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Research Paper

ENZYMATICAL ALTERATIONS AFTER LETHAL AND SUBLETHAL EXPOSURES OF ORGANOPHOSPATE PESTICIDE PHORATE 10%CG ON *Catla catla* FINGERLINGS

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Abstract

Environmental pollution resulting from industrial effluents and agricultural activities has become a global issue because of the extent damage caused to the aquatic ecosystems and the disruption in the natural food chain, by several agricultural practices such as insecticidal and herbicidal application. Phorate {O,O-diethyl-S-[(ethylthio) methyl] phosphorodithioate} is an organophosphate pesticide used in agricultural practices, primarily to control sap-feeding insects which includes various beetles, mites, grubs, and worms. This compound has been found to produce a lot of toxic effects in different fish species and is able to bring about changes in their metabolic pathways.Phorate ($C_7 H_{17} O_2 PS_3$) is commercially available as Thimet, Rampart, GranutoxAgrimet etc. and widely used as a broad-spectrum insecticide on numerous crops throughout the world and also in India. Enzymatic activities also provide quick screening methods for assessing the health of fish *Catla catla*(Hamilton)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. 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. The acute toxicity of Phorate 10% CG {O,O-diethyl-S-[(ethylthio) methyl] phosphorodithioate} previously we have studied. We have reported the Median lethal concentration values of *C. catla* for Phorate in static tests for 24, 48, 72, and 96h were 0.81, 0.76, 0.68 and 0.53 mg/L respectively. The present study is to evaluate the enzymatical alteration in *C. catla* fingerlings of sub - lethal concentrations of Phorate. The calculated mean values of LDH, SDH, MDH, ACP, AChE, AATand ALAT along with standard deviation values and percent changes over control are represented in Tables 1, 2. In the fish reared as control for 1 day, 5 days, 10 days of study enzymes contents were in LDH, SDH, MDH, ACP, AChE, AATand ALAT. In the most of the cases the liver is shown highest depletion of enzyme content in non-neurological enzymes and in nuric enzymes such as ACP and

AChE levels were decreased in brain.The LDH, SDH, MDH, ACP, AChE, AATand ALAT contents significantly increases or decreases ($p<0.05$) sub lethal and lethal exposures of Phorate 10% GC when compare to the control fish groups.
Key words: *Catla Catla*, Enzymes, LC_{50}, LDH, SDH, MDH, ACP, AChE, AATand ALAT.

INTRODUCTION

Environmental pollution resulting from industrial effluents and agricultural activities has become a global issue because of the extent damage caused to the aquatic ecosystems and the disruption in the natural food chain, by several agricultural practices such as insecticidal and herbicidal application. The increasing population has put a stress on resources, resulting in the excessive use of organophosphorus pesticides and fertilizers to meet the demand. These substances ultimately pollute the aquatic environment and cause severe damage to the aquatic life especially the non - target species. Among the different groups of pesticides organophosphates are being used commonly as insecticides due to their facilitation properties like low mammalian toxicity, less persistence and rapid biodegradability in nature [1].

Phorate {O,O-diethyl-S-[(ethylthio) methyl] phosphorodithioate} is an organophosphate pesticide used in agricultural practices, primarily to control sap-feeding insects which includes various beetles, mites, grubs, and worms. This compound has been found to produce a lot of toxic effects in different fish species and is able to bring about changes in their metabolic pathways. Phorate 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, GranutoxAgrimet etc. and its molecular formula is $C_7 H_{17} O_2 PS_3$ [2].

Acute toxicity of a pesticide refers to the chemical's ability to cause injury to an animal from a single exposure, generally of short duration. The acute toxicity test of pesticides to fish has been widely used to acquire rapid estimates of the concentrations that cause direct, irreversible harm to test organisms[3]. The most common acute toxicity test is acute lethality and LC_{50} is customary to represent the lethality of a test species in terms of mortality and time.

The production of pesticides started in India in 1952 with the establishment of a plant for the production of BHC near Calcutta, and India is now the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally [3]. There has been a steady growth in the production of technical grade pesticides in India, from 5,000 metric tons in 1958 to 102,240 metric tons in 1998. In 1996–97 the demand for pesticides in terms of value was estimated to be around Rs. 22 billion (USD 0.5 billion), which is about 2% of the total world market [4].

