to this organ by enhancing the protein synthetic potentials and other associated activities of the cell. Probably the fish could excrete or chelated the accumulated phorate over the time of exposure, there by the toxic effect of it might have been gradually decreased. It can be concluded that the degree of destruction in the kidney of the fish appeared to be linearly proportional to the period of exposure [19, 20].

4. Conclusions

On exposure to CSTP, though initially it caused a mild damage to the kidney of the fish at day1, further prolonged exposure to CSTP, the frequency and intensity of tissue lesions increased with the increasing duration of exposure. On prolonged to exposure CSTP, the fish could develop enough resistance and replenish the loss by activating the energy cycles. Thus the changes induced by CSTP in the structure and morphology of the kidney of the fish *C. carpio* are dependent on the pesticide concentration and length of the fish exposure period.

5. References

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