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Assessment of Malate dehydgrogenase activity in different tissues of the commom carp *Cyprinus carpio* on exposure to acute lethal and chronic sublethal toxicity of Phorate

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Abstract: *Phorate is an organophosphate insecticide (OPI) which is widely used throughout the world as a broad spectrum insecticide on numerous crops including paddy and groundnut. The impact of acute lethal and chronic sublethal (ALCS) toxicity of phorate was investigated on Malate dehydrogenase (MDH) activity in the vital organs of fish such as gill, liver, muscle, kidney and brain of the freshwater common carp Cyprinus carpio (C. carpio), an economically important edible fish, having great commercial value. 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 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 MDH activity was observed in the target organs after the completion stipulated exposure period. The activity of MDH was decreased significantly ($P<0.05$) at day 1 and day 4 in all the organs of the fish exposed to ALTP and that decrement was progressed from day 1 to day 4 whereas in the fish exposed to CSTP, from day 1 to day 7 there was increase in the decrement but from day 7 to day 30 it was regressed in all the organs of the fish significantly ($P<0.05$). The findings of this study have demonstrated the sensitivity of MDH activity in different tissues of C. carpio on exposure to lethal and sublethal concentrations of Phorate and organophosphates.*
Keywords: *Acute lethal, Chronic sublethal, Cyprinus carpio, Insecticide, Malate dehydrogenase.*

I. Introduction

Environmental pollution is the presence of a pollutants in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living organisms [1]. Chemical pollution in the environment is considered to be a major and serious problem and this is known to occur in different ways viz., organic and inorganic chemicals, oil refinery, radioactivity and pesticides. Recent studies on the fresh water ecosystem show the pesticides contribute much to the chemical pollution [2]. Pesticides and their mode of polluting, in addition to their intended effects are sometimes found to affect non-target organisms including humans.

Economically, fishes constitute a very important group of animals and provide a rich source of food, liver oil and a number of other bi-products. Fish are good indicators of aquatic contamination with pollutants such as pesticides, because their biochemical stress responses are quite similar to those found in mammals. A variety of fish species show uptake and accumulation of many contaminants such as pesticides [3]. Pesticides have been found to be highly toxic not only to fish but also to fish food organisms, thus threatening the life of fish [4]. Pesticides exert their effect at cellular, molecular level and on the whole organism of aquatic environment including fish [5-8].

Enzymes are biochemical macromolecules which control metabolic processes of organisms, thus a slight variation in enzyme activity would affect the organisms like fish [9]. Enzymatic activities also provide quick screening methods for assessing the health of fish and can be used to determine the incipient lethal concentration of a toxicant. Therefore, by estimating enzyme activities in an organism, it can be easily identify a disturbance in metabolism. MDH is an enzyme which catalyzes the NAD/NADH-dependent interconversion of the substrates malate and oxaloacetate. This reaction plays a key role in the malate/aspartate shuttle across the mitochondrial membrane, and in the tricarboxylic acid cycle within the mitochondrial matrix. The TCA cycle is completed when the oxidation of L-Malic acid to oxaloacetic acid is accomplished by the enzyme MDH. Pesticides accumulate in fish and produce changes in the concentration of various components of their body [10, 11] and affect human health too via ecological cycling and biological magnification. It has been reported that the activities MDH decreased in liver and muscle of fish *Clarias batrachus* exposed to a pesticide [12]. The other reports available on the effects of pesticides on different freshwater animals, also indicating noticeable changes in the activities of MDH [13-15].

Organophosphates are widely used throughout the world as an important group of pesticides because of their high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment [16]. Phorate is an organophosphate insecticide (OPI) which is widely used throughout the world as a broad spectrum insecticide on numerous crops including paddy and groundnut. Under extreme stress conditions on exposure to pesticides like organophosphates, enzymes of carbohydrate metabolism such as MDH have been known to act as the energy suppliers in metabolic pathways and biochemical reactions. Changes in the activities of the enzymes such as MDH are sensitive to environmental pollutants like pesticides [17]. Hence the present investigation is aimed to assess the impact of ALCS toxicity of phorate, which is widely used in the local area to combat pests, on MDH activity in the vital organs of the fish *C. carpio*, a representative of the aquatic environment.