The pattern of pesticide usage in India is different from that for the world in general. In India 76% of the pesticide used is insecticide, as against 44% globally [3]. The use of herbicides and fungicides is correspondingly less than theusageof insecticide. The main use of pesticides in India is for cotton crops (45%), followed by paddy and wheat.

The effects, including acute toxicity, of pesticide-contaminated water on non-target organisms (NTOs) should be determined using biological methods. The concept of *in situ* bioassays is based on exposure of test animals at field sites without disturbing contaminated sediment, and the determination of percentage survival. The process of exposing fish to test the toxicity of water is relatively simple: cages containing fish are hung in the water column or anchored at the bottom, and mortality is measured after exposure for 96 h or longer.

Organophosphorous pesticides are frequently used against pest because of their high insecticidal property low mammalian toxicity, less persistence and rapid biodegradability [5]. These also affect non-target organisms either directly or indirectly. In rice land agro ecosystem, all organisms including larvivorous fishes can be affected [6]. At lower concentrations, physiological functions including the larvivorous potential are affected and results of the present study indicate the same. Indiscriminate and prolonged use may lead to mortality and depletion of the fish population [7]. Re-establishment of fish population takes a longer duration when compared to the mosquito population. Since vector mosquito control using larvivorous fishes is considered to be an environmental friendly and safe alternative to insecticides [8], judicious use of pesticides preserving the natural habitat of these fishes is important. Integrated pest management, biological and genetical control for agricultural pests will help in the preservation of the biotic communities in the rice land ecosystem. Further, there is a need to monitor ecotoxicity whenever larvivorous fishes are used as a bio control agent in vector mosquito control programs [9].

Different concentrations of insecticides are present in water bodies and found to be toxic to aquatic organisms especially fish. Fishes are highly sensitive to the environmental contamination of water. Hence insecticides, serious pollutants may significantly damage certain physiological and biochemical processes when they enter into the organs and tissues of fish. It has been found that different kinds of insecticides can cause serious impairment to physiological and health status of fishes. Since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is important for human beings [9].

Catla catla(Hamilton) is one of the major fresh water carps native to India, Bangladesh, Myanmar, Nepal and Pakistanintroduced in many other countries as exotic species. *C.catla* is a very rich source of proteins and is reported to attain a maximum size of 182 cm and weight of about 50 Kilograms (these figures vary). It is a surface and mid-water feeder, mainly omnivorous with juveniles feeding on aquatic and terrestrial insects, detritus and phytoplankton. It has a characteristically large, upturned mouth with a prominent protruding jaw. Because of its high nutritive value, it is a highly priced food fish and of great demand in the market [10].

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. 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. Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically [11]. Changes in the activities of the enzymes like, succinate dehydrogenase, malate dehydrogenase and lactate dehydrogenase are sensitive to environmental pollutants like pesticides [12].The acute toxicity of Phorate 10% CG {O,O-diethyl-S-[(ethylthio) methyl] phosphorodithioate} was my previous study. The Median lethal concentration values of *C*. *catla* for Phorate in static tests for 24, 48, 72, and 96h were 0.81, 0.76, 0.68 and 0.53 mg/L respectively. The present study is to evaluate the enzymatical alteration in *C. catla* fingerlings of sub - lethal concentrations of Phorate.

MATERIAL AND METHODS

Healthy juvenile freshwater fish *C.catla*irrespective of the sex measuring with 7.5 ± 1.5 cm in length and 8.5 ± 0.5 gm in body weight were collected from local fish farm at Buddam village in Bapatla, mandal, Guntur district of Andhra Pradesh, India. The fishes were acclimatized to laboratory condition for two weeks in large circular plastic tubs using dechlorinated tap water (Tarsons Pvt. Ltd.), previously washed with 0.1% KMnO4 solution to free the walls of the tub from microbial infection. The physico-chemical characteristics of water was laid down by following standard protocol suggested by American Public Health Association [13] having temperature of 28 ± 2^0C, pH 6.8 ± 0.05, Dissolved Oxygen 6.9-7.4 mg/L, salinity 0 ppt, and total hardness 170 mg/L as $CaCO_3$.
The fish were exposed to organophosphorus pesticide Phorate to sublethal concentration of $1/10^{th}$ 96 h LC_{50} value i.e., 0.53 mg/l concentration for 1day, 5 day and 10 days respectively. If mortality occurred during the experimentation, the dead fish were separated immediately to avoid depletion of dissolved oxygen (DO) level which adversely affects other fish species. During the whole experiment a suitable control was also maintained to nullify any other effects that likely to affect the fish. Then the fishes were scarified immediately and isolated fresh (wet) tissue of vital organs such as brain, gill, liver, kidney and muscle were taken for biochemical estimation ofLDH, SDH, MDH, ACP, AChE, AATand ALAT.

