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A histopathological study on the liver of common carp *Cyprinus carpio* exposed to sublethal concentrations of phorate

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Abstract
The effect of chronic sublethal concentrations of phorate on the histology of liver in the common carp, *Cyprinus carpio* (*C. carpio*) was investigated in the present study. Fish were exposed to chronic sublethal toxicity (one-tenth of the LC_{50}/96 hours - 0.071 ppm/l) of phorate (CSTP) for 1, 7, 15 and 30 days and the differential toxicity tests were carried out under laboratory conditions. On exposure for a period of 1 day to CSTP, no significant changes were observed in the structure of the liver. After 7 days of exposure, some degenerative changes like nuclear hypertrophy, vacuolization and karyolysis were observed in the hepatic cells of the liver. Focal necrotic changes were seen in the liver cords with the degeneration of the hepatocytes. On exposure for a period of 15days, the disintegration of liver cords, focal necrosis, degeneration of hepatocytes and karyolysis with widespread vacuolar appearance were noticed in the liver of the fish. On exposure for a period of 30 days to CSTP, further increase in the structural degeneration with increased vacuolization was observed in the liver. A severe degree of atrophy of the liver cords and cytoplasmic disintegration appeared. The liver was disrupted due to the rupture of the cell membranes. The findings of the present study demonstrate that the frequency of pathological changes in the liver of the fish *C. carpio*, increase with the increasing exposure time to CSTP.

Keywords: Chronic sublethal, Phorate, *Cyprinus carpio*, Histology, Karyolysis, Focal necrosis, Pathological changes

1. Introduction
The frequent use of pesticides such as organophosphates (OPs) in pest control and agriculture practices pollute the soil and water bodies, reach the aquatic ecosystem and get enriched in the aquatic organisms like fishes. OPs are the potent neurotoxins, functioning by inhibiting the action of AChE, leading to the accumulation of acetylcholine in the body of animals like fishes. OPs are one of the most common causes of poisoning worldwide, and are frequently intentionally used in agrarian areas. The intake of pesticides like OPs affects the biochemical composition [1, 2] and histological aspects [3, 4] of fishes.
Phorate is an organophosphorus insecticide (OPI) and acaricide used to control sucking and chewing insects, leafhoppers, leafminers, mites, nematodes and rootworms [5, 6]. It is used in pine forests and on root and field crops including corn, cotton, paddy, groundnut, some ornamental, herbaceous plants and bulbs. Phorate is an important pesticide to which the fresh water fishes are frequently exposed due to the indiscriminate use of this pesticide by the farmers.
Research on the effects of phorate on fish is scarcely done. However, some work was carried out by Saxena and Sarin [7, 8] on desert gerbil *Meriones hurrianae*, Morowati [9-11] on the male swiss albino mouse *Mus musculus*, Jyothi and Narayan [12] on fresh water fish *Clarias batrachus* and Anand Pratap Singh *et al* [13] on snake headed fish *Channa punctatus*, about the toxic effects of phorate. It is highly toxic and extremely fast-acting on bird species, freshwater fish and aquatic invertebrates [14]. Hence the present investigation is aimed to assess the impact of CSTP, which is widely used in the local area to combat pests, on liver 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. 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.

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 including Andhra Pradesh as a broad spectrum insecticide on numerous crops. 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 and 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 [15]. Water was renewed every day to provide freshwater, rich in oxygen. 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 [16], 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 [17]. 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 liver tissues were isolated under laboratory conditions for histopathological studies after the completion of stipulated exposure period.

2.6. Histopathology

The histological sections of the liver of control and chronic toxicity exposed fish were taken by adopting the procedure as described by Humason [18]. 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 [19] 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 normal liver of the control fish consists of continuous mass of cells called hepatocytes. The hepatocytes form a rather cord-like pattern and these cords are arranged around tributaries of the hepatic vein. The liver cells are large in size, polygonal in shape with homogenous granular cytoplasm and either eccentric or centrally located distinct nuclei. Each cord of the liver was separated by the thick wall of the peripheral cells (Figure 1).

