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Phorate induced histopathological changes in the muscle of the common carp *Cyprinus carpio* (Linnaeus, 1758)

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Abstract
The present study is aimed to investigate the effect of sublethal concentrations of phorate on the histology of muscle 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 1, 7, 15 and 30 days and the chronic toxicity tests were carried out under laboratory conditions. On exposure for a period of 1 day to CSTP, mild degenerative changes were observed in the structure of the muscle of the fish. On further exposure for a period of 7 days, isolation and thinning of muscle fibers was observed. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei. Cellular degeneration and increasing cytoplasmic vacuolization were also noticed. On exposure for a period of 15 days to CSTP, further splitting and thinning of muscle fibers was observed. Degeneration of muscle fibers, nuclei and cellular necrosis were also noticed. On exposure for a period of 30 days, further degeneration in the structure of the muscle was observed. There was heavy fibrilization with the loss of total muscular integrity and appearance of muscle fibers. The nuclei become highly pyknotic and scattered without any proper organization. The structure of the muscle was lost with cellular degeneration and cytoplasmic vacuolization in the fish *C. carpio*. The findings of the present investigation suggest that the frequency of pathological changes increase with the increasing exposure time to CSTP.

Keywords: Phorate, *Cyprinus carpio*, Histology, Pyknotic nuclei, Fibrilization, Vacuolization

1. Introduction
Toxicity is the degree to which a substance can harm the animals like fish. The level of toxicity of a toxicant like pesticide can be measured in terms of its concentration or dose which kills a known number of populations of a given species within a definite period of time. Evaluation of the toxicity of a chemical like pesticide could help in knowing its potentiality. The toxicity tests on the non-target organisms like fish would help to understand the hazardous nature of pesticides and to improve the health condition of mankind on a long run. The pesticides are varying greatly in their action, toxicity and persistence. Several studies have been conducted in the evaluation of the toxicity of pesticides to the aquatic biota especially fishes [1-3].

The wide use of fishes in the toxicity tests is probably due to their adaptability to the laboratory conditions as well as their availability and their varying degree of sensitivity to the toxic substances [4]. Organophosphates are highly toxic to fish and other non-target aquatic organisms, as they inhibit AChE activity [5, 6]. Several researchers investigated the toxicity of organophosphorus pesticides in fish [7-13].

Research in the area of toxicology on the effects of phorate on fish is scarcely done. However, some work was carried out by Saxena and Sarin [14, 15] on desert gerbil *Merioneshurrianae*; Mohssen Morowati [16-18] on the male swiss albino mouse, *Musmusculus*; Jyothi and Narayan [19] on fresh water fish *Clarias batrachus* and Anand Pratap Singh *et al* [20] on snake headed fish *Channa punctatus*, about the toxic effects of phorate. Reported 96-hours LC_{50} values of phorate are 0.8 ppm in *Clarias batrachus* [19] and 0.3 mg/l in *Channa punctatus* [20].

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 [21].

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. 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 [22]. 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 [23], 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 [24]. 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 muscle tissues were isolated under laboratory conditions for histopathological studies after the completion of stipulated exposure period.

2.6 Histopathology
The histological sections of the muscle of the control and chronic toxicity exposed fish were taken by adopting the procedure as described by Humason [25]. 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 [26] 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 muscle of the normal control fish consist compactly packed muscle fibers with definite intermuscular spaces. There was no splitting in muscle fibers. The intermuscular spaces appeared to be filled with viscous fluid. Round to spindle shaped nuclei were found distributing all over the bundle length with occasional hyper chromacia (Fig.1).

3.1.1 Histopathological study in muscle
On exposure for a period of 1 day to CSTP, mild degenerative changes were observed in the structure of the muscle of the fish *C. carpio*. The muscle fibers exhibited longitudinal splitting with cellular degeneration (Fig 2a). On further exposure for a period of 7 days, isolation and thinning of muscle fibers was observed. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei. Cellular degeneration and increasing cytoplasmic vacuolization were also noticed (Fig 2b). On exposure for a period of 15 days to CSTP, further splitting in the muscle fibers followed by their thinning was observed. Degeneration of muscle fibers, nuclei and cellular necrosis were also noticed (Fig 2c). On exposure for a period of 30 days, further degeneration in the structure of the muscle was observed. There was heavy fibrilization with the loss of total muscular integrity and appearance of muscle fibers. The nuclei become highly pyknotic and scattered without any proper organization. The structure of the muscle was lost with cellular degeneration and cytoplasmic vacuolization in the fish *C. carpio* (Fig 2d).