Estimation of Lactate Dehydrogenase (LDH)

The Lactate Dehydrogenase activity (LDH) was estimated by the method of Srikanthan and Krishna Murthy (1955) [14]. 2% homogenate of the tissue were prepared in 0.25 M ice-cold sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source. The 2 mL of reaction mixture contained 0.5 mL of lithium lactate, 0.5 mL of phosphate buffer, 0.2 mL of INT or [2-(4-iodophenyl)-3(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride] and 0.2 mL of NAD (Nicotinamide adenine dinucleotide) and 0.6 mL of supernatant. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was stopped by adding 5 mL of acetic acid. Zero time controls were maintained by adding 5 mL of acetic acid prior to the addition of homogenate. The formazan dye formed was extracted overnight in 5 mL of cold toluene. The intensity of color developed was read at 495 nm against a reagent blank in a spectrophotometer. The activity was expressed as µ moles of formazan formed/mg protein/h.

Estimation of Succinate Dehydrogenase (SDH) The Succinate Dehydrogenase (SDH) activity was estimated by the method of Nachlas*et al*.(1960)[15]. The tissue homogenate (4%w/v) wasprepared in ice cold sucrose solution (0.25M)and centrifuged at 1000rpm for 15min.The supernatant acts as the enzyme source. The 2 mL of reaction mixture contained 0.6 mL of supernatant,0.5mL of phosphate buffer (pH7.2), 0.5mL of sodium succinate,0.2mL of INT or [2-(4-iodophenyl)-3(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride] and 0.2mL of distilled water was added. The reaction mixture was incubated at 37°C for 30min and reaction was stopped by adding 5mL of acetic acid. Zero time controls were maintained by adding 5mL of acetic acid prior to the addition of homogenate. The formazan dye formed was extracted overnight in 5mL of cold toluene. The intensity of color developed was read at 495nm against a reagent blank in a spectrophotometer. The activity was expressed as µ moles of formazan formed/mg protein/h.

Estimation of Malate Dehydrogenase (MDH)

The Maltase Dehydrogenase (MDH) activity was estimated by the method of Nichlas*et al*. (1960)[15]. 2%homogenate of different tissues was prepared in ice cold 0.25M sucrose solution and centrifuged at 1000 rpm for 15min. The supernatant was used as the enzyme source. The 2 of mL reaction mixture contained 0.6 mL of supernatant, 0.5 mL of phosphate buffer(pH7.2),0.5 mL of malate, 0.5 mL of INT or [2-(4-iodophenyl)-3(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride]and 0.2mL of NAD was added. The reaction mixture was incubated at 37°C for 30min. Zero time controls were maintained by adding 5mL of acetic acid prior to the addition of homogenate. The formazan dye formed was extracted overnight in 5mL of cold toluene. The intensity of color developed was read at 495nm against a reagent blank in µ moles of formazan formed/ mg protein/h.

Estimation of Acid Phosphatase (ACP)

The activity of acid phosphatase was estimated by the method of Bodasky (1932)[16]. 2% homogenates of the tissues were prepared in 0.25 M ice sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant served as the enzyme source.In acidic pH of buffer system.acid phosphatase hydrolyses α-napthyl phosphate to α-napathal and phosphate. The α-napthal is then coupled with diazotized fast red TR to form a diazo dye which has strong absorbance at 405 nm. The intensity of color developed was read at 405 nm against a reagent blank in a spectrophotometer (ELICO Model SL 207). The activity was expressed as mg pi/g protein/h.

