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Acute lethal and chronic sublethal toxic stress induced alterations in lactate dehydrogenase activity of phorate intoxicated freshwater fish *Cyprinus carpio*

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Abstract
Cyprinus carpio fish were exposed to acute lethal toxicity (LC_{50}/96 h) of phorate (ALTP) for one day and 4 days and chronic sublethal toxicity (one-tenth of the LC_{50}/96 h) of phorate (CSTP) for 1, 7, 15 and 30 days and the lactate dehydrogenase (LDH) enzyme activity was observed in the target organs of the fish such as gill, liver, muscle, kidney and brain. Relative to controls, a significant increase was observed in the activity of LDH in all the target organs of the fish exposed to both acute and chronic toxicity of phorate (ACTP). On comparison, the elevation in the LDH activity is more in all the target organs of the fish exposed to the ALTP. The results obtained demonstrate that the lethal and sublethal concentrations of phorate have some deleterious effect on the basic activities of the enzyme LDH in all the target organs of the experimental fish.

Keywords: Phorate, Lactate dehydrogenase, *Cyprinus carpio*, Acute lethal, Chronic sublethal.

1. Introduction
The environmental pollution caused by the chemical substances such as pesticides is a serious problem for living organisms including human being [1]. Water is one of the most essential needs for maintenance of life on earth. Due to rapid population growth, urbanization and industrial proliferation pollution of water bodies is increasing steadily. The contamination of water bodies with a broad spectrum of pollutants has become a matter of serious concern all over the world [2]. The aquatic environment is currently under threat by the indiscriminate use of synthetic pesticides and causing high risk to non-target organisms [3] including fish. As food is one of the most important routes by which human beings are exposed to toxic substances like pesticides, investigations on the effect of the pesticides in fish have a diagnostic significance because fishes serve as the nutritious and easily digestible food sources for human beings.

The pesticides play an important role in the production and preservation of food and other commercial crops by keeping a check on many species of harmful pests. Organophosphates (OPs) are widely used among synthetic pesticides in agriculture and in health and hygiene programs due to their high effectiveness as insecticides and less persistence in the environment, leading to one of the major causes of aquatic pollution. Phorate is an organophosphorus insecticide (OPI) which is widely used as a broad spectrum insecticide on numerous crops including paddy and groundnut. The shift from organochlorines to OPs has resulted in an increased occurrence of OPs into water bodies causing acute and chronic toxicity to fish fauna [4-6]. Pesticides are carried into the aquatic ecosystem by surface runoff from the sites of application and therefore the health of the aquatic ecosystem is being adversely affected because they serve as an ultimate sink for these pesticides [7].

Pesticides can induce noticeable changes in the activities of different enzymes in the freshwater animals like fish [8-12]. LDH is considered to be the most important enzyme of the glycolytic pathway in animals, including fishes as it is the key enzyme located at the vital point between glycolysis and TCA cycle. As the LDH is a terminal enzyme of anaerobic glycolysis, it has crucial importance to the muscle physiology, particularly in conditions of chemical stress, when high levels of energy required in a short period of time [13, 14]. It is likely that any fluctuation in the cellular environment alters the activity of LDH and the changes in its activity indicate the damage to any or all organs producing this enzyme such as liver and

Kidney [15]. The changes in the activity of LDH provide a direct and indirect evidence of the toxic mechanism of the pesticides.

Pesticides become easily available in the food chain and subsequent accumulation in both aquatic and terrestrial flora and fauna [16] with possible unquantifiable disastrous consequences on the ecosystem [17]. Due to the residual effects of pesticides, important organ like liver and kidney are damaged in fishes [18]. The intake of insecticides like phorate affects the biochemical composition of fishes [19, 20]. Hence, the role of any pesticide can be well understood by analyzing the tissue level changes in an animal species. Thus, the present study is aimed to assess the changes in the levels of LDH activity in the tissues of vital organs of fish such as gill, liver, muscle, kidney and brain of the edible fresh water teleost fish, *C. carpio* exposed to acute lethal and chronic sub-lethal toxicity of phorate.

