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Original Research Article

Impact of Acute Lethal and Chronic Sublethal toxicity of Phorate on Succinate dehydrogenase activity in the Fresh Water Fish *Cyprinus carpio*

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Abstract

The present study is aimed to investigate the acute and chronic toxicity stress of phorate, induced alterations in the activity levels of Succinate dehydrogenase (SDH), a vital enzyme of citric acid cycle that catalyses the reversible oxidation of succinate to fumarate. Freshwater fish *Cyprinus carpio* (*C. carpio*) 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. After the completion of stipulated exposure period the SDH enzyme activity levels were estimated in the vital organs of fish such as gill, liver, muscle, kidney and brain. The activity of SDH in all the organs of the fish exposed to phorate decreased at 1 and 4 days of exposure in acute toxicity in the order, day 1>4. The decrease was more at day 4 than at day 1. In the fish exposed to chronic toxicity of phorate (CTP), in the activity of SDH 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, day 1>7<15<30. The differences in the SDH activity between controls and experimental were found to be statistically significant ($P<0.05$). The present study indicates the phorate induced alterations in the activities of carbohydrate metabolic enzyme caused significant metabolic effect on the physiological consequences.

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

1. Introduction

Pesticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are useful in recognizing acute or chronic toxicity of insecticides [1,2] like Phorate and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. Phorate is an organophosphate insecticide (OPI) that is widely used throughout the world and in India and Andhra Pradesh as a broad-spectrum insecticide on numerous crops including paddy and groundnut.

Enzymes are biochemical catalysts which control metabolic processes of organisms, thus a slight variation in enzyme activity would affect the organisms [3]. 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 identified a disturbance in metabolism. The studies related to the activities of enzymes in the presence of pollutants like pesticides in water became a routine practice in clinical medicine to diagnose certain diseases and the extent of tissue or organ damage [4].

Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically [5]. Changes in the activities of the enzymes like, SDH are sensitive to environmental pollutants like pesticides [6]. SDH is one of the important key enzymes and exhibits an important function in energetics which catalyses the reversible oxidation of succinate to fumarate and serves as a link between electron transport system and oxidative phosphorylation [7]. Pesticides alter the mitochondrial structure and decrease the SDH activity in the organs of animals [8,9]. The activity of SDH can be taken to reflect the rate of operation of TCA cycle in different organs of the fish under the situation of stress [10].

SDH is the only enzyme that participates in both the citric acid cycle and the electron transport chain and acts as indicator of aerobic respiration. Since the activity of SDH in mitochondria is greater than the other enzymes of TCA cycle, an insight into the alterations of this enzyme activity may be taken as index to assess the function of TCA cycle in different organs of the fresh water animals [11]. SDH is unique among the Krebs cycle enzymes, in that it is tightly bound to the inner mitochondrial membrane. Any alterations in its activity indicate changes in the structure and function of

mitochondria. Different organs of fishes have different functional specializations; hence the degree of participation of these organs in energetics may also differ during toxic stress [12].

2. 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 [13]. 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 lethal concentration=LC_{50} of Phorate) and batch II was exposed for Chronic toxicity of Phorate (exposed to sub lethal concentration = $1/10^{th}$ of LC_{50} - 0.071 ppm/1). 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 Succinate dehydrogenase (Succinate:Acceptor) oxidoreductase, EC: 1.3.99.1) activity

SDH activity in the organs of fish was estimated using the colorimetric method of Nachlas *et al*., [14]. A 5% homogenate (w/v) of the tissues was prepared in 0.25M ice cold sucrose solution, centrifuged at 3000 rpm for 10 minutes and the supernatant was taken as the source of the enzyme. The incubation mixture consisted of 0.2 ml of 0.4M phosphate buffer (pH 7.7), 0.2 ml of 0.2M sodium succinate, 1.0 ml of 0.004M 2-(p-indophenol)-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT), 0.1 ml of 0.005M phenazine methosulphate and 0.5 ml of enzyme preparation. The mixture was incubated at 37^0 C for 30 minutes and the reaction was stopped by adding 6.0 ml of glacial acetic acid. The formazone formed was extracted into 6.0 ml of toluene by keeping overnight at 0^0 C and the optical density of the colour 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/hr.

