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Brain histopathology of the fish *Cyprinus carpio* exposed to lethal concentrations of an organophosphate insecticide phorate

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Abstract
Cyprinus carpio (*C. carpio*) fish were exposed to acute lethal toxicity of phorate (ALTP) and its effect on the histopathological responses of the brain of the fresh water fish 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, mild degenerative changes were observed in the neural cells of the brain of fish. Initiation of the degeneration in the neural cells and structural degeneration were observed. On exposure for a period of 4 days, there was a further increase in the structural damage in the brain. Necrosis of neurons, intracellular oedema, congestion and degeneration of neural cells with the cytoplasmic vacuolization were observed. On exposure to ALTP, though initially it caused a mild damage to the brain of the fish at day 1 but further exposure to ALTP for 4 days, it caused a pronounced damage. The frequency and intensity of tissue lesions in the brain depend on the concentration of the pesticide as well as the length of the fish exposure period to the pesticide.

Keywords: *Cyprinus carpio*, acute lethal toxicity, phorate, necrosis, intracellular oedema, vacuolization.

1. Introduction
Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease or damage. Toxicological histopathology gives useful data concerning the changes induced by chemicals like pesticides at the tissue and cellular level [1-8]. All the tissues and organs in the body of an animal may be potential targets for the toxic effects of any chemical compound such as a pesticide. A histopathological assessment throws light on the nature of tissue alteration and the extent of damage which in turn indicates the toxic nature of the compound. Therefore, histopathology gives a useful insight in to the tissue lesions to prove the external manifestations of the deleterious effects of toxic chemicals [9].

Pesticides possess high toxicity not only to the target organisms but also to the non-target organisms [10-17]. These substances find their way in to the places far from application site and lead to alterations in metabolic activities of living organisms like fish by bio-accumulation [18-22]. Pesticide residues in the tissues of animals cause serious physiological alterations even at low levels [23, 24]. Johnson [25] and Verma *et al* [26] pointed out that a prolonged period of exposure to chemical compounds with very low concentration, results in the accumulation of more pesticide in the organs. Phorate is an organophosphorus insecticide (OPI) and acaricide used to control sucking and chewing insects, leafhoppers, leafminers, mites, some nematodes and rootworms [27, 28]. It is an important OPI to which the fresh water fishes are frequently exposed due to the indiscriminate use of it by the farmers. Hence the present investigation is aimed to assess the impact of ALTP, which is widely used in the local area to combat pests, on the brain histopathological responses 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 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, Anantapuramu, 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 [29]. 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. [30], 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 [31]. 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 and the group II & III were experimental groups. The group II was exposed to ALTP (LC_{50} of Phorate= 0.71 ppm/l) for one day and the group III for 4 days. Then the fishes were sacrificed and the tissues of brain 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 ALTP exposed fish were taken by adopting the procedure as described by Humason [32]. The 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, stained with hematoxylin [33] 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 lesion and any morphological change in the brain of control fish (Fig 1).

Histopathological study in brain
On exposure for a period of one day to ALTP, compared to the control, mild degenerative changes were observed in the neural cells of the brain of the fish *C. carpio*. Initiation of the degeneration of neural cells and structural degeneration were observed (Fig 2a). On exposure for a period of 4 days to ALTP, a further increase in the structural damage of the brain was observed. Necrosis of neurons, intracellular oedema and congestion of neural cells were noticed. Degeneration of neural cells with the cytoplasmic vacuolization was also observed (Fig 2b).