II. Materials And Methods

2.1 Material

2.1.1 Test Species

The Indian major carp *Cyprinus carpio* (Linnaeus, 1758) has been selected as 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 and Andhra Pradesh 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 Probit method of Finney [18]. LC_{50}/96 hours (0.71 ppm/l) of phorate was taken as lethal concentration to study acute toxicity and one-tenth of the LC_{50}/96 hours (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. Batch I was exposed for acute toxicity of Phorate (exposed to LC_{50} of Phorate) and batch II was exposed for chronic toxicity of Phorate (exposed to sub lethal concentration = 1/10th of LC_{50} - 0.071 ppm). In 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 batch II**,** group 1 was considered as 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 Malate dehydrogenase (L-Malate NAD-oxidoreductase, EC: 1.1.1.37) activity

MDH activity in the organs of the fish *C. carpio* was estimated by the method of Nachlas et al., [19] with slight modification as suggested by Prameelamma and Swami (1975). A 10% tissues homogenate was prepared in 0.25M ice-cold sucrose solution and centrifuged at 2500 rpm for 15 minutes. The supernatant was taken as the source of the enzyme. The reaction mixture in a final volume of 2.0 ml contained 100 µ moles of phosphate buffer (pH 7.4) + 4 µ moles of INT + 50 µ moles of sodium malate + 0.1 µ moles of NAD + enzyme. This mixture was incubated at 37^0 C for 30 minutes and the reaction was stopped by adding 5.0 ml of glacial acetic acid. Then the colour was extracted into 5.0 ml of toluene by keeping overnight at 5^0C and the optical density of the colour developed was read in a spectrophotometer at a wavelength of 495 nm. 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/hr.

2.2.4 Statistical analysis

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

III. Results and Discussion

3.1. Results

The data on the activity of MDH in the organs of the fish such as gills, liver, muscle, kidney and brain of *C. carpio* at 1 and 4 days on exposure to acute toxicity of phorate (ATP) and 1, 7, 15 and 30 days on exposure to chronic toxicity of phorate (CTP), 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 acute and chronic toxicity study of phorate, 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.

3.2 Activity of Malate dehydrogenase

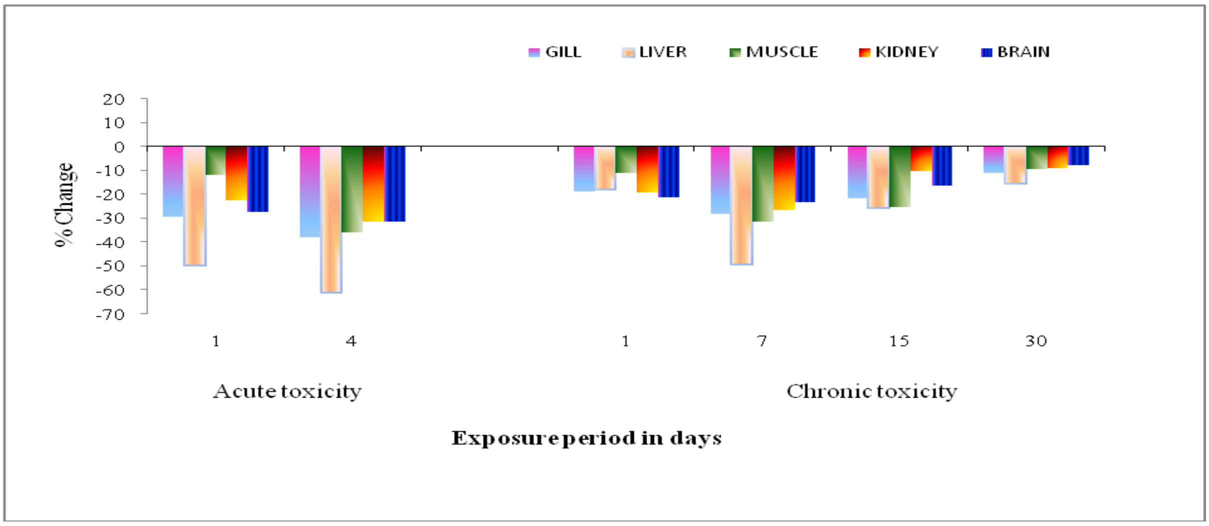
From the data presented in the Table-1 and Figure-1 relative to controls, the activity of MDH in all the organs of the fish exposed to phorate decreased at 1 and 4 days of exposure in acute toxicity in the order of day 1>4 and the differences in the activity between controls and experimental were also found to be statistically significant (P<0.05). The decrease was more at day 4 than at day 1 in all the organs of the fish. Suppression of this enzyme progressed from day 1 to day 4 in all the organs of the fish exposed to ATP. In the fish exposed to CTP, in the activity of MDH, from day 1 to day 7 there was increase in the decrement but from day 7 to day 30 it was regressed in all the organs of the fish in the order of day 1>7<15<30. All the values were found to be statistically significant (P<0.05).

