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RESEARCH ARTICLE

TOXIC EFFECT OF PHORATE ON THE HISTOPATHOLOGY OF MUSCLE IN THE FRESH WATER FISH *CYPRINUS CARPIO* EXPOSED TO LETHAL CONCENTRATIONS

***Lakshmaiah, G.**

Department of Zoology, Sri Krishnadevaraya University, Anantapuramu-515003, A.P, India

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ABSTRACT

Fingerlings of *Cyprinus carpio* (*C. carpio*) 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 toxic effect of acute lethal concentrations of phorate on the histopathology of muscle was investigated in the present study. Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the probit method of Finney and the LC_{50} of phorate was taken as lethal concentration for acute toxicity study. On exposure for a period of 1 day to ALTP, splitting of muscle fibers followed by their thinning was observed in the muscle of the exposed fish. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei. On exposure for a period of 4 days, a further increase in the longitudinal splitting of muscle fibers with pyknotic nuclei and cellular degeneration were observed. Karyolysis seems to have occurred as granular debris. The isolation of muscle fibers from one and another was noticed with necrotic changes. The findings of this study demonstrated that the histopathological changes induced by ALTP in the muscle of *C. carpio* were not only dependent on the concentration of the pesticide but also on the length of the fish exposure period.

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INTRODUCTION

The pollution of water bodies such as rivers and streams with chemical contaminants like pesticides has become one of the most critical environmental problems in the present days. As these pollutants are transported from the industrial and agricultural areas into the aquatic environment, many freshwater ecosystems are facing problems with high levels of chemical substances like pesticides. The intake of pesticides such as organophosphates (OPs) affect the biochemical composition (Jebokumar *et al*., 1990; Prasad *et al*., 2002) and histological aspects (Ram Nayan Singh, 2012; Aswin *et al*., 2016) of fishes and other aquatic organisms present in the water bodies polluted with pesticides. Since fishes are the nutritious and easily digestible food sources for human beings and the other animals, the assessment of their health condition is very important. Basal metabolism in fishes is relatively constant under constant environmental conditions. Pesticides are known to interfere with the cellular metabolisms of fishes and may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (John, 2007; Banaee *et al*., 2011a). Pesticides can cause serious impairment to physiological and health status of fishes (Begum, 2004; Monteiro *et al*., 2006; Siang *et al*., 2007).

****Corresponding author: Lakshmaiah, G.***
Department of Zoology, Sri Krishnadevaraya University, Anantapuramu-515003, A.P, India.

Phorate is an important organophosphorus insecticide (OPI), to which the fresh water fishes are frequently exposed due to the indiscriminate use of it by the farmers, is used to control sucking and chewing insects, leafhoppers, leafminers, mites, nematodes and rootworms (Wagner, 1989; Gallo and Lawryk, 1991). The common carp, *C. carpio* is a very important staple fish generally found in rivers, ponds, and reservoirs. 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 (Sreenivasan and Swaminathan, 1967). Nutritional value of fish like *C. carpio*, depends on their biochemical composition, physiological and health status, that are affected by the water pollution (Prado *et al*., 2009) with pollutants like pesticides. Hence the present study is aimed to assess the impact of ALTP, on muscle histopathology in the fresh water fish *C. carpio*, a representative of the aquatic environment.

MATERIALS AND METHODS

Test Species

The Indian major carp *C. carpio* (Linnaeus, 1758) has been selected as test species for the present study. It is an economically important edible fish, having great commercial value.

Test Chemical

Pesticide selected for this study is phorate (O, O-diethyl S-ethylthiomethyl phosphorodithioate) an OPI which is widely used throughout the world, especially in Andhra Pradesh, India as a broad spectrum insecticide on numerous crops. Commercial names of phorate are thimet, rampart, granutox, agrimet etc and its molecular formula is $C_7 H_{17} O_2 PS_3$.

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 (Doudoroff *et al*., 1951). Water was renewed every day to provide fresh water, rich in oxygen. During experimentation water was aerated once a day to prevent hypoxic conditions, if any (Khorram and Knight, 1977). 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 (Sivaramakrishna *et al*., 1991), precautions were taken throughout this investigation to control all these factors as far as possible.

Acute toxicity procedures

Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the probit method of Finney (Finney, 1971). LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration to study acute toxicity of phorate (ATP).

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 ATP (LC_{50} of phorate= 0.71 ppm/l) for 1 day and the group III for 4 days. Then the fishes were sacrificed and muscle tissues were isolated under laboratory conditions for histopathological studies after the completion of stipulated exposure period.

Histopathology

The histological sections of the muscle of control and acute toxicity exposed fish were taken by adopting the procedure as described by Humason (Humason, 1972). 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 (Harris, 1900) 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.