2.2.4 Statistical analysis

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

3. Results and Discussion

3.1. Results

The data on the activities of SDH in the organs such as gills, liver, muscle, kidney and brain of the fish *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 CTP, besides controls, are presented in the Table I. For comparison, the differences obtained in relation to the controls in each organ of the fish at the above said exposure periods in acute and chronic toxicity of phorate (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.

3.1.1 Activity of Succinate dehydrogenase

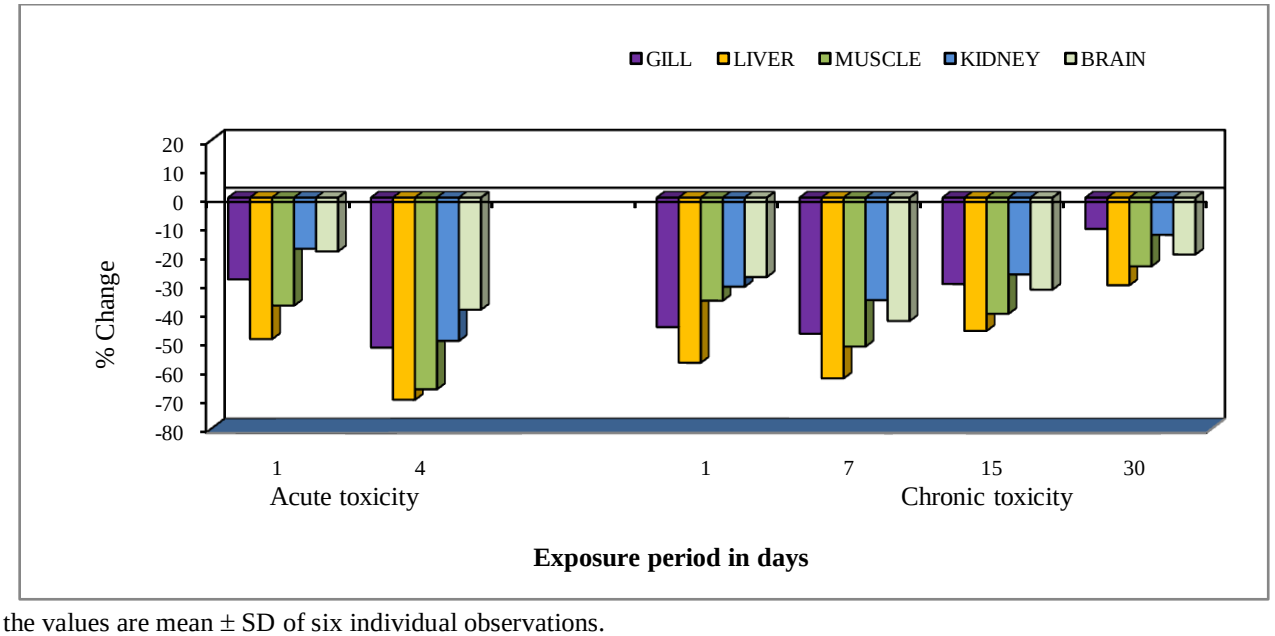
From the data presented in the Table-1 and Figure-1 relative to controls, the activity of SDH in all the organs of the fish exposed to phorate decreased at 1 and 4 days of exposure in acute toxicity in the order of day 1>4 and the differences in the activity between controls and experimental were also 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 SDH 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.