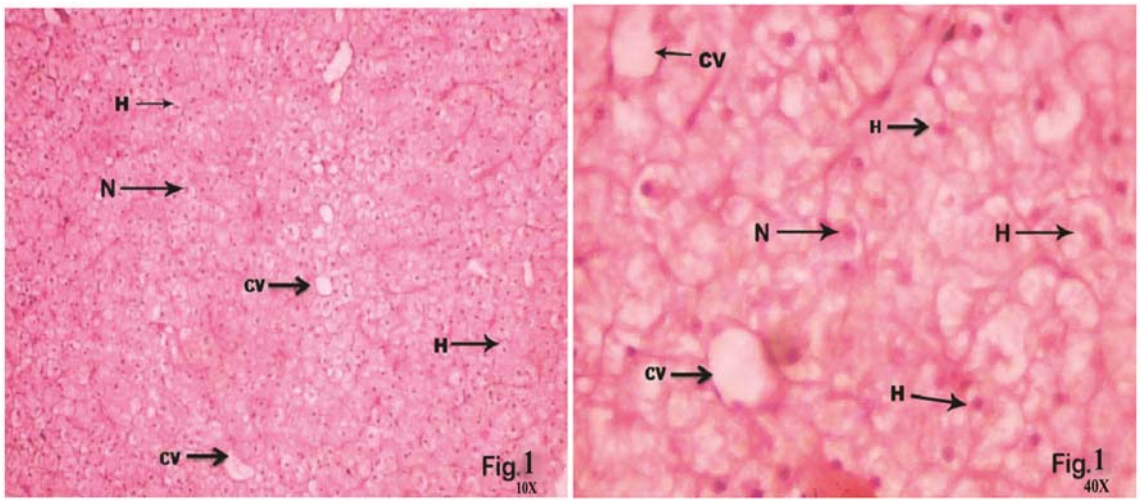

Fig 1: The normal architecture of the control fish liver tissue showing continuous mass of polygonal cells called hepatocytes (H), eccentric or centrally located distinct nuclei (N) and central vein (CV) with lower (10X) and higher magnification (40X).

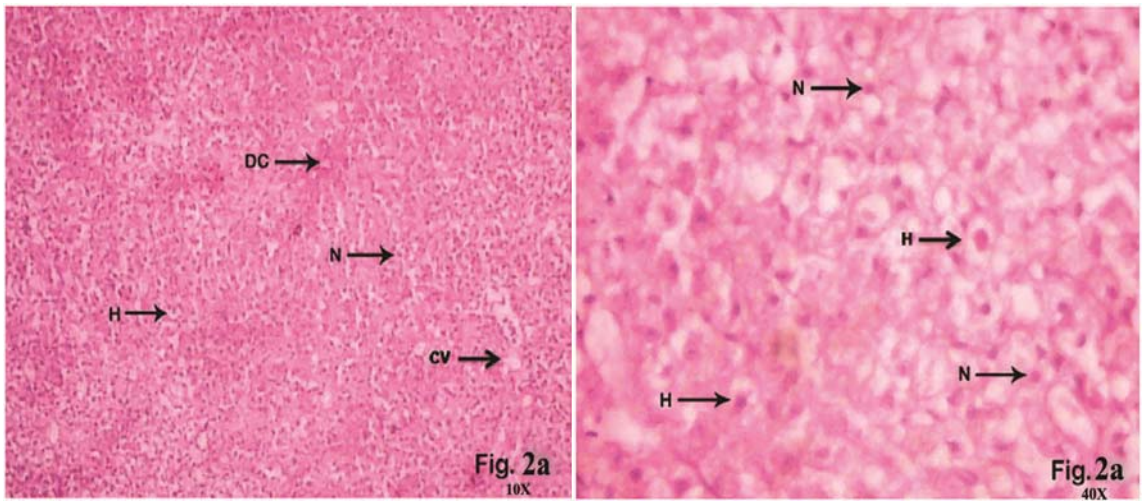

Fig 2a: The liver of the fish exposed to CSTP for 1 day showing hepatocytes (H), nuclei (N) and central vein (CV) with the initiation of degenerative changes (DC) in normal cytoarchitecture with lower (10X) and higher magnification (40X).