2. Materials and Methods

2.1 Material

2.1.1 Test species

The Indian major carp *Cyprinus carpio* (Linnaeus, 1758) has been selected as a test species for the present investigation. It is an economically important edible fish, having great commercial value. The animals were starved for 24 hours prior to each estimation to avoid any influence of differential feeding.

2.1.2 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 $C_7 H_{17} O_2 PS_3$.

2.2. Methods

2.2.1 Acute and chronic toxicity procedures

Lethal concentration (LC_{50}) of phorate to *C. carpio* was determined by the Probit method of Finney [21]. LC_{50}/96 h (0.71 ppm/l) of phorate was taken as lethal concentration to study acute toxicity and one-tenth of the LC_{50}/96 h (0.071 ppm/l) concentration of phorate was taken as the sub-lethal concentration for chronic toxicity study.

2.2.2. Experimental design

160 fishes were divided into two batches, again batch I was divided into 3 groups and batch II into 5 groups comprising of 20 fishes each. The batch I was exposed for ALTP (exposed to LC_{50} of Phorate) and the batch II for CSTP (exposed to sub lethal concentration = 1/10th of LC_{50} - 0.071 ppm/l). In the batch I, group 1 was considered as normal control, group 2 and 3 were experimental groups. The fishes of group 2 were exposed for 1 day and group 3 for 4 days. In the batch II**,** group 1 was considered as a normal control group, group 2, 3, 4 and 5 were experimental groups. The fishes of group 2 were exposed for 1 day, group 3 for 7 days, group 4 for 15 days and group 5 for 30 days.

2.2.3 Estimation of Lactate dehydrogenase (L-Lactate NAD oxidoreductase, EC: 1.1.27) activity

LDH activity in the organs of the fish was estimated using the method of Srikanthan and Krishnamoorthi [22].as modified by Govindappa and Swami (1965). A 5% homogenate (w/v) of the tissues was prepared in 0.25 M ice-cold sucrose solution, centrifuged at 2500 rpm for 15 min and the supernatant was taken as the source of the enzyme. The incubation mixture consisted of 0.2 ml of 0.4 M phosphate buffer (pH 7.4), 0.5 ml of 0.1 M lithium lactate, 1.0 ml of 0.0001M nicotinamide adenine dinucleotide (NAD), 1.0 ml of 0.004 M 2-(p-indophenol)-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT) and 0.5 ml of enzyme preparation. The mixture was incubated at 37 ^{0}C for 30 min and the reaction was stopped by adding 6.0 ml of glacial acetic acid. The formazone formed was extracted in 6.0 ml of toluene by keeping overnight at 0 ^{0}C and the optical density of the color developed was read at 495 nm in a spectrophotometer. A blank taking 0.5 ml of distilled water and control by taking 0.5 ml of boiled enzyme were also run similarly. INT standards were prepared alongside for comparison. The enzyme activity was expressed as µ M of formazone formed/mg protein/h.

2.2.4 Statistical analysis

Duncan's Multiple Range (DMR) test had been employed for the statistical analysis of the LDH activity level data. P value (level of significance) is significant at < 0.05.

3. Results

The data on the activity of LDH in the organs of the fish such as gills, liver, muscle, kidney and brain of the fish *C. carpio* at 1 and 4 days of exposure to ALTP and 1, 7, 15 and 30 days of exposure to CSTP, besides controls, are presented in the Table 1. For comparison, the differences obtained in relation to the controls of each organ of the fish at the above said exposure periods in ACTP, were converted as percentages of the corresponding controls and those percent values are also presented in the same table and was plotted a graph of percent changes against exposure periods in Figure 1.

