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RESEARCH ARTICLE

BIOCHEMICAL CHANGES INDUCED BY PHENTHOATE, AN ORGANOPHOSPHATE COMPOUND TO SUBLETHAL CONCENTRATIONS IN FRESHWATER FISH *LABEO ROHITA* (HAMILTON)

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ABSTRACT

The present study was carried out to investigate the toxicity of commercial grade Phenthoate effects on biochemical parameters in an Indian major carp *Labeo rohita* under the controlled laboratory conditions. The median lethal concentration value (96 h LC_{50}) of Phenthoate was found to be 2.1 mgl^{-1} by using Finney's Probit Method. Fish were exposed to sublethal concentration of $1/10^{th}$ 96 h LC_{50} (0.21 mgl^{-1}) for the period of 1 and 10 days respectively and the changes were assessed by using various methods. A significant increase in aminotransferase (AST), activity was observed in muscle (83.77% - highest), kidney (47.53% - lowest), ALT activity was noticed in muscle (61.65% - highest) and in kidney (1.84% - lowest), the ACP activity level elevated was observed in intestine (21.36% - highest) and in gill (0.78% - lowest). The acetyl cholinesterase (AChE) enzyme decreased was observed in gill (53.33% - highest) and in kidney (1.20% - lowest) for 1day. Similarly, during 10 days sublethal exposure, AST activity elevated in kidney (69.10% - highest) and muscle (18.82% - lowest), ALT activity also increased in muscle (36.77% - lowest) and (131.74% - highest) in intestine tissues. The ACP percent change was noticed in brain (36.44% - highest) and in muscle (20.20% - lowest). The AChE activity decreased in muscle (70.04% - highest) and (47.68 % - lowest) in brain compared to the control group fish ($P < 0.05$) during the experimentation. Hence, the percent change of biochemical constituent's has gradually increased or decreased due to disruption of internal organ in all tissues. This might be due to enhanced enzyme turnover under pesticide stress. The decreased levels of AChE enzyme possibly due to the activity of the organophosphorus compound with the active site of AChE followed by phosphorylation of the phosphorus of the organophosphate to the oxygen of the hydroxyl group of serine at the active site.

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INTRODUCTION

Freshwater is a finite essential resource component for developmental activities of agriculture, aquaculture and industry and even for human existing. Sustainable development is mostly depends upon the availability of adequate quality and quantity of efficient water resources management (Odoemelam *et al*., 2014). The rapid growth of population, unplanned industrialization, urbanization, poor agricultural practices are the major contributory factors responsible for water pollution by discharging of various chemical pesticides and heavy metals, which causes serious threats to aquatic life like fishes and other organisms. It is obvious that the adverse effects of toxic pollutants can have great impacts on members of the aquatic food chain in the environment such as hazards to human health, harm to living resources, hindrances to aquatic activities and reduction in its aesthetic values. During the last few decades the indiscriminate applications of various pesticides and fertilizers has created havoc in the environment and which has become a reason for different ailments in human beings and other fauna and flora (Arockia Rita and John Milton, 2014). Due to their lower persistence and rapid biodegradability in the environment, organophosphates are used judiciously to regulate a wide variety of agricultural pests as well as ectoparacites in fish in aquaculture. It also has

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become the most commonly used class of insecticides in the world for domestic and industrial purposes (Das and Mukherjee (2000). The exposure risk and impacts of aquatic ecosystems to these pesticides are often difficult to assess because they degrade very quickly (Barry and Logan, 1998), can be absorbed onto the sediments in the environment (Peterson and Batley, 1993; Hamer *et al*., 1999).

Among the aqua fauna, fish form an important group attributes due their rich nutritive value. However, the study of these insecticides is very important, because they are highly poisonous to aquatic organisms. The fish species are very sensitive to biochemical, enzymatic as well as hormone destruction, which might be due to the stressful conditions and toxicants effect in the tissues. Organophosphates are powerful neurotoxic chemicals as they inhibit acetyl cholinesterase (AChE) reported by no. of researchers (Kabeer and Rao, 1980; Klaverkamp and Hobden, 1980; Lieske *et al*., 1980; Rath and Mishra, 1981). When the chemical pesticides come in contact with internal organs, unrepairable changes in metabolic activities, many pesticides have been reported to generate a number of serious biochemical responses in the fish at sublethal and lethal levels, which measurements shows as index of pollution.