Estimation of Acetyl cholinesterase Enzyme (AChE)

AChE enzyme assay was performed spectrophotometrically by the method of Ellaman et al (1961) [17]. The reactions performed at 37^0 C were initiated by adding small aliquots of varying concentrations of the substrate (acetyl-choline iodide) to yield a final volume of 3 ml. The absorbance was recorded were recorded continuously at 412nm for 5 min. Corresponding blanks lacking AChE were subtracted to yield the enzymatic activity rate. The typical runs for all experiments used were 2.7 ml buffer, 0.1 M phosphate buffer (pH 8), 50 µl (0.16mM) DTNB, 100 µl (1 mg/ml) protein and 100 µl substrate.

Estimation of aminotransferase (AAT) activity

The amino transferase activity was estimated by the method of Mohan and cook (1957) [18]. The 1.5 ml of reaction mixture contains: 1 ml of phosphate buffer (pH 7.4), 0.1 ml of L- aspartate (L-Asparticacid), and 0.1 ml of α-ketoglutaric acid and 0.3 ml of supernatant as enzyme source. The reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by adding 1 ml of 2,4-dinitrophenyl hydrazine(DNPH) solution prepared in 0.1 N Hcl and was allowed to stand for 20 minutes at room temperature. The rest of the details were the same as for alanine aminotransferase. The activity levels were expressed as µ moles of pyruvate formed/mg protein/h

Estimation of Alanine Aminotransferase (ALAT) activity

Alanine aminotransferase activity was estimated by the method of Mohun and Cook (1957) [18]. 10% homogenate of different tissues was prepared in 0.33 M sucrose solution and centrifuged at 1000 rpm for 15 minutes. The supernatant obtained was used as the enzyme source. The reaction mixture of 1.5 mL contains 1 mL phosphate buffer (pH 7.4), 0.1 mL of L-alanine, 0.1 mL of α-ketoglutarate and 0.3 mL of

supernatant as enzyme source. The contents were incubated at 37°C for 30 minutes. After incubation, 0.3 mL of the enzyme was added to the control. Then 1 mL of 2,4-DNPH reagent was added and kept at room temperature for 20 minutes. The reaction was stopped by addition of 10 mL of 0.4 N NaOH, vortexed and kept at room temperature for 5 minutes. The color developed was read at 540 nm in a spectrophotometer against a reagent blank. The AST activity was expressed as µ moles of pyruvate formed/mg protein/h.

Statistical analysis

The analyzing data was subjected to unpair t-test using SPSS-version 20. The significant difference was set up at ($p< 0.05$ and $p< 0.001$) for all parameters in the experimental group.

RESULTS

The calculated mean values of LDH, SDH, MDH, ACP, AChE, AATand ALATalong with standard deviation values and percent changes over control are represented in Tables 1, 2. In this study the fish was reared as control and exposures for 1 day, 5 days, 10 days for enzymes alternations in LDH, SDH, MDH, ACP, AChE, AATand ALAT. In the most of the cases the liver is shown highest depletion of enzyme content in non-neurological enzymes and in nuric enzymes such as ACP and AChE levels were decreased in brain.The LDH, SDH, MDH, ACP, AChE, AATand ALATcontents significantly increases or decreases ($p<0.05$) sub lethal and lethal exposures of Phorate 10% GC when compare to the control fish groups.

Table 1. Alterations in the specific activities of LDH, SDH, MDH, ACP, AChE, AAT and ALAT,and % change over control in different tissues of fish *Catla Catla* treated with Lethal and 1 Day sublethal doses of Phorat.

Tissues	Control (mg/g)	Sub-lethal (mg/g)	% change	Lethal (mg/g)	% change
LDH					
Gill	0.85±0.12	0.91±0.14	7.05	1.13±0.33	32.94
Liver	1.25±0.31	1.36±0.32	8.80	1.38±0.20	10.40
Kidney	1.11±0.25	1.23±0.26	10.81	1.30±0.19	17.11
Brain	0.76±0.10	0.79±0.05	3.94	0.88±0.09	15.78
Muscle	1.29±0.09	1.33±0.23	3.10	1.41±0.22	9.30
SDH					
Gill	0.75±0.04	0.63±0.02	-16.00	0.59±0.01	-21.33
Liver	1.15±0.06	0.74±0.10	-35.65	0.60±0.07	-47.82
Kidney	0.87±0.11	0.57±0.05	-34.48	0.50±0.04	-42.52
Brain	0.59±0.09	0.51±0.08	-13.55	0.45±0.02	-23.37
Muscle	0.77±0.05	0.70±0.02	-9.09	0.62±0.06	-19.48
MDH					
Gill	0.69±0.03	0.58±0.08	-11.59	0.56±0.04	-18.84
Liver	1.06±0.21	0.88±0.10	-16.98	0.80±0.09	-24.52
Kidney	0.94±0.10	0.79±0.05	-15.96	0.67±0.06	-28.72
Brain	0.84±0.08	0.66±0.07	-21.42	0.56±0.02	-33.33
Muscle	0.73±0.06	0.61±0.08	-16.43	0.50±0.07	-31.50
ACP					