3.1 Activity of Lactate dehydrogenase

From the data presented in the Table 1 and Figure 1 relative to controls, a significant increase was observed in the activity of LDH in all the target organs of the fish exposed to both ACTP. The LDH activity in the liver of both controls and experimental fish is higher than in the other organs of the fish. The percent increase was also more in the liver when compared to the other organs of the fish exposed to both ACTP. In the fish exposed to ALTP, the increase in the activity of LDH was more at day 4 than at day 1 in all the target organs of the fish in the order of the day 1<4. In the fish exposed to CSTP, there was a gradual increase in the activity of LDH from day 1 to day 7 but from the day 15 to day 30 a decrease in the increment was observed in the order of the day 1<7>15>30. The differences in the activity of LDH between controls and experimental were also found to be statistically significant (P<0.05).

4. Discussion

Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically [23]. Changes in the activity of the enzymes like LDH are sensitive to environmental pollutants like pesticides [24]. In the present investigation the non-oxidative enzyme LDH showed an elevation in its activity in all the osmoregulatory (gill and kidney) and non-osmoregulatory (liver, muscle and brain) tissues of the fish *C. carpio*, which indicates the suppression of oxidative metabolism in the fish exposed to both ACTP [25]. In support of present investigation, several investigators reported

Table 1: LDH activity in different organs of the fish *C. carpio*.

Organ		Exposure period in days							
		Acute toxicity			Chronic toxicity				
		Control	1	4	Control	1	7	15	30
Gill	Mean S.D. ± % change	0.183^{a} 0.0026	0.234^{b} 0.0069 +28.15	0.308^{c} 0.0028 +68.61	0.183^{a} 0.0026	0.219^{c} 0.0029 +20.01	0.244^{e} 0.0025 +33.54	0.229^{d} 0.0034 +25.45	0.206^{b} 0.0017 +12.99
Liver	Mean S.D. ± % change	0.348^{a} 0.0344	0.472^{b} 0.0125 +35.91	0.604^{c} 0.0042 +73.65	0.348^{a} 0.0344	0.477^{d} 0.0030 +37.20	0.521^{e} 0.0026 +49.76	0.446^{c} 0.0026 +28.40	0.424^{b} 0.0017 21.95
Muscle	Mean S.D. ± % change	0.162^{a} 0.0039	0.233^{b} 0.0032 +44.05	0.263^{c} 0.0062 +62.37	0.162^{a} 0.0039	0.203^{c} 0.0034 +25.44	0.223^{e} 0.0028 +38.10	0.212^{d} 0.0021 +31.19	0.193^{b} 0.0020 +19.32
Kidney	Mean S.D. ± % change	0.121^{a} 0.0027	0.153^{b} 0.0060 +26.54	0.167^{c} 0.0034 +38.55	0.121^{a} 0.0027	0.140^{c} 0.0021 +16.08	0.153^{e} 0.0023 +26.81	0.146^{d} 0.0017 +21.37	0.134^{b} 0.0018 +11.55
Brain	Mean S.D. ± % change	0.212^{a} 0.0063	0.277^{b} 0.0079 +30.89	0.304^{c} 0.0035 +43.54	0.212^{a} 0.0063	0.250^{c} 0.0028 +18.19	0.277^{e} 0.0030 +30.94	0.259^{d} 0.0016 +22.48	0.234^{b} 0.0020 +10.84

All the values are mean ± SD of six individual observations. Values with different superscripts within the column are significantly different from each other at $P<0.05$ according to DMR test.