RESULTS AND DISCUSSION

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).

Histopathological study in the muscle

On exposure for a period of 1 day to ALTP, splitting of muscle fibers followed by their thinning was observed in the muscle of the fish *C. carpio*. The muscle fibers exhibited longitudinal splitting with pyknotic nuclei (Fig. 2a). On exposure of for a period of 4 days, a further increase in the longitudinal splitting of muscle fibers with pyknotic nuclei and cellular degeneration were observed. Karyolysis seems to have occurred as granular debris. The isolation of muscle fibers from one and another was noticed with necrotic changes (Fig. 2b).

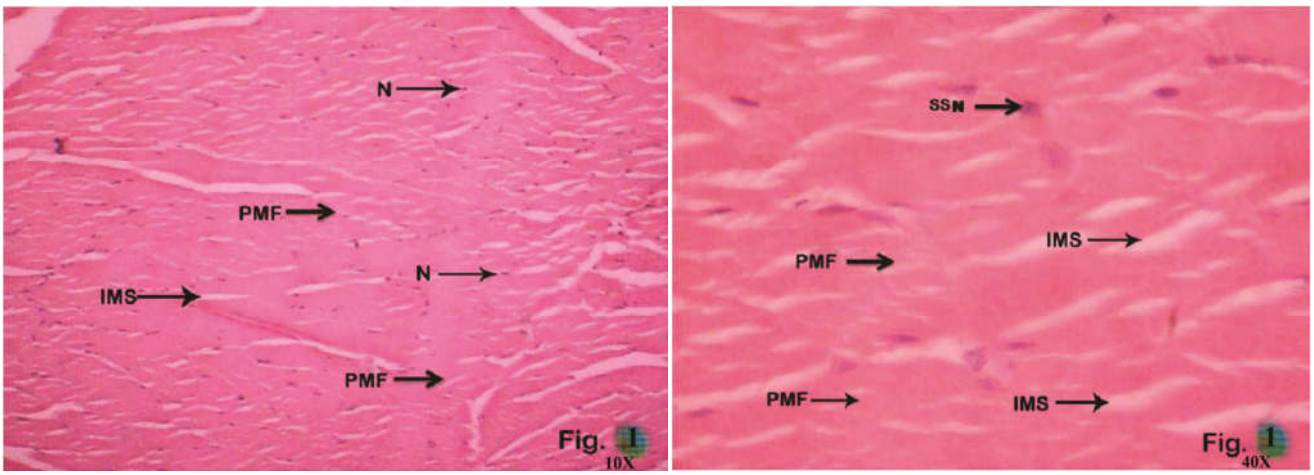

Fig. 1. The normal architecture of the control fish muscle tissue showing compactly packed muscle fibers (PMF), nucleus (N), inter muscular spaces (IMS) and round to spindle shaped nucleus (SSN) with lower (10X) and higher magnification (40X)

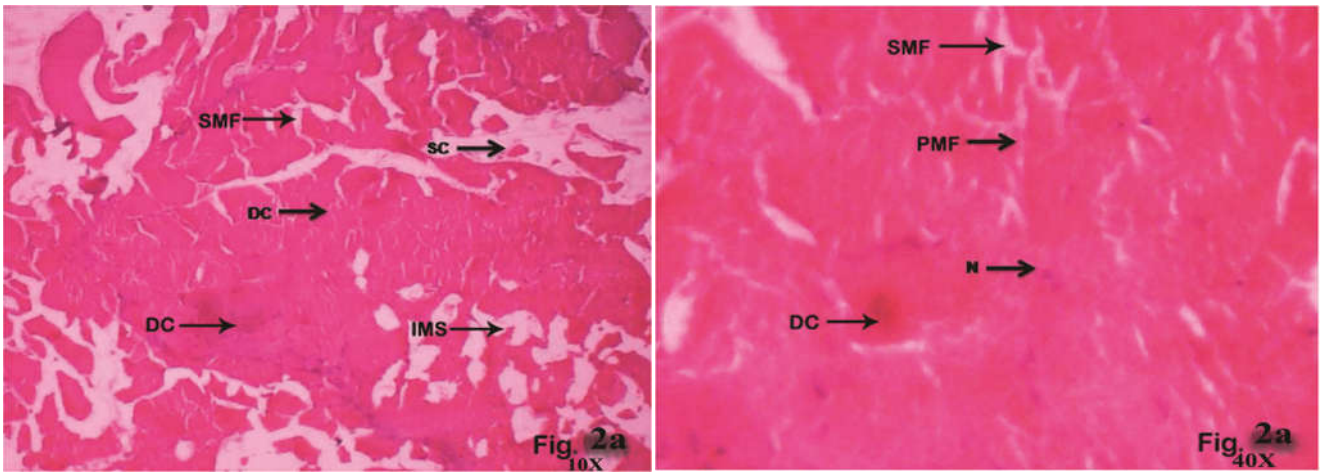

Fig. 2a. The muscle of the fish exposed to ALTP for 1 day showing packed muscle fibers (PMF), nuclei (N) and degenerative changes (DC) such as initiation of structural changes (SC), splitting of muscle fibers (SMF) and increasing inter muscular spaces (IMS) with lower (10X) and higher magnification (40X)

