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Effect of phorate lethal concentrations on the histological aspects of liver in common carp *Cyprinus carpio* (Linnaeus, 1758)

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Abstract
The hazardous effect of acute lethal concentrations of phorate on the histology of liver in the fresh water fish *Cyprinus carpio* (*C. carpio*) was investigated in the present study. Fish were exposed to acute lethal toxicity (LC_{50}/96 hours - 0.71 ppm/l) of phorate (ALTP) for one day and 4 days and the acute toxicity tests were carried out under laboratory conditions. On exposure for a period of 1 day to ALTP, mild degenerative changes were observed in the liver of the fish, but the structure of the liver was distinct. Further on exposure for a period of 4 days to ALTP, severe pathological changes were observed in the liver of the fish. The results obtained demonstrated that the histopathological changes induced by ALTP in the liver of *C. carpio* were not only dependent on the concentration of the pesticide but also on the length of the exposure period.

Keywords: Phorate, *Cyprinus carpio*, Acute lethal toxicity, Histology, Pathological changes

1. Introduction
Environmental and chemical stress can interfere with physiological and biochemical functions such as growth, development, reproduction and circulatory system in fish. Numerous biochemical indices of stress have been proposed to assess the health of non target organisms exposed to toxic chemicals in aquatic ecosystem [1]. It has been reported that apart from nervous tissue, tissues like liver and gills also contribute information in the detection of toxic symptoms caused by certain groups of pesticides [2]. Pesticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical and histological tests are useful in recognizing acute or chronic toxicity of insecticides [3-5] like phorate and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish.
Due to the severity of the damage to the tissues, particularly liver, synthesis of many biochemical substances reduce significantly in cells, which can decrease some biochemical factors in blood of fish exposed to pesticides. These changes were observed in *Channa punctatus* [6], *Oncorhynchus mykiss* [7], *Oreochromis niloticus* [8], *Heteropneustes fossilis* [9], *Clarias batrachus* [10], *Cyprinus carpio* [11], *Oncorhynchus mykiss* [12] and *Colisa fasciatus* [13] which were exposed to monocrotophos, bifenthrin, carbaryl, cypermethrin, cypermethrin, diazinon, malathion and carbaryl respectively.
Toxicity is the degree to which a toxin or poison can harm the animals like fishes. It is the sum of adverse effects or the degree of danger posed by a substance like pesticide to living organisms. The edible freshwater fishes constitute one of the major sources of nutritious food for humans. Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bioindicators of environmental pollution. Among the aquatic species, the fish are the major targets of toxicants like pesticides. Hence, pollutants such as pesticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes [14, 15].

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 [16].

2.2. Test Chemical
Pesticide selected for this study is phorate (O, O-diethyl S-ethylthiomethyl phosphorodithioate) an organophosphorous 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 ± 2 gm and were maintained in static water without any flow [17]. 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 [18], 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 [19]. LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration to study 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 group III for 4 days. Then the fish were sacrificed and tissues of liver 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 acute toxicity exposed fish were taken by adopting the procedure as described by Humason [20]. 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 [21] 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 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).

3.1.1. Histopathological study in liver
On exposure for a period of 1 day to ALTP, mild degenerative changes were observed in the liver of the fish *C. carpio*, but the structure of the liver was distinct (Fig 2a). Further on exposure for a period of 4 days to ALTP, the pathological changes observed in the liver of the fish were severe degree atrophy of the liver cords, degeneration of hepatocytes and cytoplasmic disintegration. Cloudy swelling of hepatocytes, nuclear hypertrophy and nuclear degeneration were also noticed along with focal necrosis (Fig 2b). The liver was mostly disrupted due to the rupture of the cell membranes of the hepatocytes.

