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A Study on the effect of organophosphorus insecticide phorate on brain histopathology of the common carp *Cyprinus carpio*

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Abstract
The present study is aimed to investigate the effect of sublethal concentrations of phorate on the histopathology of brain in the common carp, *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 chronic toxicity tests were carried out under laboratory conditions. On exposure for a period of one day to CSTP, necrosis of neurons, formation of minor vacuoles with degeneration of neural cells and distended sinusoids were observed in the brain of the fish. Significant hemorrhage was also noticed at some places. On exposure for a period of 7 days to CSTP, further structural damage, intracellular oedema and pycnotic nuclei were observed in the brain of the fish. After 15 days of exposure period there was an increase in necrosis of neurons, cytoplasmic vacuolization and lesions in the brain of the fish. After the exposure period of 30 days, reorganized neuronal tissue with indistinct nuclei was observed in the brain of the fish. The findings of the present study suggest that the frequency of pathological changes increase with the increasing exposure time to CSTP.

Keywords: Histopathology, *Cyprinus carpio*, Phorate, Necrosis, Sinusoids, Hemorrhage, Oedema.

1. Introduction
The 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. Tissue injuries and damages caused by pesticides in the organs of the fish can result in their reduced survival, growth and fitness [1-3]. Frequency and intensity of tissue lesions depend on the concentration of pesticides and the length of the fish exposure period to pesticides [4-7].
Susceptibility of a chemical injury varies greatly in the tissues and cells of the same animal and in different animal groups. However, the location of the major damage may be determined by the mode of action of the chemical. Some of the chemicals exert their effect locally at the portal of entry and some other toxic compounds do not cause damage at the portal of entry but affect the organs systematically in which they are accumulated. The extent of severity of tissue damage is a function of the concentration and potentiality of toxic compound accumulated in the tissues as it is time dependent [4-7].
Several reports are available on the cytoarchitectural damage in different organs of various animals exposed to pesticides [3, 7, 8-11]. Few reports of histological changes due to pesticides exposure on common carp are available [3, 12-14]. But the literature on phorate toxicosis on the commercially important fish, *C. carpio* is extremely sparse. Phorate is an organophosphate insecticide (OPI), which is widely used in agricultural fields on different 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. Thus the present work is an effort to assess the impact of CSTP on the histology of brain of the common carp, *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 for toxic studies [15].

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. 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 [16]. 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 [17], 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 [18]. 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 one day, group III for 7 days, group IV for 15 days and group V for 30 days. Then the fish were sacrificed and brain tissues were isolated under laboratory conditions for histopathological studies after the completion of stipulated exposure period.

2.6. Histopathology
The histological sections of the brain of the control and chronic toxicity exposed fish were taken by adopting the procedure as described by Humason [19]. 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, stained with hematoxylin [20] 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 normal brain of the control fish consists of clear neural cells with distinct nuclei. There was no discolouration, no significant lesion and any morphological change in the brain of control fish (Fig 1).

3.1.1. Histopathological study in brain
On exposure for a period of one day to CSTP, necrosis of neurons, formation of minor vacuoles with degeneration of neural cells and distended sinusoids were observed in the brain of the fish. Significant hemorrhage was also noticed at some places (Fig 2a). Further on exposure for a period of 7 days to CSTP, significant changes were observed in the structure of the brain of the fish compared to the fish brain at day one. Further structural damage, necrosis in neurons, intracellular oedema and pycnotic nuclei were observed (Fig 2b). On exposure of the fish *C. carpio* for a period of 15 days to CSTP, there was an increase in the necrosis of neurons and cytoplasmic vacuolization. Severe lesions were also noticed in the brain of the fish (Fig 2c). After the exposure period of 30 days, reorganized neuronal tissue with indistinct nuclei were observed in the brain of the fish *C. carpio* (Fig 2d).