Gill	2.62±0.58	2.98±0.50	13.74	3.32±0.76	26.71
Liver	3.15±1.10	4.05±0.79	28.57	4.85±1.39	53.96
Kidney	2.89±0.75	3.24±1.21	12.11	4.31±1.00	49.13
Brain	2.44±0.58	3.84±1.41	57.37	3.87±0.88	58.60
Muscle	3.00±0.39	4.16±1.14	38.67	4.76±1.21	58.67
ALAT					
Gill	6.38±0.78	8.15±2.10	27.74	8.98±1.23	40.75
Liver	5.12±0.65	6.77±0.48	32.22	7.55±1.45	47.46
Kidney	6.13±0.75	7.89±1.02	28.71	8.13±0.96	32.62
Brain	4.98±0.38	5.73±0.76	15.06	6.10±0.76	22.48
Muscle	4.74±0.49	8.11±1.11	71.09	9.10±2.10	40.50
AAT					
Gill	2.86±0.41	2.41±0.28	-15.73	2.11±0.38	-26.22
Liver	3.25±0.56	2.87±0.19	-11.69	2.56±0.24	-21.23
Kidney	2.97±0.72	2.49±0.70	-16.16	2.23±0.19	-24.91
Brain	3.89±0.19	3.42±0.56	-12.08	3.10±0.44	-20.30
Muscle	5.14±0.79	4.21±0.74	-18.09	3.59±0.64	-30.15
AChE					
Gill	3.67±0.39	3.19±0.83	-13.07	2.83±0.33	-22.88
Liver	5.44±1.21	3.70±0.19	-31.98	3.50±0.59	-35.66
Kidney	5.10±1.38	3.17±0.74	-37.84	2.98±0.40	-41.56
Brain	4.10±1.10	2.39±0.45	-41.17	2.26±0.37	-44.87
Muscle	5.13±1.22	2.98±0.80	-41.91	2.64±0.66	-48.53

Table 2. Alterations in the specific activities of LDH, SDH, MDH, ACP, AChE, AAT and ALAT and %change over control in different tissues of fish *Catla Catla* treated with 5 day and 10 Day sublethal doses of Phorate.

Tissues	5 Day			10 Day		
	Control (mg/g)	Sub-lethal (mg/g)	% change	Control (mg/g)	Sub-lethal (mg/g)	% change
LDH						
Gill	0.73±0.06	0.84±0.12	15.06	0.58±0.04	0.67±0.06	15.51
Liver	0.89±0.21	0.98±0.13	10.11	0.72±0.09	0.86±0.08	19.44
Kidney	0.85±0.13	1.05±0.22	23.52	0.70±0.05	0.81±0.04	15.71
Brain	0.65±0.09	0.78±0.05	20.00	0.49±0.02	0.57±0.09	16.32
Muscle	0.51±0.06	0.66±0.01	29.41	0.36±0.01	0.43±0.02	19.44
SDH						
Gill	0.50±0.11	0.43±0.11	-16.20	0.45±0.03	0.29±0.05	-35.55
Liver	1.19±0.31	0.73±0.07	-38.65	0.85±0.05	0.56±0.07	-34.11
Kidney	1.12±0.23	0.82±0.04	-26.78	0.79±0.08	0.61±0.12	-22.78
Brain	0.54±0.08	0.46±0.10	-14.81	0.50±0.06	0.37±0.03	-26.00
Muscle	0.74±0.05	0.56±0.09	-24.32	0.64±0.14	0.53±0.08	-17.18
MDH						
Gill	0.77±0.10	0.61±0.05	-20.78	0.65±0.06	0.56±0.04	-13.84
Liver	1.14±0.23	0.83±0.02	-27.19	0.89±0.02	0.76±0.07	-14.61
Kidney	0.84±0.07	0.72±0.08	-14.28	0.71±0.09	0.58±0.08	-18.30
Brain	0.71±0.04	0.65±0.04	-8.45	0.64±0.07	0.47±0.05	-26.56
Muscle	0.80±0.11	0.69±0.06	-13.75	0.73±0.04	0.64±0.02	-12.32