These analysis produce early warning signs before other toxicological points, including death are evident (Sabiha Khan and Neelam (2012); and Livingstone, 1998) Hence in the present study an attempt is made to examine the toxicological impacts of phenthoate 50% EC on certain biochemical changes (AST, AAT, AChE and ACP) in the tissues of Indian major carp *Labeo rohita* exposed to sublethal concentrations for 1 and 10 days respectively. The percent change of enzymatic activities found to be declined when compared to over control of fish along with standard deviation.

MATERIALS AND METHODS

Healthy juvenile freshwater fish *L.rohita* 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 (APHA, 2005) having temperature of 28 ± 20^0C, 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 Phenthoate to sublethal concentration of $1/10^{th}$ 96 h LC_{50} value i.e., 0.21 mg/l concentration for 1day 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 (Schreck and Brouna, 1975). 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, intestine and muscle were taken for biochemical estimation of AST, ALT, ACP and AChE.

Estimation of Acid Phosphatase

The activity of acid phosphatase was estimated by the method of Bodasky (1932). 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 colour 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 enzyme assay were performed spectrophotametrycally by the method of Ellaman *et al.* (1961). 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 absorbances of 412 nm were recorded continuously 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 aminotransferase (AST and ALT) activity

The amino transferage activities were estimated by the method of Mohan and cook (1957). The reaction mixture of 1.5 ml 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 370 C for 30 minutes. The reaction was stopped by adding 1 ml of 2, 4-dinitrophenyl hydrazine 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

Statistical analysis

The analyzing data was subjected to unpaired t-test using Graphpad-software 2015 version and standard bar graphs were plotted by following (MS-Excel-2007). The significant difference was set up at ($P < 0.05$ and $P < 0.001$) for all parameters in the experimental group.

RESULTS AND DISCUSSION

The calculated biochemical values of AST, ALT, ACP activity and AChE inhibition and their percent changes over control along with standard deviation are given in Table. 1 - 3 and graphically represented in Figure 1 - 3 for 1 day and 10 days sublethal concentrations on exposure to Phenthoate

respectively. The biochemical variables were studied in different organs like intestine, liver, brain, kidney, muscle and gill in the experimental group fish *Labeo rohita* treated with the organophosphate pesticide, Phenthoate (50% EC).

AST Activity

The calculated mean values of aspartate amino transeferase (AST) activity along with the standard deviation, mean values and the percent change over control are represented in Table 1 and 2 and its comparative account of AST activity in different tissues can be seen in Figure 1 and 2 for 1day lethal and sublethal exposure group fish. The 10 day sublethal exposure group mean and standard deviation values shown in Table 3 and AST activity in different tissues can be noticed in Figure 3 during Phenthoate exposure. In control fish group the AST activity in different tissues is in the following order: Kidney > Gill > Liver > Muscle > Brain > Intestine.

In the fish reared as control for 1 day and 10 days, the AST activity levels were highest in kidney (3.65 and 3.94 µ moles of pyruvate formed/mg of protein/h), followed by gill (3.54 and 3.56 µ moles of pyruvate formed/mg of protein/h) and liver (3.37 and 3.34 µ moles of pyruvate formed/mg of protein/h). Moderate values were observed in brain (3.15 and 3.19 µ moles of pyruvate formed/mg of protein/h), very low in brain (2.26 and 2.17 µ moles of pyruvate formed/mg of protein/h) and least in intestine (1.82 and 1.66 µ moles of pyruvate formed/mg of protein/h) was noticed respectively.

