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Effect of phorate on histopathological changes in the kidney of common carp *Cyprinus carpio* exposed to lethal concentrations

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Abstract
The effect of acute lethal toxicity of phorate (ALTP) on the histopathology of kidney of the fresh water fish *Cyprinus carpio* (*C. carpio*) was investigated in the present study. Fish were exposed to ALTP (LC_{50}/96 hours - 0.71 ppm/l) for one day and 4 days and the differential acute toxicity tests were carried out under laboratory conditions. On exposure for a period of 1 day to ALTP, contraction in the glomerulus renal tubule, expansion of space inside the Bowman's capsule and initiation of the degeneration of hematopoietic tissue were noticed in the kidney of the fish. Further on exposure for a period of 4 days to ALTP, there was a further increase in the glomerular shrinkage and dilation in Bowman's capsule. The other pathological changes that were observed are the appearance of periglomerular space, formation of pycnotic nuclei in the hematopoietic tissue, necrosis in tubular epithelium, destruction in the renal tubules and vacuolation in the cytoplasm. Complete damage in the structural integrity of the kidney was noticed. On exposure to ALTP, though initially it caused a mild damage to the kidney of the fish at day 1, further exposure for 4 days, it caused an irreversible damage.

Keywords: Phorate, *Cyprinus carpio*, Bowman's capsule, Hematopoietic tissue, Periglomerular space, Pycnotic nuclei

1. Introduction
Due to rapid growth in the human population and industrialization, the pollution of water bodies such as lakes, rivers and streams has become a universal phenomenon in recent days [1]. The important sources of water pollution are domestic sewage, industrial effluents, drainage and chemical substances such as pesticides [2]. Pesticides like organophosphates are known to cause several structural and functional changes in the aquatic organisms like fish and destroy the ecological balance between animal species and ecosystems [3]. Pesticides are carried into the aquatic ecosystems by surface runoff from the sites of application and therefore the health of the aquatic ecosystem is being adversely affected which leads to high risk to the non-target organisms like fish [4, 5].

Phorate is an organophosphorus insecticide (OPI) and acaricide used to control sucking and chewing insects, leafhoppers, leafminers, mites, some nematodes, and rootworms [6, 7]. It is used in pine forests and on root and field crops including paddy and groundnut. It is an important OPI to which the fresh water fishes are frequently exposed due to the indiscriminate use of it by the farmers. It is highly toxic and extremely fast-acting on bird species, freshwater fish and aquatic invertebrates [8]. Increased use of pesticides like phorate leads to the excess inflow of toxic chemicals into the aquatic ecosystem [9] and affect the aquatic flora and fauna when they enter into their systems by bringing about external and internal damages [10, 11].

Teleost fish have proved to be good models to evaluate the toxicity and effects of pollutants such as pesticides on animals, as their biochemical responses are similar to those of mammals and other vertebrates. The Indian major carp *C. carpio* is an economically important edible fish, having great commercial value. It is abundantly available in the fresh water tanks and ponds in and around Anantapur. Besides its wide availability and commercial importance, this carp fish is known for its adaptability to laboratory conditions and appear to be suitable test animal to toxic studies [12]. Thus, the objective of the present study was to investigate the acute toxic effects of phorate in the common carp *C. carpio* with emphasis on the histopathological changes in the kidney.

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 OPI 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 ± 2 gm and were maintained in static water without any flow [13]. 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 [14], precautions were taken throughout this investigation to control all these factors as far as possible.

2.4 Acute toxicity procedures
Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the probit method of Finney [15]. LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration to study the acute toxicity of phorate.