ACP						
Gill	1.98±0.19	2.56±0.11	29.29	2.31±0.37	2.76±0.44	19.48
Liver	2.36±0.56	3.12±1.21	32.20	2.58±0.56	2.30±0.18	-10.85
Kidney	2.11±0.38	2.98±0.45	41.23	2.34±0.43	2.10±0.57	-10.25
Brain	1.76±0.74	2.53±0.31	43.75	1.81±0.24	1.90±0.63	4.97
Muscle	2.34±0.33	2.74±0.52	17.09	3.10±0.78	2.50±0.35	-19.35
ALAT						
Gill	3.89±0.38	4.10±0.27	5.39	3.74±0.70	3.89±0.26	4.01
Liver	4.13±0.74	4.56±0.18	10.41	4.05±0.84	4.56±0.18	12.59
Kidney	5.47±0.75	5.89±0.64	7.67	5.13±0.69	5.45±0.77	6.23
Brain	4.12±0.24	4.69±0.50	13.83	3.92±0.38	4.20±0.46	7.14
Muscle	3.11±0.22	3.76±0.13	20.90	2.89±0.44	3.27±0.37	13.14
AAT						
Gill	1.98±0.23	1.45±0.18	-26.76	1.75±0.31	1.35±0.13	-22.85
Liver	2.68±0.53	2.10±0.33	-21.64	2.56±0.56	1.69±0.30	-33.98
Kidney	1.98±0.22	1.49±0.13	-24.74	1.79±0.32	1.44±0.11	-19.55
Brain	2.49±0.16	1.98±0.19	-20.48	2.31±0.43	1.87±0.19	-19.04
Muscle	2.59±0.41	1.74±0.20	-32.81	2.36±0.51	1.72±0.22	-27.11
AChE						
Gill	3.56±0.39	3.12±0.33	-12.35	3.2±0.85	2.56±0.31	-20.00
Liver	5.12±0.59	4.56±0.75	-10.93	4.51±0.73	3.22±0.52	-28.60
Kidney	4.82±0.74	3.98±0.26	-17.42	4.20±0.66	3.59±0.45	-14.52
Brain	3.58±0.18	2.10±0.45	-41.34	3.29±0.50	1.59±0.21	-51.67
Muscle	4.76±0.28	3.24±0.39	-31.93	3.19±0.42	2.84±0.16	-10.97

DISCUSSION

The edible freshwater fishes constitute one of the major sources of nutritious food for humans. Fish are largely being used for the assessment of the quality of aquatic environment and as such can serve as bio indicators of environmental pollution. Among the aquatic species, fishes are the major targets of toxicants contamination. 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 organism. These parameters are the indicators of the response of the animal to the environmental effects and can also serve as markers for toxicantexposure and effect in fish. The biochemical parameters in fish are valid for physiopathological evaluation and sensitive for detecting potential adverse effects andrelatively early events of pollutant, like pesticide damage. Enzymes are biochemical macromolecules which control metabolic processes of organisms, thus a slight variation in enzyme activity would affect the organisms [19].

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. 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. Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically. Changes in the activities of the enzymes like, succinate

dehydrogenase, malate dehydrogenase and lactate dehydrogenase are sensitive to environmental pollutants like pesticides [12].

On exposure to Phoratethe liver, kidney,brain and gill showed highly elevated LDH levels during 10 day sub lethal followed by lethal and 1, 5 day sub lethal periods.Increased LDH activity suggests a significant increase in the conversion of pyruvate to lactic acid, thereby leading to the accumulation of lactic acid. Lactate form is an important gluconeogenic substrate which can be used to cope with the high and rapid demand of energy due to stress. There was a remarkable increase in LDH activity in the liver and kidney during 10 day sub lethal exposure of Phorate. The increase in LDH level indicated metabolic changes i.e., the glycogen catabolism and glucose shift towards the formation of lactate occurred in stressed fish [20].