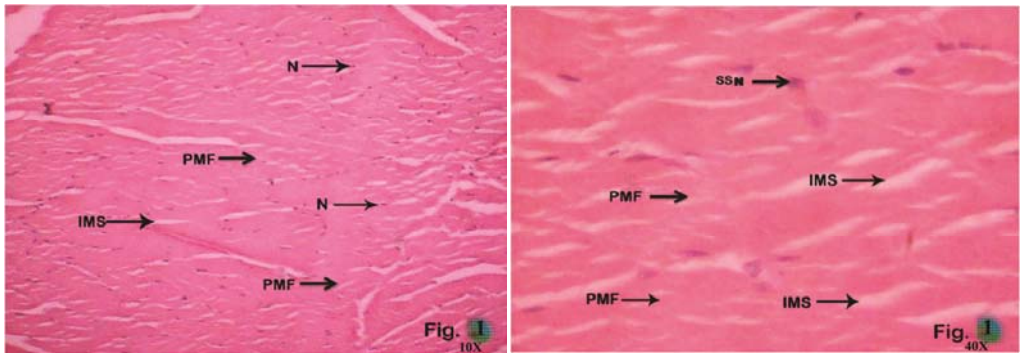

Fig 1. The normal architecture of the control fish muscle tissue showing compactly packed muscle fibers (PMF), nucleus (N), inter muscular spaces (IMS) and round to spindle shaped nucleus (SSN) with lower (10X) and higher magnification (40X).

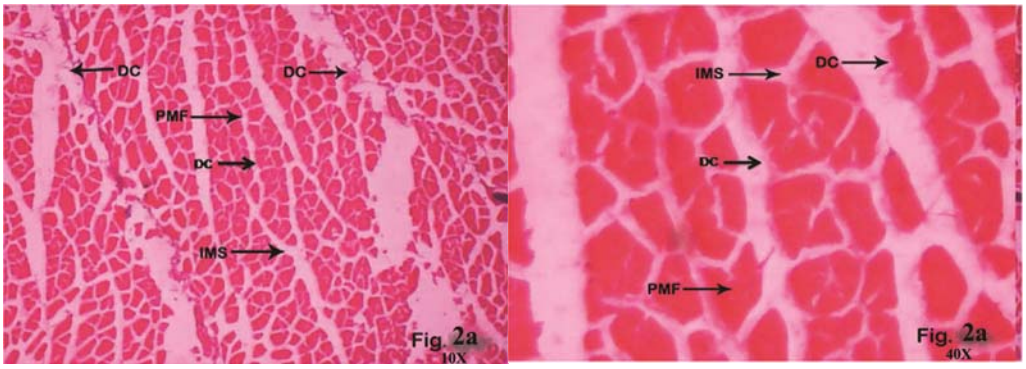

Fig 2a. The muscle of the fish exposed to CSTP for 1 day showing packed muscle fibers (PMF) and inter muscular spaces (IMS) with mild degenerative changes (DC) in normal cytoarchitecture with lower (10X) and higher magnification (40X).

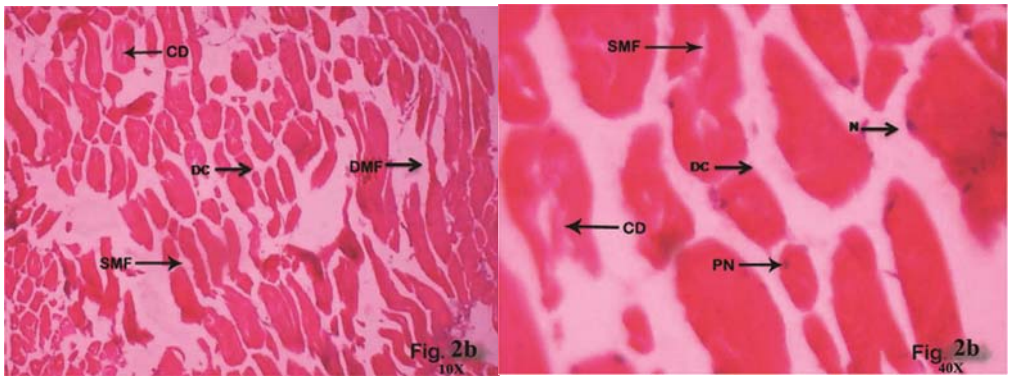

Fig 2b. The muscle of the fish exposed to CSTP for 7 days showing nuclei (N), degenerative changes (DC) such as splitting of muscle fibers (SMF), pycnotic nucleus (PN), cellular degeneration (CD) and degeneration of muscle fibers (DMF) with lower (10X) and higher magnification (40X).