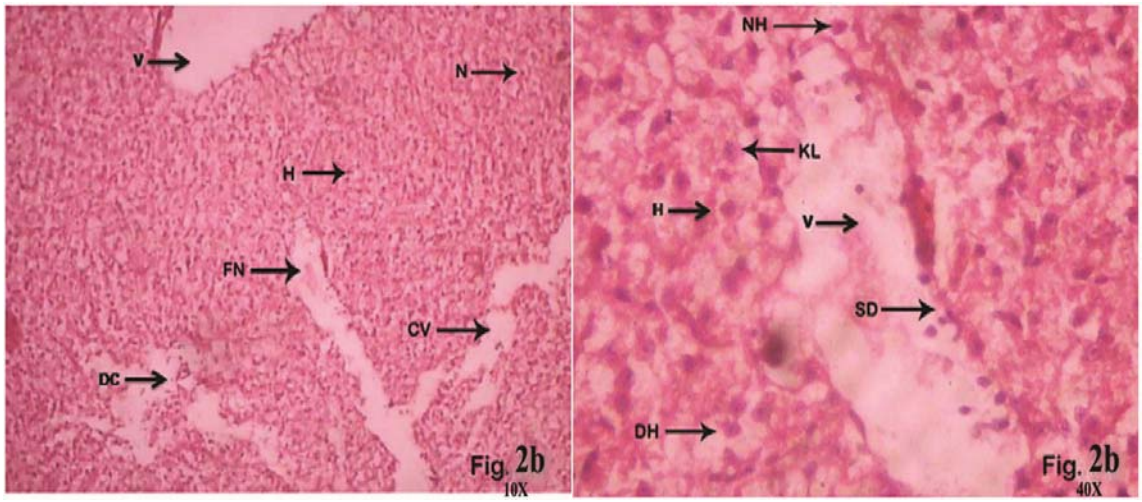

Fig 2b: The liver of the fish exposed to CSTP for 7 days showing nuclei (N), degenerative changes (DC) such as degeneration of hepatocytes (DH), nuclear hypertrophy (NH), karyolysis (KL) with focal necrosis (FN), structural degeneration (SD), cytoplasmic vacuolization (CV) and formation of vacuoles (V) with lower (10X) and higher magnification (40X).

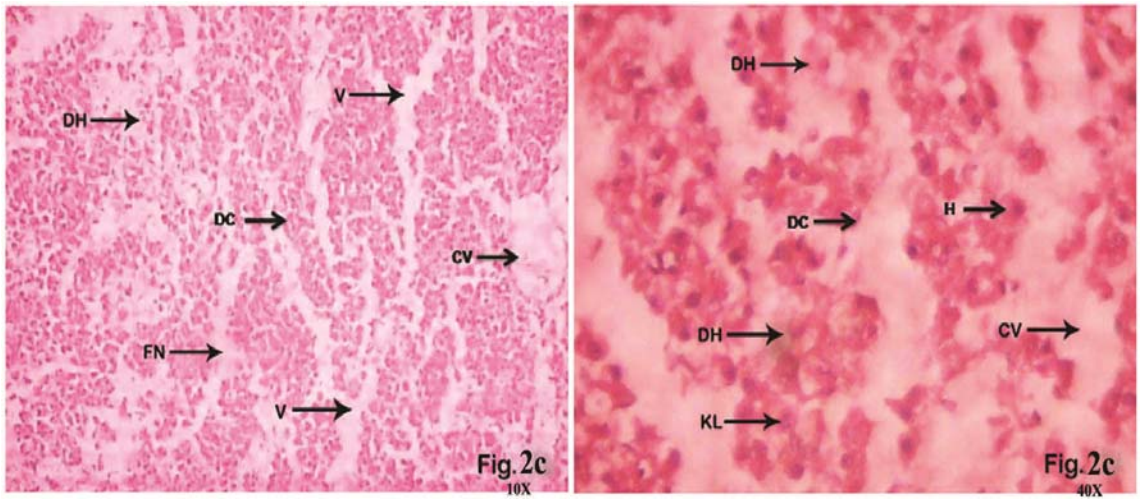

Fig 2c. The liver of the fish exposed to CSTP for 15 days showing hepatocytes (H), degenerative changes (DC) such as degeneration of hepatocytes (DH), karyolysis (KL) with focal necrosis (FN), cytoplasmic vacuolization (CV) and formation of vacuoles (V) with lower (10X) and higher magnification (40X).