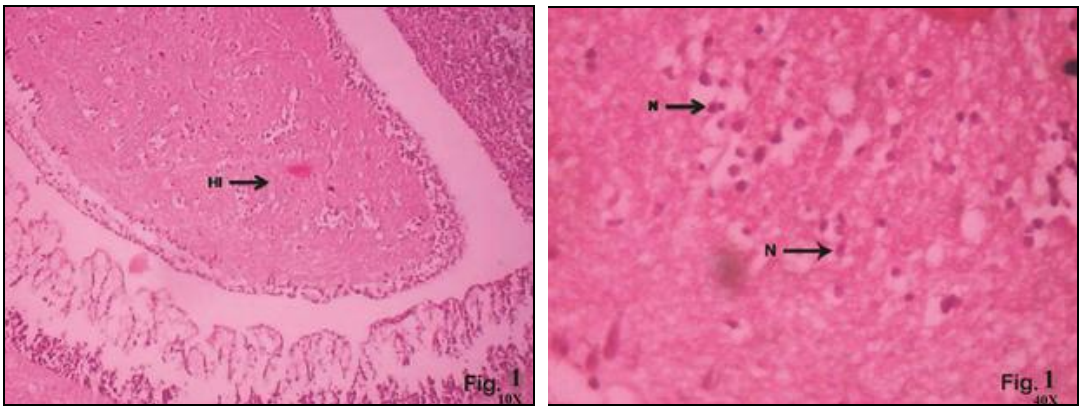

Fig 1: The normal architecture of the control fish brain tissue showing clear hippocampus (HI) and neural cells with distinct nuclei (N) with lower (10X) and higher (40X) magnifications. There was no discolouration, no lesion and any morphological change in the brain.

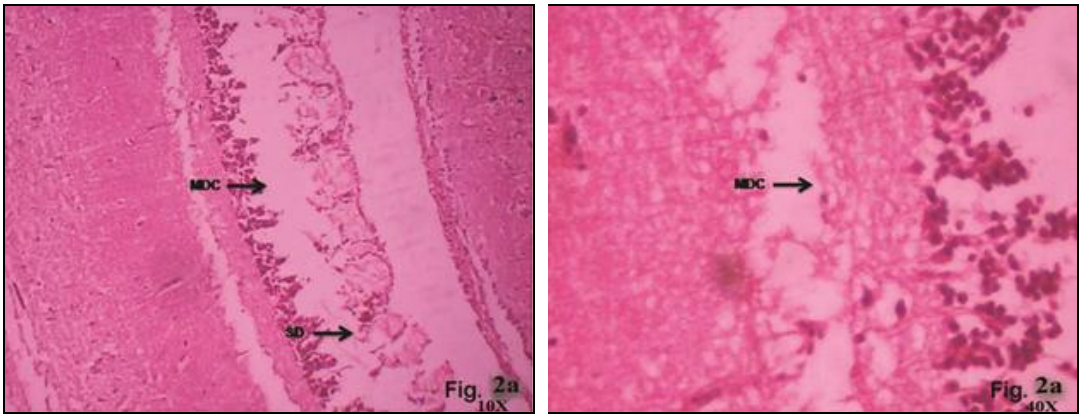

Fig 2a: The brain of the fish exposed to ALTP for one day showing clear neural cells and distinct nuclei with mild degenerative changes (MDC) and mild structural damage (SD) in the normal cytoarchitecture with lower (10X) and higher (40X) magnifications.