2.5 Experimental Design
60 fishes were divided into 3 groups comprising of 20 fishes each. The group I was considered as normal control, group II and III were experimental groups. The group II was exposed to ALTP (LC_{50} of Phorate= 0.71 ppm/l) for 1 day and the group III for 4 days. Then the fish were sacrificed and the tissues of kidney 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 [16]. The tissues were isolated from control and the 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 [17] 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 photomicrographing 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 ALTP, compared to the control, some structural changes that were observed in the kidney of the fish *C. carpio* are contraction in the glomerulus renal tubule, expansion of space inside the Bowman's capsule and initiation of the degeneration of hematopoietic tissue (Fig 2a). On exposure for a period of 4 days to ALTP, further increase in the glomerular shrinkage and dilation in Bowman's capsule was observed. The other pathological changes that were observed are appearance of periglomerular space, formation of pycnotic nuclei in the hematopoietic tissue, necrosis in tubular epithelium, destruction in the renal tubules and vacuolation in the cytoplasm. Complete damage in the structural integrity of the kidney was noticed (Fig 2b).

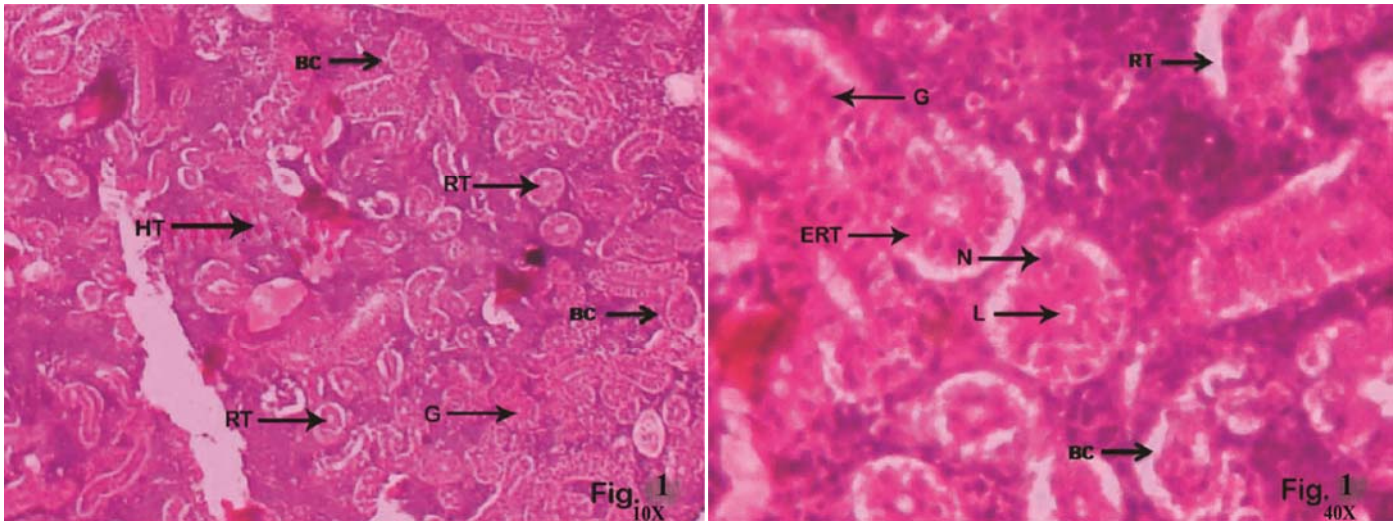

Fig 1: The normal architecture of the control fish kidney showing a clear Bowman's capsule (BC), glomerulus (G), renal tubules (RT), lumen of the renal tubules (L), nuclei of renal tubules (N), epithelial cells of renal tubules (ERT) and hematopoietic tissue (HT) with lower (10X) and higher magnification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

3.2 Discussion
As histology is the study of the microscopic anatomy of cells and tissues, it has been successfully employed as a diagnostic tool in different fields such as medical and veterinary science. Exposure of animals like fish to contaminated water causes severe pathological changes at the tissue level. Interaction between pollutants and cellular components results in pathological changes in cellular and sub-cellular level and can