Table-1: MDH activity (µ moles of formazone formed/mg protein/hr) in different organs of the fish *C. carpio* at different periods of exposure to ACTP. The values below the mean are percent changes over the respective control.

ORGAN		EXPOSURE PERIOD IN DAYS							
		ACUTE TOXICITY			CHRONIC TOXICITY				
		CONTROL	1	4	CONTROL	1	7	15	30
GILL	Mean S.D. ± % change	0.253c 0.0029	0.178b 0.0031 -29.26	0.157a 0.0032 -37.78	0.253e 0.0029	0.205c 0.0013 -18.65	0.181a 0.0021 -28.18	0.198b 0.0016 -21.70	0.225d 0.0037 -10.88
LIVER	Mean S.D. ± % change	0.373c 0.0036	0.185b 0.0043 -49.76	0.145a 0.0050 -61.01	0.373e 0.0036	0.281c 0.0021 -18.03	0.188a 0.0018 -49.42	0.277b 0.0025 -25.56	0.315d 0.0043 -15.54
MUSCLE	Mean S.D. ± % change	0.128c 0.0041	0.113b 0.0025 -11.70	0.082a 0.0042 -35.85	0.128e 0.0041	0.114c 0.0029 -11.07	0.087a 0.0013 -31.51	0.095b 0.0018 -25.27	0.116d 0.036 -9.51
KIDNEY	Mean S.D. ± % change	0.165c 0.0051	0.128b 0.0037 -22.53	0.113a 0.0031 -31.50	0.165d 0.0051	0.133b 0.0015 -19.15	0.121a 0.0013 -26.70	0.148c 0.0021 -10.45	0.150c 0.0017 -9.06
BRAIN	Mean S.D. ± % change	0.193c 0.0032	0.140b 0.0053 -27.41	0.132a 0.0043 -31.29	0.193e 0.0032	0.152b 0.0016 -21.36	0.148a 0.0018 -23.27	0.162c 0.0036 -16.19	0.178d 0.0021 -7.81

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

Fig-1: MDH activity (µ moles of formazone formed/mg protein/hr) in different organs of the fish *C. carpio* at different periods of exposure to ACTP.



All the values are mean ± SD of six individual observations.

IV. Discussion

Analysis of biochemical parameters could help to identify the level of toxicity to target organs as well as the general health status of animals. It may also provide an early warning signal in stressed organisms such as fishes [20]. These parameters are the indicators of the response of the animal to the environmental effects and can also serve as markers for toxicant exposure and effect in the animals like fish. The biochemical parameters in fish are valid for physiopathological evaluation and sensitive for detecting potential adverse effects and relatively early events of pollutant, like pesticide damage [21-23].