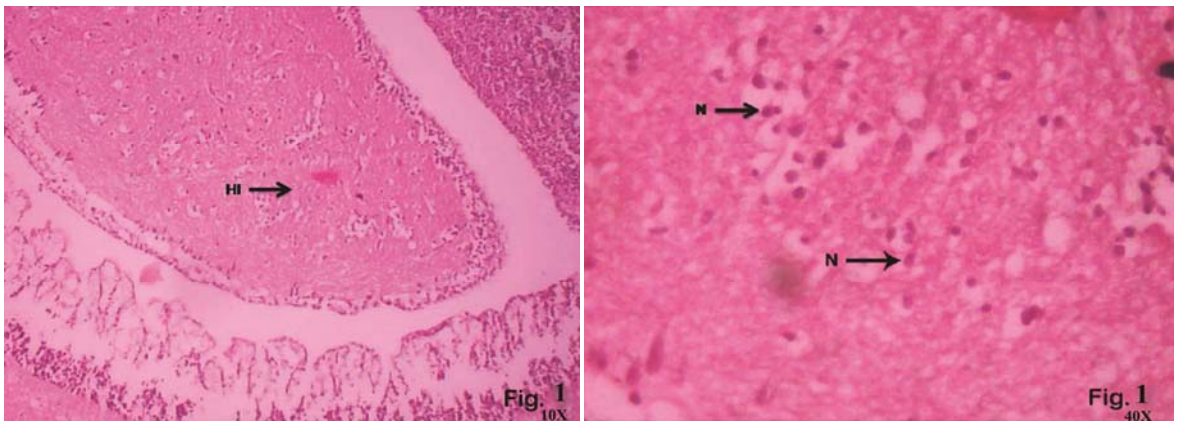

Fig 1: The normal architecture of the control fish brain tissue showing clear hippocampus (HI) and neural cells with distinct nuclei (N) with lower (10X) and higher magnification (40X). There was no discolouration, no lesion and any morphological change in the brain.

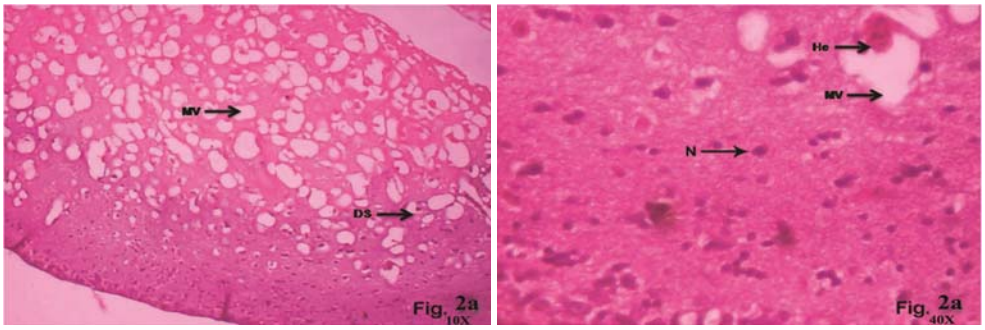

Fig 2a: The brain of the fish exposed to CSTP for one day showing nuclei (N), degenerative changes such as hemorrhage (He) distended sinusoids (DS) and minor vacoulation (MV) in normal cytoarchitecture with lower (10X) and higher magnification (40X).

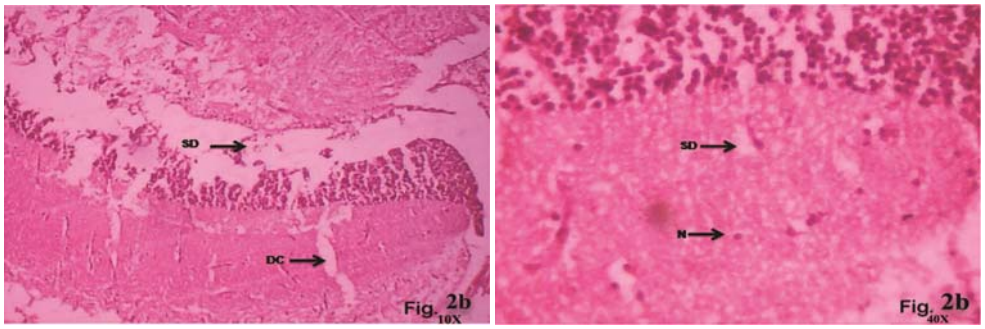

Fig 2b: The brain of the fish exposed to CSTP for 7 days showing nuclei (N), degenerative changes (DC) and structural degeneration (SD) with lower (10X) and higher magnification (40X).