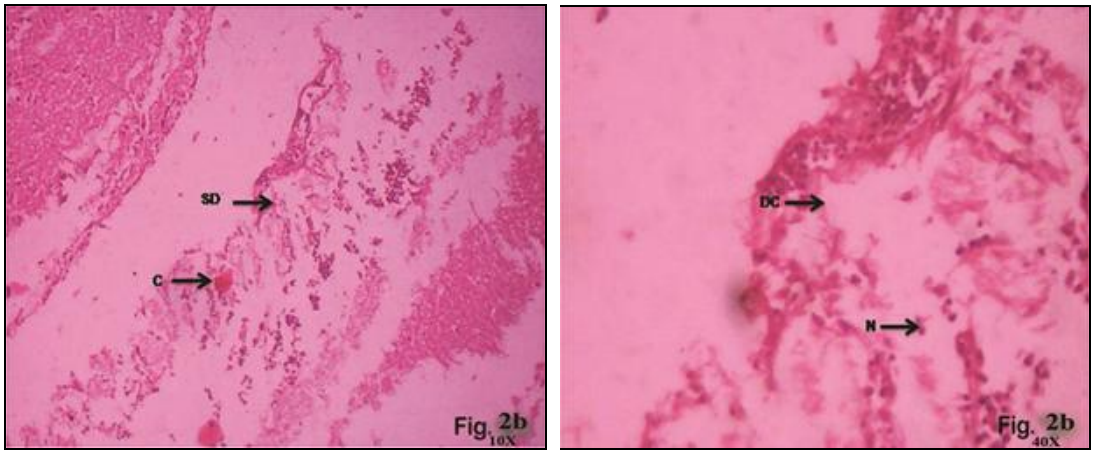

Fig 2b: The brain of the fish exposed to ALTP for 4 days showing nuclei (N), degenerative changes (DC) such as structural degeneration (SD) and congestion (C) of neural cells with lower (10X) and higher (40X) magnifications.

3.2. Discussion

As histopathology is the microscopic study of diseased or damaged tissue, it is an important tool of anatomical pathology since accurate diagnosis of diseases usually requires histopathological examination of samples. Even though biochemical studies may give an idea of the pathological state of the animal, a clear picture of cytoarchitectural changes produced during the chemical intoxication can be traced by histopathological studies. These studies would help in assessing the extent of pollution in the ecosystem by the pollutants such as pesticides and offer an exceptional opportunity to detect the effect of pollutants in various organs and organ systems of an organism. In the present study, it is clearly indicated that phorate has induced pronounced pathological changes in the brain of the fish *C. carpio* exposed to ALTP (Fig 2a and 2b). The histopathological responses of the fish reveal the degree of damage caused by this pesticide to the brain of the fish. The extent of damage caused and the degenerative changes that were occurred in the brain of the fish due to phorate toxicity were progressive over the period of exposure, suggest that the histopathological responses depend not only on the concentration of pesticide but also on the length of the fish exposure period to the pesticide.

Several authors have reported different histopathological alterations in the brain of fishes after exposing to different chemical substances [34-37]. Basanta Kumar Das and Subhas Chandra Mukherjee [34] observed mild vacuolar changes in the cerebrum with empty spaces after 0.35 ppm hexachlorocyclohexane exposure, whereas at 1.73 ppm they have observed severe necrosis of neuronal cells of cerebrum and loss of Nissl substance in the brain of the Indian major carp (*Labeo rohita*), exposed to hexachlorocyclohexane. Ayoola and Ajani [35] reported mononuclear infiltration, neuronal degeneration and severe spongiosis in the brain of the fish *Clarias gariepinus* after exposing to lethal concentrations of cypermethin. There was a severe congestion, mononuclear infiltration, haemorrhage and generalized spongiosis in the brain of the fish *Oreochromis niloticus* exposed to lethal concentrations of glyphosate [36]. The experimental fish brain showed disintegration and severe damage in the brain cells and broke down of neural bundles after exposing to different concentrations of malathion [37]. Various regions in fish brain are concerned with different kind functions. The impairment of tissue of a region in the brain by these pathological changes may lead into the curtailment of the particular function in fishes. This alters the physiological and behavioural functions of the fish. This is evidenced in the behaviour of the fish in the form of respiratory distress, loss of equilibrium and erratic swimming. Bradbury *et al* [38] observed tremors and convulsions in rainbow trout due to the toxic effect on brain on exposure to fenvalerate. Cope *et al* [39] and Sajitha Bhaskar [40] observed vascular dilation in fish brain on exposure to 2, 4-D and endosulfan respectively. Pugazhvendan *et al* [37] observed scatterly arranged cells, severe necrosis and loss of differentiation in the brain cells in *Ophiocephalus punctatus* exposed to malathion pesticide. Thus 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 due to further accumulation of phorate in the brain of the fish, it caused destruction in this organ structures.

4. Conclusions

On exposure to ALTP, though initially it caused a mild damage to the brain of the fish at day1, further exposure for 4 days it caused a pronounced damage. Thus the histopathological changes induced by ALTP 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 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.

5. References

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