In the present investigation the oxidative enzymes like succinate dehydrogenase(SDH) and malate dehydrogenase (MDH) showed a reduction and elevation in non-oxidative enzyme like LDH in their activity in all the osmoregulatory (gill and kidney) and non-osmoregulatory (liver, muscle and brain) tissues of the fish *Cyprinuscarpio,* which indicates the suppression of oxidative metabolism in the fish exposed to acute and chronic toxicity of phorate [21].The decrease in the MDH activity is in line with the decreased SDH activity indicating suppressed oxidative metabolism. As SDH and MDH are the oxidative enzymes involved in Krebs cycle, any disturbance in these enzyme activities will affect the Krebs 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 theactivity of SDH and MDH and increase in the LDH activity after exposingto differentpesticides. 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 [1]

Suneetha, (2012)miss observed a decrease in the SDH activity in the brain, gill, kidney,liver and muscle of the freshwater fish *Labeorohita*after exposing to lethal and sub lethalconcentrations of two pesticides, such as endosulfan and fenvalerate for 24 hrs and 15 days. Khemani et al., (1989) 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 [22]

It is known that SDH and MDH acts as indictors of aerobic respiration, their inhibitionand with subsequent elevation of LDH indicates the prevalence of anaerobic conditionsimposed by the stress factor of phorate toxicity. As SDH and MDH are the key enzymes inTCA cycle, it is logical to assume that with the inhibition of SDH and MDH and elevation ofLDH activities, the metabolic pathway might have turned to anaerobic to meet the increasedenergy demands during the phorate exerted toxic stress.

The decrease in SDH and MDH activity also indicates the impairment of oxidativemetabolism in the mitochondria as a consequence of hypoxic conditions under pesticideexposure, most probably by disrupting the oxygen binding capacity of the respiratory pigment. The decrease in SDH and MDH activity may be due to the disorganization of mitochondria affecting enzymes of TCA cycle. The decrease in these enzyme activities might be probably due to mitochondrial damage and decreased state of respiration. The fall in these enzyme activities 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. Any alteration in the respiratory area decreases the oxygen absorption capacity of the gill due to its close contact with polluted water. It may also be one of the reasons for the diminished activity of SDH and MDH[2]
Nagarathnamma, (1982) and Srinivasulu Reddy and RamanaRao, (1986) reported that disruption in gill lamellae caused by the organophosphate pesticides adversely affected theabsorption of oxygen from the surrounding medium in fish and prawn respectively. Decrease in SDH and MDH activity may be due to damage in the mitochondrial structural integrity in the organs of the fish. The decrease in the activity of MDH may suggests the lower level of functioning of TCA cycle due to inadequate supply of substrate or decreased oxygen uptake at the tissue level during phorate toxicity stress. Another probable reason for the low level of MDH activity may be the reduced SDH activity which in turn lowers fumarate-malate conversions[2].
Aminotransferases are widely accepted for their significance in protein metabolism by virtue of their ability to control both synthesis and degradation of amino acids. Alterations in the aminotransferase activities are often associated with changes in several other metabolic functions and thus represent widespread alterations in the organism's physiological state. AST and ALT are two essential enzymes mainly involved in the inter-conversion of important compounds such as pyruvate, oxaloacetate, α-ketoglutarate and amino acids thus bringing the protein and carbohydrate metabolism on one hand and aspartate, alanine and glutamic acid on the other hand.
In the present study, the assessment of AAT and ALAT enzymes are the best indicators of organophosphate and chloroacetanilide herbicide pollution. Alterations in AAT and ALAT enzyme activity in fish havebeen used frequently as potential stress biomarker in the contaminated aquatic ecosystem. The activities of the aspartate and alanine amino transferase serve as strategic links between protein and carbohydrate metabolism under several physiological and pathological conditions [23].
Significant elevation in the activities AAT and ALAT in different tissues on exposure to both the pesticides in the experimental groups may be due to incorporation of ketoacids into the TCA cycle via generation of glutamate through tissue transamination followed by their conversion of α-ketoglutarate through oxidative deamination to favor gluconeogenesis or energy production. Similar elevation in activity was observed in gill, liver and muscle of *Cyprinuscarpio* and *Channapunctatus* freshwater fishes were exposed to lethal and sublethal concentration for different durations due to cypermethrin stress and Thiamethoxam stress [24].
AAT and ALAT are placed in both mitochondrial and cytosol fractions of the cell. A close relation seems to exist between the mitochondrial integrity and transaminase levels and any modification in the organization of mitochondria is bound to alter the enzyme systems associated with it [25]. The increased activities of AAT and ALAT as observed in the present study may also be due to the mitochondrial disruption and damage as a result of Phorate induced stress.
The ACP is a hydrolytic lysosomal enzyme released by lysosomes for the hydrolysis of foreign materials and increase in its activity is probably related to the cellular damage. The acid phosphatase has a role in eliminating certain toxins by the detoxification function. However it is difficult to tolerate the decrease in ACP activity with necrosis. Increase in acid phosphatase and alkaline phosphatase activities can be interpreted as a shift, which emphasize on energy breakdown pathway from normal ATPase system which includes phosphorylation. The phosphatases, ACP and ALP are active at specific pH and are usually called as phosphomonoesterases. The toxicity of pesticide, increases the ACP and ALP activity in fishes [2].