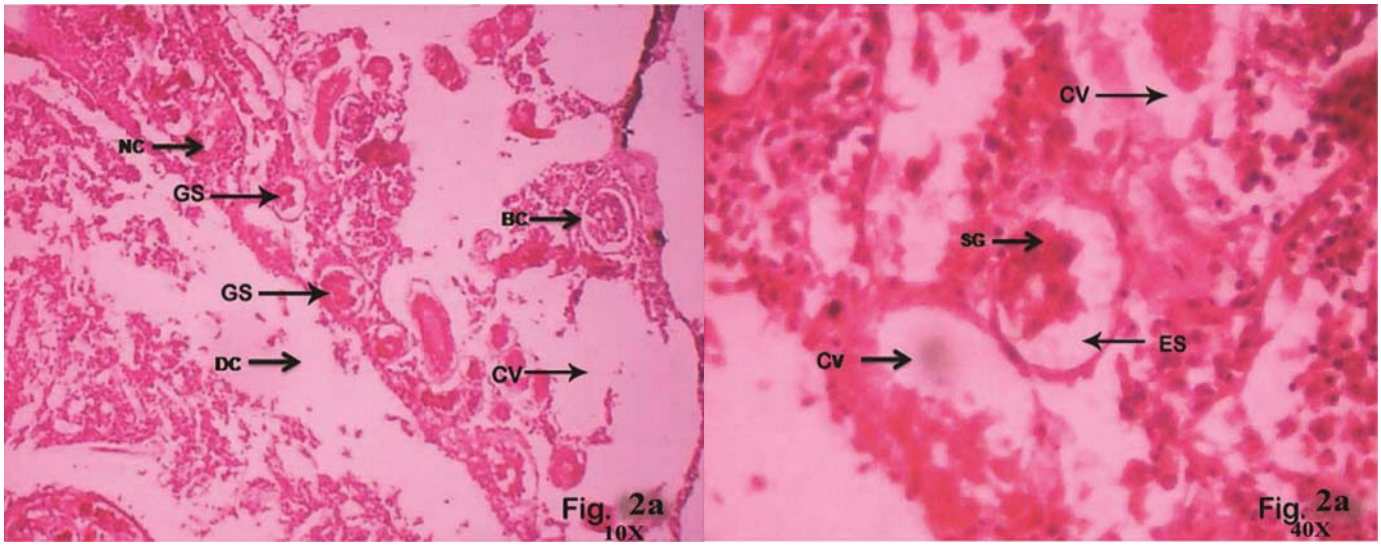

Fig 2a: The kidney of the fish exposed to ALTP for 1day showing degenerative changes (DC) such as glomerular shrinkage (GS), expansion of space inside the Bowman's capsule (ES), degeneration of Bowman's capsule (BC), necrotic changes (NC) and cytoplasmic vacuolization (CV) with lower (10X) and higher magnification (40X).