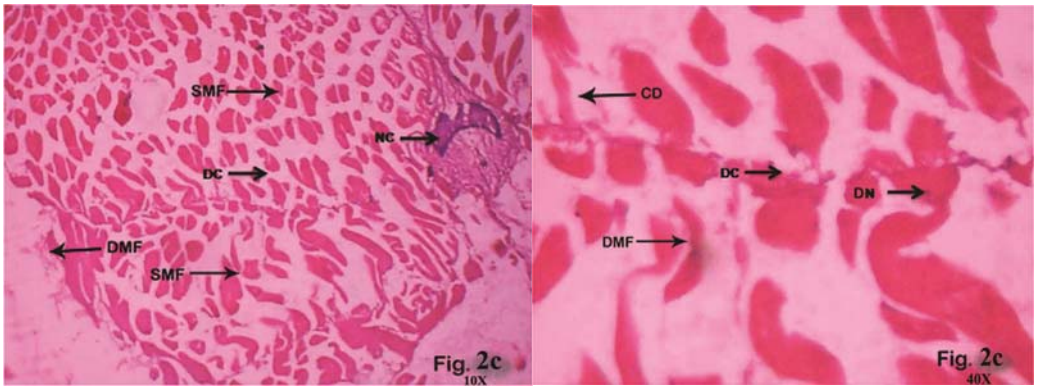

Fig 2c. The muscle of the fish exposed to CSTP for 15 days showing degenerative changes (DC) such as splitting of muscle fibers (SMF), necrotic changes (NC), degeneration of nucleus (DN), cellular degeneration (CD) and degeneration of muscle fibers (DMF) with lower (10X) and higher magnification (40X).

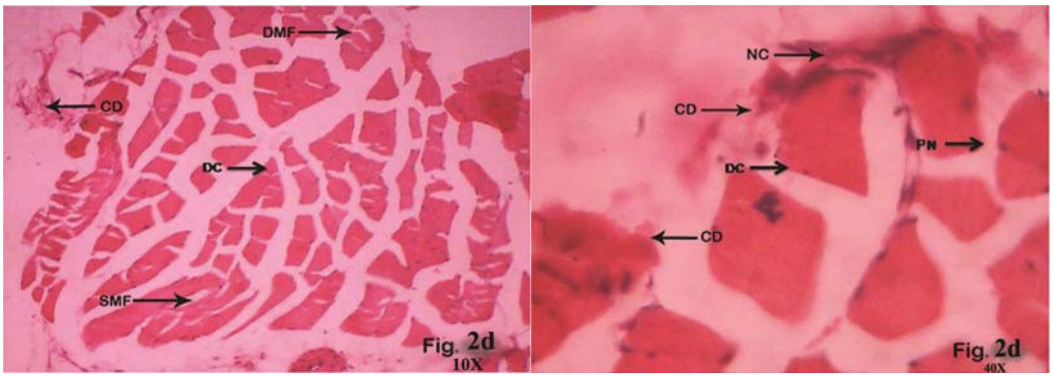

Fig: 2d. The muscle of the fish exposed to CSTP for 30 days showing further increase in the degenerative changes (DC) such as splitting of muscle fibers (SMF), necrotic changes (NC), pyknotic nuclei (PN), cellular degeneration (CD) and degeneration of muscle fibers (DMF) with lower (10X) and higher magnification (40X).

3.2 Discussion

Histopathological investigations on the tissues of fish are valuable tools for toxicology studies, as these investigations can provide information about the health and functionality of organs of the animals like fish. In the present study, it is clearly indicated that the phorate has induced pronounced pathological changes in the muscle of the fish *C. carpio* exposed to CSTP (Fig 2a to 2d). The histopathological responses of the fish in the present study reveal the degree of damage caused by this pesticide to the muscle tissues of the fish. The extent of damage caused by phorate to the muscle of the fish and the degenerative changes that were occurred in the muscle were progressive over the period of exposure to CSTP suggest that the histopathological responses depend on the concentration of pesticides and also on the length of the fish exposure period to pesticides.

The pathological changes like in the present study were observed by several investigators in the muscle of fish on exposure to different substances. After exposing to 1/10 and 1/5 sub-lethal doses of hexachlorocyclohexane, Basanta Kumar Das and Subhas Chandra Mukherjee [27] observed the histopathological changes in the muscle of fish such as marked thickening, separation of muscle bundles and severe intramuscular oedema in the Indian major carp (*Labeo rohita*),

during a 45-day trial period. Mild lesions, necrosis, inclusion bodies, inflammation and cellular degenerations were observed by Ayoola Simeon Oluwatoyin [28] in the muscle of Nile tilapia (*Oreochromis niloticus*) juveniles exposed to lethal concentrations (96 h LC_{50}) of aqueous and ethanolic extracts of *Ipomoea aquatica*leaf.

In the present study the initial stimulus of phorate, induced hyperactivity and excitability that was observed in the muscular behaviour of the fish *C. carpio*, resulted a subsequent release of lactic acid and muscular fatigue. All these changes were clearly evident as clinical signs at the initial stage of the investigation and were subsequently reflected through histopathological changes in muscle tissue [29]. The histological changes that were taken place in the present study, at the initial period of exposure in the muscle of the fish on exposure to CSTP might be a part of defense mechanism of the fish. On prolonged exposure due to further accumulation of phorate in the muscle of the fish, it caused destruction in the organ structures. The slight structural reorganization of the muscle 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 muscle 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 muscle of the fish appeared to be linearly proportional to the period of exposure [30, 31].

4. Conclusions

On exposure to CSTP, though initially it caused a mild damage to the muscle of the fish at day 1, further exposure for 7, 15 and 30 days it caused to the destruction in this organ. 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 muscle 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 as well as the length of the fish exposure period to pesticides.

5. References

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