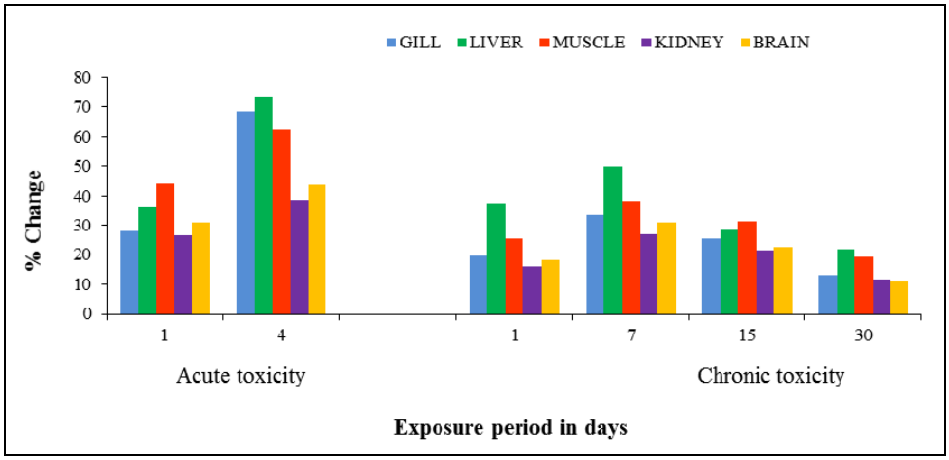

Fig 1: LDH activity in different organs of the fish *C. carpio*.

an increase in the LDH activity after exposing to different pesticides. An increase in the LDH activity was observed by Sastry and Siddiqui [26] in the liver and brain of *Channa punctatus* exposed to sublethal concnentrations of sevin. Ghosh [27] has reported an increase in the activity of LDH in brain, liver and muscle and decreased activity in kidney and intestine of *Clarias batrachus* exposed to sublethal concentrations of Tara 909, Suquin and Croton 36.

The increase in the LDH activity in the organs of the freshwater animals exposed to pesticide toxicity was also observed by Koundinya and Ramamurthi [28], Siva Prasad Rao and Ramana Rao [29] in *Tilapia mossambica,* exposed to lethal (LC_{50}) concentration of sumithion, sevin and sublethal concentration of methyl parathion respectively. Dayananda Reddy [30], Bhagyalakshmi *et al* [31] and Venkateswara Rao [32] have also reported an increase in the LDH activity in different organs of the exposed animals.

The elevation of LDH indicates the prevalence of anaerobic conditions imposed by the stress factor of phorate toxicity. With elevation of LDH activity, the metabolic pathway might have turned to anaerobic to meet the increased energy demands during the phorate exerted toxic stress. The increase in the LDH activity also indicates the impairment of oxidative metabolism in the mitochondria as a consequence of hypoxic conditions under pesticide exposure, most probably by disrupting the oxygen binding capacity of the respiratory pigment. Nagarathnamma [33], Srinivasulu Reddy and Ramana Rao [34] have reported that the absorption of oxygen from the surrounding medium was adversely affected due to disruption in gill lamellae caused by the OP pesticides in fish and prawn respectively.

The pesticide induced elevation in the activity of LDH in all the organs of the fish can be correlated to the binding of pesticide to the active sites of the oxidative enzymes and/or impairment in mitochondrial organization [35, 36]. In the present study such impairment might have gradually increased with the increase in the period of exposure in chronic toxicity study up to day 7 due to slow accumulation of pesticide in the tissues of the fish. May be due to the domination of the detoxification of pesticide over its accumulation, the fish may have slowly regained the oxidative metabolic activity by activating the oxidative enzymes from day 15 to day 30 in the present study, the elevation in the LDH activity was decreased slowly at day 15 and day 30 in all the target organs of the fish exposed to CSTP. The fish might have relied both on energetically more efficient oxidative metabolism and less efficient anaerobic glycolysis during this period of exposure as more energy is required either for detoxification or for the degradation of pesticide. So on prolonged exposure to CSTP the fish could develop resistance to phorate and could adapt slowly to the new environment [37].

5. Conclusions

On an overall assessment the results of this study show that the fish *C. carpio* were stressed after being exposed to ACTP and the pesticide phorate altered the activity of the carbohydrate metabolic enzyme significantly, thus resulted in the instable physiological state of the fish. Therefore, lethal and sublethal concentrations of phorate have some deleterious effect on the basic activities of the enzyme LDH in the gill, liver, muscle, kidney and brain of the experimental fish, *C. carpio*. On comparison, in the activities of enzyme, the elevation in LDH activity is more in the tissues of the fish exposed to the ALTP due to more pesticide stress.

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