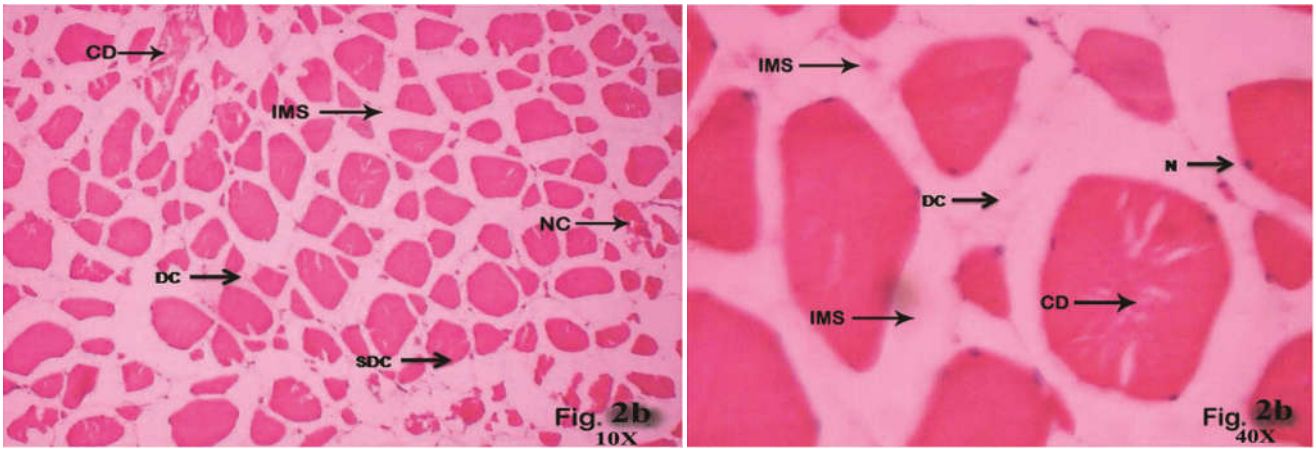

Fig. 2b. The muscle of the fish exposed to ALTP for 4 days showing nuclei (N), structural degenerative changes (SDC), necrotic changes (NC), degenerative changes (DC), cellular degeneration (CD) and increasing inter muscular spaces (IMS) with lower (10X) and higher magnification (40X)

DISCUSSION

A histological assessment in the toxicological studies, demonstrates the nature of tissue alterations and the extent of damage to the organs, in the exposed animals like fishes. This in turn indicates the toxic nature of the compound used. Therefore, the histology gives useful insight into the tissue lesions prior to the external manifestations of the deleterious effects of a chemical substance (Jayantha Rao *et al*., 1984). 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 ALTP (Fig 2a and 2b). The extent of damage caused by phorate to the muscle and the degenerative changes that were occurred in the muscle of the fish were progressive over the period of exposure to the ALTP, suggest that the histopathological responses depend on the concentration of pesticides as well as 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. Basanta Kumar Das and Subhas Chandra Mukherjee (2000) observed the dystrophic changes with marked thickening, separation of muscle bundles and severe intramuscular oedema in the Indian major carp (*Labeo rohita*), exposed to 1/10 and 1/5 sub-lethal doses of hexachlorocyclohexane during a 45-day trial period. Ayoola Simeon Oluwatoyin (2011) observed mild lesions, necrosis, inclusion bodies, inflammation and cellular degenerations 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 (Das, 1998). The pathological changes that were observed in the muscle of the fish suggest that significant concentration of pesticide accumulation in this organ. These changes affect the contractile ability and cause for disfunctioning of muscle fibers. The histological changes that were taken place in the present study, at the initial period of exposure for 1 day in the muscle of the fish might be a part of defense mechanism. On prolonged exposure for 4 days due to the further accumulation

of phorate in the muscle of the fish, it caused destruction in the organ structures.

Conclusion

On overall assessment, on exposure to ALTP, though initially it caused a mild damage to the muscle of the fish at day 1, but further exposure for 4 days it caused an irreversible damage to the muscle organ. Thus the changes induced by ALTP 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 exposure period. Frequency and intensity of tissue lesions depend on the concentrations of pesticides and the length of the fish exposure period to pesticides.

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