The AST activity significantly increased ($p < 0.05$) during sublethal and lethal exposures of Phenthoate and Butachlor when compared with the control. The percent depletion of AST activity in test tissues of the fish, *L. rohita* under 50% EC of Phenthoate is in the following order:

Phenthoate Sublethal for 1 Day: Gill >Liver > Muscle >Brain > Intestine >Kidney
Phenthoate Lethal for 1 Day: Muscle > Gill > Liver >Brain > Intestine > Kidney
Phenthoate Sublethal for 10 Days: Kidney > Gill > Muscle > Liver >Brain >Intestine

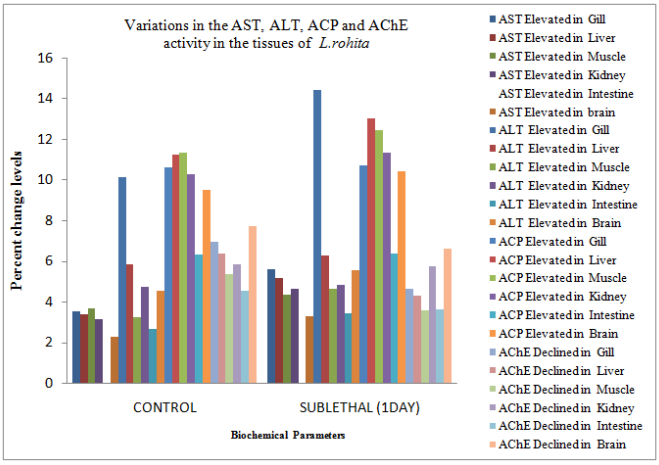

Figure 1. The % change in the specific activities of aminotransferase (AST and ALT), acid phosphatase (ACP) and acetyl cholinesterase enzyme and % change over control in different tissues of fish *L.rohita* treated with sublethal doses of Phenthoate for 1 day

Table 1. Alterations in the specific activities of aminotransferase (AST and ALT), acid phosphatase (ACP) and acetyl cholinesterase enzyme and % change over control in different tissues of fish *L.rohita* treated with sublethal doses of Phenthoate for 1 day

Biochemical Constituents	Tissues	Control (X±SD)	Sublethal (1Day) (X±SD)	%Change (1Day)	Regression
AST	Gill	3.54 ± 0.032	5.63±0.287	58.72	Y=2.09 x + 1.45
	Liver	3.37± 0.019	5.16±0.018	52.21	Y = 1.79 x + 1.58
	Kidney	3.65±0.22	4.34±0.013	18.82	Y= 0.69x + 2.96
	Muscle	3.15±0.024	4.64±0.015	47.27	Y = 1.49x + 1.66
	Intestine	1.82±0.011	2.23±0.736	22.37	Y = 0.41x +1.41
	brain	2.26±0.025	3.28±0.019	44.87	Y = 1.02x + 1.24
ALT	Gill	10.16±0.015	14.43±0.017	42.05	Y = 4.27x + 5.89
	Liver	5.85 ±0.033	6.26±0.0171	7.03	Y = 4.12X + 5.44
	Kidney	3.25 ±0.027	4.65 ±0.008	42.99	Y = 1.42x+ 1.85
	Muscle	4.74±0.028	4.83 ±0.017	1.84	Y = 0.09x + 4.65
	Intestine	2.65±0.023	3.45±0.0129	30.38	Y = 0.8x +1.85
	brain	4.55 ±0.033	5.54 ±0.021	21.76	Y = 0.99x + 3.56
ACP	Gill	10.65±0.020	10.74±0.015	0.78	Y= 0.09x +10.56
	Liver	11.25±0.019	13.04 ±0.025	15.95	Y= 1.79x +9.46
	Kidney	11.36±0.029	12.46 ±0.041	9.66	Y = 1.1x +10.26
	Muscle	10.32±0.007	11.36±0.0126	10.03	Y =1.04x + 9.28
	Intestine	6.33 ±0.016	6.40 ±0.019	1.07	Y= 0.07x + 6.26
	brain	9.53 ±0.03	10.44 ±0.022	9.49	Y = 0.91x + 8.6
AChE	Gill	6.94 ±0.031	4.65 ±0.022	33.02	Y= -2.29x+ 9.2
	Liver	6.37 ±0.021	4.32 ±0.017	32.06	Y= -2..05 x + 8.42
	Kidney	5.36 ±0.029	3.56 ±0.017	33.64	Y= - 1.8x + 7.16
	Muscle	5.83 ±0.027	5.76 ±0.022	1.2	Y= - 0.07x + 5.9
	Intestine	4.54 ±0.028	3.62 ±0.005	20.19	Y= - 0.92x + 5.46
	brain	7.75 ±0.031	6.20 ±0.062	20	Y= - 1.13x + 8.88

Values are the mean of five observations and standard deviation is indicated as (X±SD).
The values are significant at $p < 0.05$. NS – Not Significant.