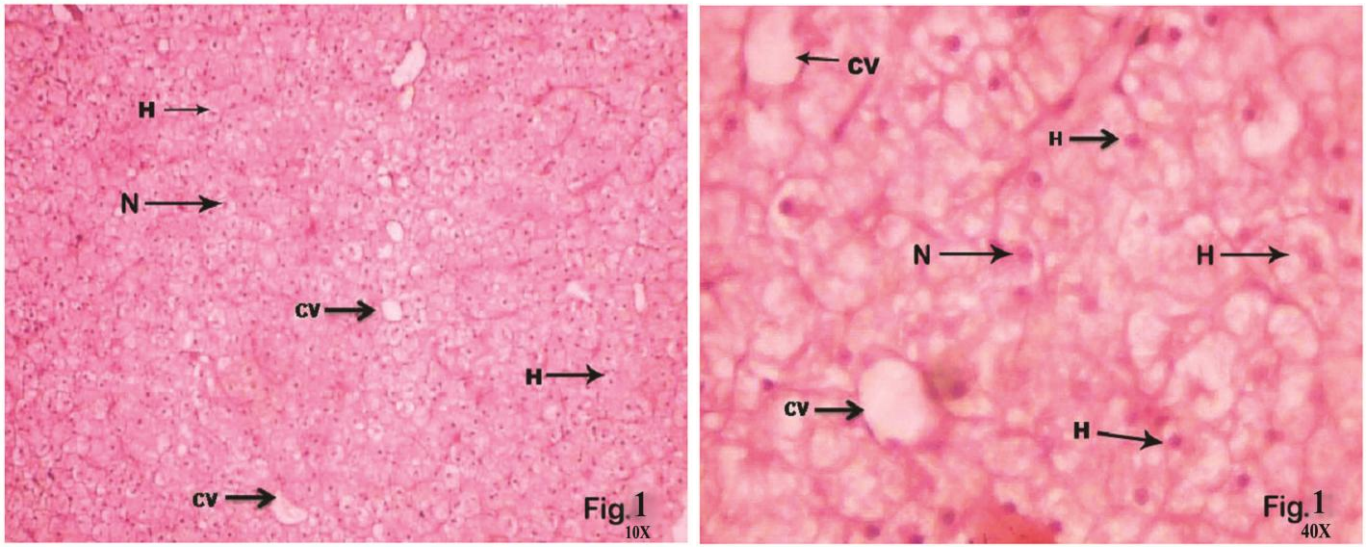

Fig1: The normal architecture of the control fish Liver tissue showing continuous mass of polygonal cells called hepatocytes (H), eccentric or centrally located distinct nuclei (N) and central vein (CV) with lower (10X) and higher magnification (40X).

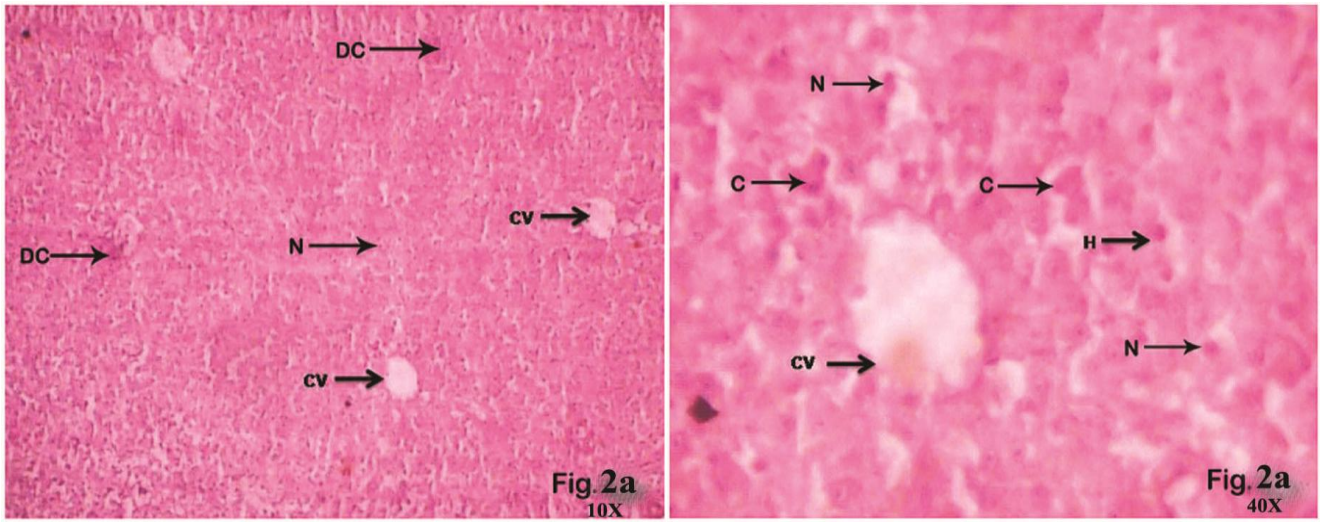

Fig 2a: The liver of the fish exposed to ALTP for one day showing hepatocytes (H), nuclei (N) and central vein (CV) with congestion (C) and mild degenerative changes (DC) in normal cytoarchitecture with lower (10X) and higher magnification (40X).