Increased ACP enzyme activity at all the concentrations of Phorate might be due to increase in protease activity which caused damage to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytoplasm. Changes in the enzyme activity are due to adverse effect of xenobiotics on the cell and its organelles. In the present study, the mean value of ACP activity in the brain, muscle and gill of *C. catla*increased during the long time of exposure. This increased phosphatase activity was due to the cellular damage caused by hepatotoxins or a response to overcome toxicity of Phorate. The significant difference in phosphatases activities between the control and experimental groups of fish species might be considered due to the damage of hepatic tissue with disfunctions of organs. The elevation in ACP activity proposes an increase in the lysosomal mobilization and cell necrosis due to the pesticide toxicity [26].

The elevation of ACP activity in all the tissues of fish has been noticed in the present experiment. Many researchers have supported the increase in ACP activity levels by studying various species exposed under different toxicants and pesticides [27]. Thus in the present study, the pesticides intoxication produced elevation in the activity levels of ACP in all the tested tissues of the fish.

The results of the present experiment are in correlation with the previous work done on various fish species exposed to different toxicants where ACP levels were increased [28]. The statistical analysis indicated the significant increase ($p< 0.05$) in ACP activity levels in all the tissues but the increase is not significant ($p< 0.05$) in the kidney and brain tissues during 1day sub lethal exposure.

In the present study the AChE activity was inhibited in all the tissues. This might be due to the pesticidal activity induced on the brain in the fish. Since, the compounds are neurotoxic the activity levels of AChE were inhibited. The residues of the Phorate in brain tissues were maximumwhere the inhibition of activity was also maximum, as the exposed fish is continuously swimming in the pesticide medium throughout the exposure period.

Several reports on AChE inhibitory effects due to various pesticides in different fish species are in corroboration with the present findings [29]. Inhibition of AChE is responsible for the depletion of acetylcholine which will result in excessive stimulation of cholinergic nerves. Several factors seem to be involved in affecting the AChE activity caused by toxicants such as environmental temperature, species, sex, age, length to time and exposure concentrations . Inhibition of AChE impairs cholinergic nerve impulses and may result in death of organisms [30].

Responses to organophosphate insecticides by aquatic organisms are broad ranging which are depending on the compound, exposure time, water quality and the species. Organophosphate insecticides are known to inhibit acetylcholinesterase,which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate. The inhibitory effects of organophosphate insecticides are dependent on their binding capacity to the enzyme active site and by their rate of phosphorylation in relation to the behavior and age [31].

Recently, Marigoudar*et al*. revealed that the cypermethrin inhibits AChE activity at sublethal concentration in functionally different organs of *L. rohita* [32]. It has been reported that similar observations were noticed in *L. rohita* when it treated with the sublethal concentration of Endosulfan pesticide [33]. In the present study AChE activity levels in brain was inhibited by the pesticide.Tilak*et al*. reported that similar an inhibition of AChE activity in *C. catla, L. rohita*and *C. mrigala*exposed to Chlorpyrifos also supports the present work [34].

However *C. catla* is more sensitive to the toxicity of Phorate compared to control group fish. Accordingly, there is an increasing need to minimize the adverse impacts of these pesticides on the environmental quality by the controlled application of such hazardous chemicals.

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