In the present investigation the oxidative enzyme, MDH showed a reduction in its activity in all the target organs of the fish *C. carpio,* which indicates the suppression of oxidative metabolism in the fish exposed to acute [24] and CTP. As MDH is the oxidative enzyme involved in Kreb's cycle, any disturbance in this enzyme activity will affect the Kreb's cycle. Since this cycle represents a central oxidative pathway for carbohydrates, fats and amino acids, if there is any disturbance in this cycle the whole metabolism is likely to be affected. In support of present investigation, several reports are available on a decrease in the activity of MDH after exposing to different pesticides. A decrease in the activity of MDH was observed by Thakore et al., [25] in the liver of BHC treated mice. The MDH activity in the tissues of the fish *Tilapia mossambica* was decreased on exposure to malathion [26]. Srinivas [13] studied the impact of endosulfan on carbohydrate metabolism in the fresh water fish *Clarias batrachus* and reported decreased MDH activity. Mishra and Shukla [14] studied endosulfan effects on muscle MDH of the freshwater catfish *Clarias batrachus* and reported that endosulfan treatment reduced the activity of cytoplasmic MDH (cMDH) and mitochondrial MDH (mMDH) in the muscle of the fish. Sarma et al., [27] in liver and muscle of the fish *Channa punctatus* exposed to endosulfan and Suneetha [15] in the brain, gill, kidney, liver and muscle of *Labeo rohita* after exposing to lethal and sublethal concentrations of two pesticides, endosulfan and fenvalerate, were also observed a decrease in the MDH activity in the respective target organs.

It is known that the oxidative enzymes like MDH act as indictors of aerobic respiration, the inhibition of MDH indicates the prevalence of anaerobic conditions imposed by the stress factor of phorate toxicity. As MDH is the key enzymes in TCA cycle, with the inhibition of MDH, the metabolic pathway might have turned to anaerobic to meet the increased energy demands during the phorate exerted toxic stress. The decrease in MDH 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. The decrease may be also due to the disorganization of mitochondria, affecting enzymes of TCA cycle and decreased state of respiration [28]. The fall in this enzyme activity might be related to the close contact of pesticide with cell organelle and their subsequent disorganization accompanied by increased histopathology of gill area and shifting of the aerobic to anaerobic metabolism as reported in other teleosts [29, 30]. Any alteration in the respiratory area decreases the oxygen absorption capacity of the gill due to its close contact with polluted water [31, 32]. It may also be one of the reasons for the diminished activity of MDH.

The pesticide induced suppression in the activity of MDH 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 [33]. 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 detoxification over accumulation, the fish slowly regained the oxidative metabolic activity by activating the MDH from day 15 to day 30, the suppression in the activity of MDH was decreased slowly at day 15 and day 30 in the tissues of the fish on exposure to CTP. 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 CTP the fish could develop resistance to phorate and could adapt slowly to the new environment [34]. More suppression in MDH activity in the liver might be greater interference of pesticide with the activities of oxidative metabolic enzymes in liver as the liver is important centre for metabolism, inter conversion and detoxification as it plays a vital role during toxic stress in fishes.

V. Conclusion

The results of this study show that the pesticide phorate altered the activity of the carbohydrate metabolic enzyme MDH significantly thus result in the instable physiological state of the fish. Therefore, lethal and sublethal concentrations of phorate have some harmful effects on the basic activities of enzyme of carbohydrate metabolism in the gill, liver, muscle, kidney and brain of the experimental fish, *C. carpio*. On comparison, the suppression of MDH activity is more in the tissues of the fish exposed to ATP due to more pesticidal stress. It may be concluded that the phorate induced alterations in the activity of carbohydrate metabolic enzyme MDH caused significant metabolic effect on the physiological consequences. The physiological conditions are directly related to the bioavailability of the pesticide.

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