Table-1 SDH 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)	0.174± 0.0074^{c}	0.124± 0.0091^{b} (-28.27)	0.083± 0.0044^{a} (-51.91)	0.174 ± 0.0074^{d}	0.096± 0.0013^{a} (-44.82)	0.092± 0.0016^{a} (-47.12)	0.122± 0.0018^{b} (-29.88)	0.155± 0.0029^{c} (-10.80)
Liver	Mean± S.D. (% change)	0.579± 0.0065^{c}	0.295± 0.0034^{b} (-48.97)	0.173± 0.0030^{a} (-70.00)	0.579± 0.0065^{e}	0.248± 0.0025^{b} (-57.16)	0.216± 0.0013^{a} (-62.57)	0.312± 0.0021^{c} (-46.12)	0.403± 0.0047^{d} (-30.31)
Muscle	Mean± S.D. (% change)	0.152± 0.0047^{c}	0.095± 0.0026^{b} (-37.38)	0.051± 0.0037^{a} (-66.36)	0.152± 0.0047^{e}	0.098± 0.0016^{c} (-35.67)	0.073± 0.0013^{a} (-51.53)	0.091± 0.0017^{b} (-40.21)	0.116± 0.0021^{d} (-23.78)
Kidney	Mean± S.D. (% change)	0.146± 0.0037^{c}	0.120± 0.0051^{b} (-17.67)	0.073± 0.0032^{a} (-49.59)	0.146± 0.0037^{e}	0.101± 0.0034^{b} (-30.82)	0.094± 0.0015^{a} (-35.47)	0.107± 0.0023^{c} (-26.57)	0.127± 0.0017^{d} (-12.86)
Brain	Mean± S.D. (% change)	0.189± 0.0034^{c}	0.154± 0.0029^{b} (-18.60)	0.115± 0.0038^{a} (-38.79)	0.189± 0.0034^{e}	0.137± 0.0013^{c} (-27.48)	0.108± 0.0011^{a} (-42.70)	0.128± 0.0021^{b} (-31.84)	0.152± 0.0024^{d} (-19.66)

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.

Figure-1: SDH 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.

3.2. Discussion

In the present investigation the oxidative enzyme, SDH showed a reduction 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 ACTP [15]. As the SDH 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 authors reported a decrease in the activity of SDH after exposing to different pesticides. It has been reported earlier by several investigators that pesticides alter the mitochondrial structure and decrease the SDH activity in the organs of many animals [3,4, 16-18]. Suneetha, (2012) observed a decrease in the SDH activity in the brain, gill, kidney, liver and muscle of the freshwater fish *Labeo rohita* after exposing to lethal and sublethal concentrations of two pesticides, endosulfan and

fenvalerate for 24 hrs and 15 days. Khemani *et al*., [19] reported a significant inhibition in the SDH activity in the tissues of rat treated with dieldrin and suggested that one of the reasons for the observed inhibition of SDH activity could be diminished availability of the soluble cofactors within the subcellular structures. Sastry and Siddiqui [20] reported decreases in SDH activity of liver and brain of *Channa punctatus* exposed to sublethal concnentrations of sevin.

It is known that SDH acts as indictor of aerobic respiration, the inhibition of SDH indicates the prevalence of anaerobic conditions imposed by the stress factor of phorate toxicity. As SDH is the key enzymes in TCA cycle, it is logical to assume that with the inhibition of SDH activity, the metabolic pathway might have turned to anaerobic to meet the increased energy demands during the phorate exerted toxic stress. The decrease in SDH 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 in SDH activity may be due to the disorganization of mitochondria, affecting enzymes of TCA cycle. The decrease in this enzyme activity might be probably due to mitochondrial damage and decreased state of respiration [21].

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 [22,23]. Any alteration in the respiratory area decreases the oxygen absorption capacity of the gill due to its close contact with polluted water [24,25]. It may also be one of the reasons for the diminished activity of SDH. The decrease in the activity of SDH during pesticide toxic stress is associated with the inhibition of mitochondrial respiratory mechanism or dearrangement in ultra structure, architectural integrity and permeability of mitochondria [16] which leads to the prevention of transfer of electrons to molecular oxygen, resulting in the inhibition of SDH activity and shifting the aerobic metabolism to anaerobiosis [17].

The present work indicates the phorate induced alterations in the activities of carbohydrate metabolic enzyme caused significant metabolic effect on the physiological consequences. The physiological conditions are directly related to the bioavailability of the pesticide. The alteration in the enzyme activity can be taken as good marker or indicator of the pesticide induced stress.

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