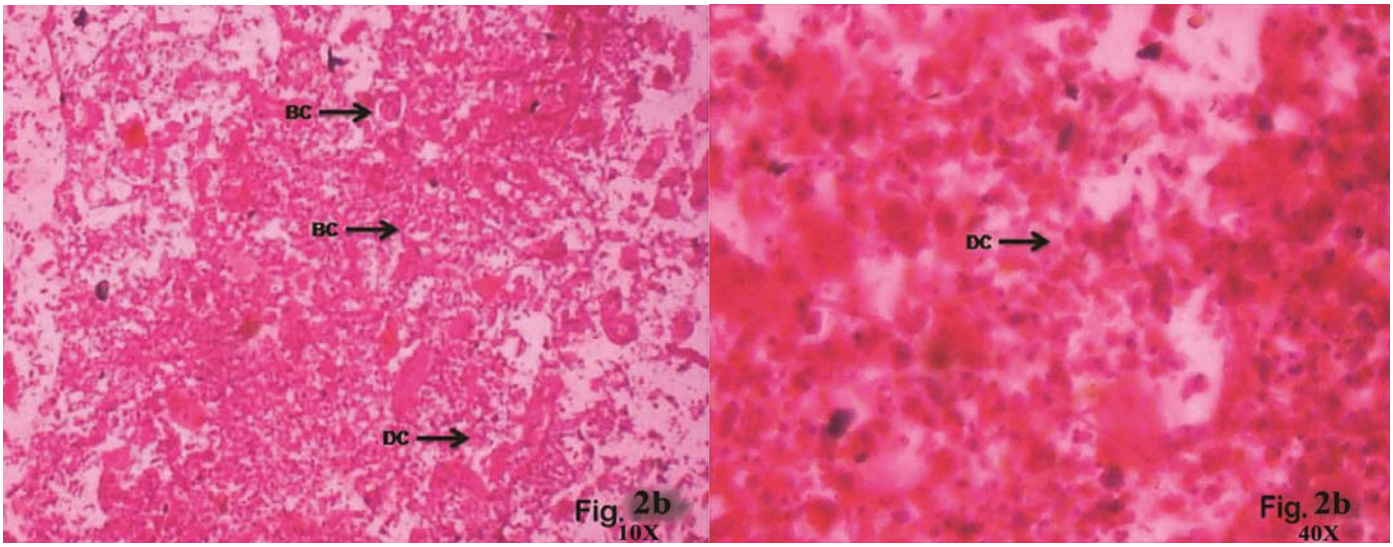

Fig 2b: The kidney of the fish exposed to ALTP for 4 days showing congestion, degenerative changes (DC) in Bowman's capsule (BC) and hematopoietic tissue with lower (10X) and higher magnification (40X).

be observed through histopathological analysis [18]. The histopathological investigations can provide information about the health and functionality of organs in the animals exposed to pesticides. Various histopathological responses occurred during the acute and chronic toxicity of pesticides could bring a relationship between the level of accumulation of the pesticide and physiological and biochemical activities of the animal. 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 ALTP (Fig 2a and 2b). These histopathological responses of the fish in the present study reveal the degree of damage caused by this pesticide to the kidney of the fish. The extent of damage caused by phorate to the kidney of the fish and the degenerative changes that were occurred in this organ were progressive over the period of exposure to the ALTP, suggest that the histopathological responses depend on the concentration of pesticides and the length of the fish exposure period to pesticides.

Kidney serves as a major route of excretion of metabolites of xenobiotics and receives the largest proportion of postbranchial blood. Therefore, it is more likely to undergo histopathological alterations under pesticide stress [19]. Thus the renal lesions might be expected to be good indicators of environmental pollution [19]. The cellular damage which occurred in the kidney due to the toxicity of phorate might impaired the osmoregulatory function of the fish as the kidney is not only the excretory organ but also functions as osmoregulatory organ of the fish. Several investigators have been found histological alterations earlier at the level of the tubular epithelium and glomerulus in the kidney of fishes. Dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells were observed by Das and Mukherjee [20] in the fish *Labeo rohita* exposed to hexachlorocyclo-hexane. Tilak *et al* [21] reported severe necrosis, cloudy swelling in the renal tubules, cellular hypertrophy, granular cytoplasm and vacuolization in kidney tissues of *Ctenopharyngodon idellus* exposed to fenvalerate.

Cengiz [22] 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 the 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 [23] observed 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 reported that duration of exposure appears to have profound effect on the kidney. The histological changes that were taken place in the present study at the initial period of exposure to ALTP at day 1 in the kidney of the fish might be a part of defense mechanism of the fish. On prolonged exposure for 4 days to ALTP, due to further accumulation of phorate in the kidney of the fish, it caused destruction in the organ structure. The degree of destruction in the kidney of the fish appeared to be linearly proportional to the period of exposure [23, 24].

4. Conclusions

On exposure to ALTP, though initially it caused a mild damage to the kidney of the fish at day 1, further exposure for 4 days it caused an irreversible damage to this organ. Thus the changes induced by ALTP in the structure and morphology of the kidney of the fish *C. carpio* are not only dependent on the concentration of the pesticide but also on the length of the fish exposure period. Frequency and intensity of tissue lesions depend on the concentration of pesticides as well as the length of the fish exposure period to pesticides.

5. References

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