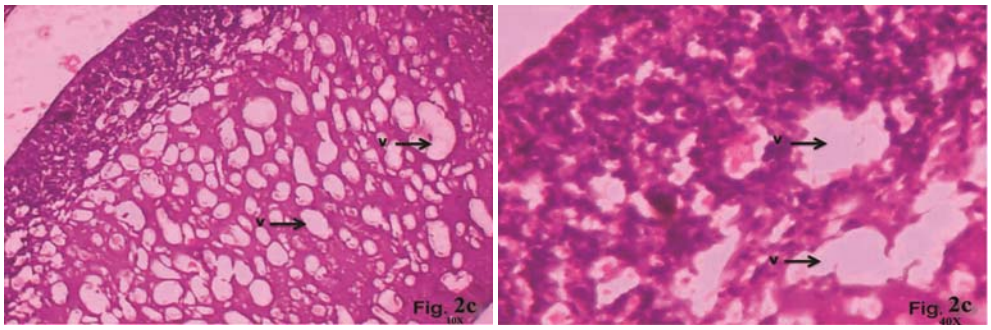

Fig 2c: The brain of the fish exposed to CSTP for 15 days showing degeneration of neural cells and formation of vacuoles (V) with lower (10X) and higher magnification (40X).

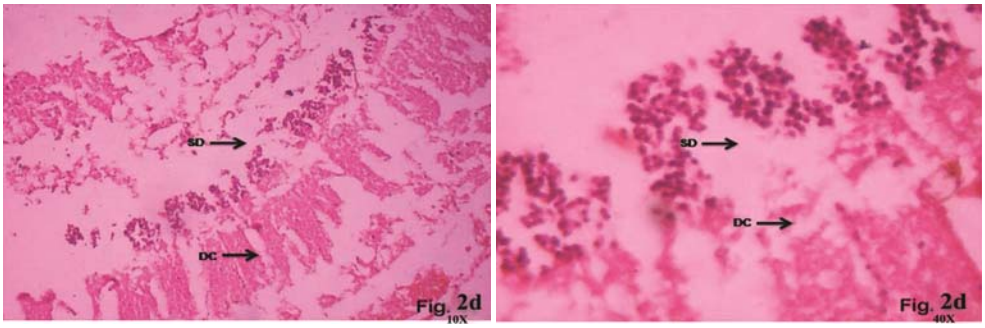

Fig 2d. The brain of the fish exposed to CSTP for 30 days showing degenerative changes (DC) such as degeneration of neural cells and structural damage (SD) with lower (10X) and higher magnification (40X).

3.2. Discussion

Various histopathological responses during the acute and chronic toxicity of pesticides 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, it is clearly indicated that the phorate has induced pronounced pathological changes in the brain of the fish *C. carpio* exposed to CSTP (Fig 2a to 2d). The histopathological responses of the fish *C. carpio* in the present study reveal the degree of damage caused by this pesticide to the brain of the fish. The extent of damage and the degenerative changes that were occurred in the brain of the

fish were progressive over the period of exposure to the CSTP suggest that the histopathological responses depend on the concentration of pesticide as well as the length of the fish exposure period to pesticides [3, 5, 7]. Several authors have reported earlier different histopathological alterations in the brain of fishes after exposing to different chemical substances [4-6, 21].

As brain is the controlling centre for all functions and movements in the body of organisms like fish, it serves as a relay station. The pesticides present in water reaches the fish body through water taken in with food and mucosa of the mouth or gills and they may reach the liver, muscle, kidney and brain through blood circulation. As the various regions in fish brain are concerned with many functions, the impairment of tissue of a particular region in the brain by the pesticides may lead into the curtailment of the particular function. This alters the physiological and behavioural functions of the fish. This is evident by respiratory distress, loss of equilibrium, erratic swimming and impairment in the fish behavior in the present study. This indicates that phorate has exerted a neurotoxic effect and impairment of neural conductivity in the central and peripheral nervous system. Bradbury *et al* [22] observed tremors and convulsions in rainbow trout due to the toxic effect of fenvalerate on the brain. Cope *et al* [23] and Sajitha Bhaskar [24] observed vascular dilation in fish brain on exposure to 2, 4-D and endosulfan respectively. Pugazhvendan *et al* [6] observed loss of differentiation, scattered arrangement and severe necrosis in the brain cells in *Ophiocephalus punctatus* exposed to malathion pesticide.

The histological changes that were taken place in the present study, at the initial period of exposure in the brain of the fish, might be a part of defense mechanism. On prolonged exposure for a period of 15 and 30 days due to further accumulation of phorate in the brain of the fish, it caused destruction in the brain structure. The slight structural reorganization of the brain of the fish observed at day 30 of exposure 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 brain 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 brain of the fish was linearly proportional to the period of exposure [3, 7].

4. Conclusions

On exposure to CSTP, though initially it caused a mild damage to the brain of the fish, further exposure for a period of 15 and 30 days it caused a pronounced damage. On prolonged exposure to 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 brain of the fish *C. carpio* are not only dependent on the concentration of the pesticide but also on the length of the 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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