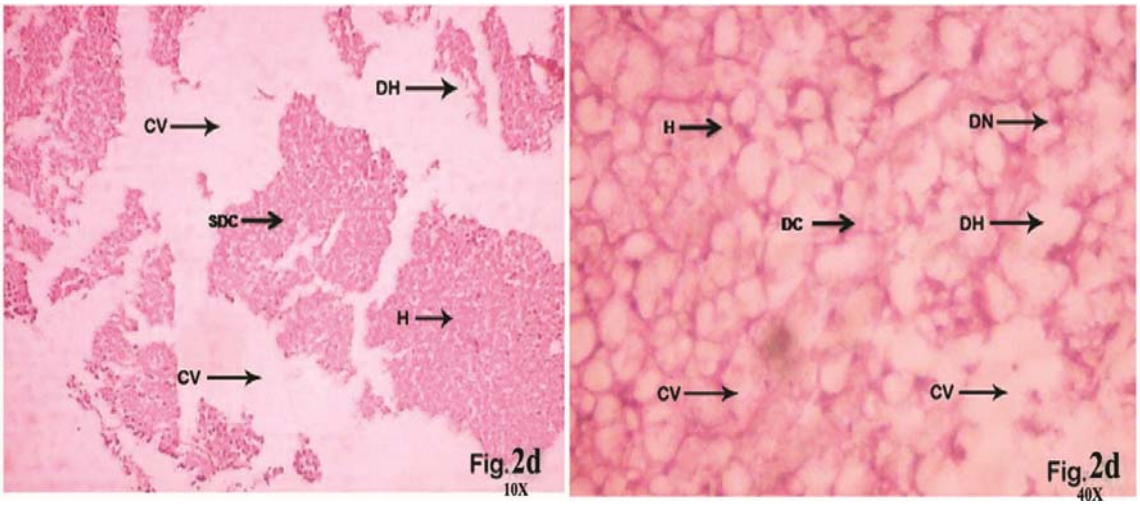

Fig 2d: The liver of the fish exposed to CSTP for 30 days showing hepatocytes (H), degenerative changes (DC) such as degeneration of hepatocytes (DH), structural degenerative changes (SDC), degeneration of nucleus (DN) and cytoplasmic vacuolization (CV) with lower (10X) and higher magnification (40X).

3.1.1. Histopathological study in liver

On exposure for a period of 1 day to CSTP, no significant changes were observed in the structure of the liver of the fish *C. carpio* (Fig 2a). After 7 days of exposure to CSTP, some degenerative changes like nuclear hypertrophy, vacuolization in the hepatic cells and karyolysis were observed in the liver of the fish. The parenchymatous tissue was disrupted and the liver cords were seen disarranged. Focal necrotic changes were seen in the liver cords with the degeneration of the hepatocytes (Fig 2b). On exposure for a period of 15 days, the disintegration of liver cords was observed. Focal necrosis, degeneration of hepatocytes and karyolysis with widespread vacuolar appearance were noticed in the liver of the fish. Few hepatocytes lost their polygonal shape as they were hypertrophied and the cell membranes found to be degenerated (Fig 2c). On exposure for a period of 30 days to CSTP, further increase in the structural degeneration with increased vacuolization was observed. A severe degree of atrophy of the liver cords and cytoplasmic disintegration appeared. The liver was disrupted due to the rupture of the cell membranes of the hepatocytes (Fig 2d).

3.2. Discussion

Histopathological investigations on different tissues of fish are valuable tools for toxicology studies and monitoring water pollutions. The histopathological investigations can provide information about the health and functionality of organs in the animals like fish. In the present study, it is clearly indicated that the phorate has induced pronounced pathological changes in the liver 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 liver tissues of the fish. The extent of damage caused by phorate to the liver of the fish is progressive over the period of exposure to CSTP suggest that the histopathological responses are linearly proportional to the period of exposure [3, 20].

The liver is the main organ for detoxification [21] that suffers serious morphological alterations in fish exposed to pesticides [22]. Alterations in the liver may be useful as a marker that gives prior indication of pathological alterations on exposure to environmental stressors. The pathological changes like in the present study were observed by several investigators in the liver of fish on exposure to different pesticides. Fanta *et al* [23] observed cloudy swelling, focal necrosis, atropy and vacuolization in the liver of *Corydoras paleatus* exposed to methyl parathion. Sarkar *et al* [24] reported hyperplasia, vacuolation, disrupted hepatocytes, focal coagulative necrosis, disorganized hepatic canaliculi in the liver of *Labeo rohita* exposed to cypermethrin. Cengiz and Unlu [25] observed hepatic lesions in the liver tissue of fish *Gambusia affinis* such as hypertrophy of hepatocytes, increase of Kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis and narrowing of sinusoids on exposure to deltamethrin. Sandipan Pal *et al* [20] observed nuclear and cellular hypertrophy, cellular atrophy, irregular contour of cells and nucleus, cytoplasmic and nuclear degeneration, cytoplasmic vacuolation, cellular rupture, pyknotic nucleus, necrosis and melanomacrophages aggregations in the liver of common carp, *C. carpio*, intoxicated with sub-lethal concentrations of chlorpyrifos pesticide for a period of 14 days.

The histological changes that were taken place at the initial period of exposure to CSTP in the present study might be a part of the defense mechanism of the fish. On prolonged exposure to CSTP, due to further accumulation of phorate in the liver of the fish, it caused destruction in the liver. The slight structural reorganization in the liver 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 the liver 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. The degree of destruction in the liver of the fish was linearly proportional to the period of exposure [3, 20].

4. Conclusions

On exposure to CSTP, though initially it caused a mild damage to the liver of the fish at day 1, further exposure for 7, 15 and 30 days it caused a profound damage to the liver. On prolonged exposure to CSTP, the fish could develop

enough resistance and replenish the loss by activating the energy cycles. The changes induced by CSTP in the structure and morphology of the liver 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 and the length of the fish exposure period to pesticides.

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