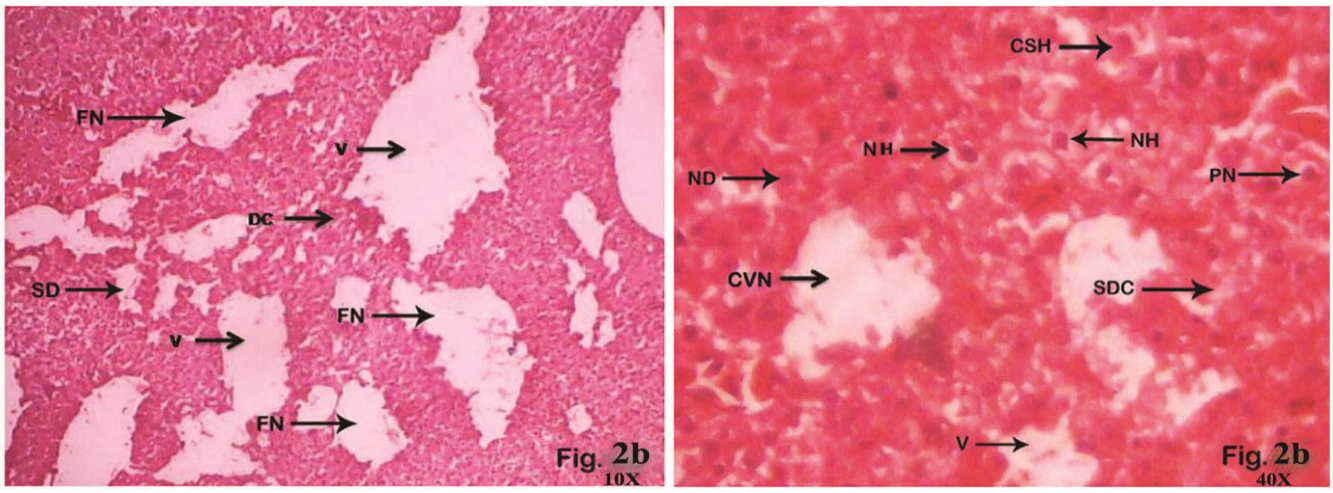

Fig 2b: The liver of the fish exposed to ALTP for 4 days showing structural degenerative changes (SDC) such as cloudy swelling of hepatocytes (CSH) nuclear hypertrophy (NH), nuclear degeneration (ND), pycnotic nucleus (PN), structural degeneration (SD), vacuolization with focal necrosis (FN) and cytoplasmic vacuolization (CVN) with lower (10X) and higher magnification (40X).

3.2. Discussion

Liver is the main organ for detoxification [22] that suffers serious morphological alterations in fish exposed to pesticides [23]. Alterations in the liver may be useful as a marker that gives prior indication of pathological alterations on exposure to environmental stressors such as pesticides. Tissue injuries and damages caused by pesticides in the organs of the fish can result in the reduced survival, growth, fitness and the low reproductive success or increase of susceptibility to pathological agents. Various histopathological responses in exposed fish could bring a relationship between the level of accumulation of the pesticide and various physiological and biochemical activities of the animal.

In the present study, phorate has induced pronounced pathological changes in the liver of the fish *C. carpio* exposed to ALTP (Fig 2a and 2b). Similar types of pathological changes were observed by many researchers on exposure to lethal concentrations of different pesticides. Ayoola and Ajani [24] observed fatty infiltration, hemosiderosis and congested central vein at the concentration of 1.9 to 9mg/l, severe infiltration of leukocytes, pyknotic and hepatic necrosis at 21 and 45mg/l concentrations in the liver of the fish *Clarias gariepinus* after exposing to lethal concentrations of cypermethrin. They also observed severe necrosis, hemorrhage and vacuolation in the liver of cypermethin exposed fish. The liver of fish exposed to glyphosate, showed an infiltration of leukocytes, increasing hepatocyte size with pyknotic nuclei, vacuolation of hepatocytes and necrosis in Nile tilapia, *Oreochromis niloticus* exposed to lethal concentrations [25].

The histopathological responses of the fish *C. carpio* exposed to ALTP in the present study reveal the degree of damage caused by this pesticide to the liver tissues of the fish. The degenerative changes that were occurred in the liver of the fish were progressive over the period of exposure to the ALTP suggest that the histopathological responses depend not only on the concentration of pesticides but also on the length of the fish exposure period to pesticides [25]. The histological changes that were taken place in the present study at the initial period of exposure at day 1 in the liver of the fish might be a part of defense mechanism. On further exposure for 4 days due to accumulation of phorate in the liver of the fish, it caused destruction in the organ structures. The degree of destruction in the liver of the fish appeared to be linearly proportional to the period of exposure [26].

4. Conclusions

On exposure to ALTP, though initially it caused a mild damage to the liver of the fish at day 1, further exposure to ALTP for 4 days, it caused irreversible damage to the liver of

the fish. The histopathological changes induced by ALTP 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 period of fish exposure to pesticides.

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