ALT Activity

The calculated mean values of alanine amino transeferase (ALT) activity along with the standard deviation, mean values and the percent change over control are represented in Table.1 and 2 and its comparative account of ALT activity in different tissues can be observed in Figure 1 and 2 for 1 day lethal and sublethal exposure group fish. The 10 day sublethal exposure group mean and standard deviation values shown in Table 3 and ALT activity in different tissues can be observed in Figure 3 during Phenthoate exposure.

In the fish reared as control for 1 day and 10 days, the ALT activity levels were the highest in gill (10.16 and 10.02 µ moles of pyruvate formed/mg of protein/h), followed by liver (5.85 and 5.31 µ moles of pyruvate formed/mg of protein/h) and muscle (4.74 and 4.48 µ moles of pyruvate formed/mg of protein/h). Moderate values were observed in brain (4.55 and 4.46 µ moles of pyruvate formed/mg of protein/h), very low in kidney (3.25 and 3.17 µ moles of pyruvate formed/mg of protein/h) and least in intestine (2.65 and 2.63 µ moles of pyruvate formed/mg of protein/h) was observed respectively.

Table 2. Alterations in the specific activities of aminotransferase (AST and ALT), acid phosphatase (ACP) and acetyl cholinesterase enzyme (AChE) and % change over control in different tissues of fish *L.rohita* treated with lethal doses of Phenthoate for 1 day

Biochemical Constituents	Tissues	Control (X±SD)	Lethal (1day) (X±SD)	% Change (1day)	Regression
AST	Gill	3.54 ± 0.032	5.86 ±0.100	65.41	Y= 2.3x + 1.22
	Liver	3.37± 0.019	5.40 ± 0.35	60.39	Y = 2.03 + 1.34
	Kidney	3.65±0.22	4.45±0.03	21.97	Y= 0.8x + 1.34
	Muscle	3.15±0.024	5.430±0.018	69.1	Y= 2.28x+ 0.78
	Intestine	1.82±0.011	2.34±0.028	28.56	Y=0.52x +1.3
	brain	2.26±0.025	3.46±0.010	52.83	Y=1.2x + 1.06
ALT	Gill	10.16±0.015	14.64±0.008	44.09	Y= 4.48xs +5.68
	Liver	5.85 ±0.033	6.94 ±0.017	18.66	Y=1.09+ x 4.76
	Kidney	3.25 ±0.027	5.26 ± 0.029	61.65	Y=2.01x+1.24
	Muscle	4.74±0.028	5.36± 0.017	13.23	Y=0.62x +4.12
	Intestine	2.65±0.023	3.64±0.022	37.43	Y=0.91x +5.42
	brain	4.55 ±0.033	6.56±0.036	44.17	Y=2.01x+1.54
ACP	Gill	10.65±0.020	12.06±0.017	13.17	Y=1.41x+9.24
	Liver	11.25±0.019	13.65±0.013	21.36	Y=2.4x +8.85
	Kidney	11.36±0.029	12.87±0.029	13.29	Y=1.51x+9.85
	Muscle	10.32±0.007	11.57±0.005	12.13	Y=1.25x+9.07
	Intestine	6.33 ±0.016	7.24±0.013	14.45	Y=0.91x+5.42
	brain	9.53 ±0.03	11.06±0.022	16.05	Y= 1.53x+8
AChE	Gill	6.94 ±0.031	3.24 ±0.022	53.33	Y= -3.7x +10.64
	Liver	6.37 ±0.021	3.842±0.027	39.65	Y= -2.528x+8.89
	Kidney	5.36 ±0.029	2.74 ±0.029	48.79	Y= - 2.62x+ 7.98
	Muscle	5.83 ±0.027	4.87 ±0.026	16.54	Y= - 0.6x +6.97
	Intestine	4.54 ±0.028	3.13 ±0.018	31.09	Y= - 1.41x +5.95
	brain	7.75 ±0.031	5.22±0.024	32.68	Y= -2.53 x+10.28

Values are the mean of five observations and standard deviation is indicated as (X±SD).
The values are significant at $p < 0.05$. NS – Not Significant.

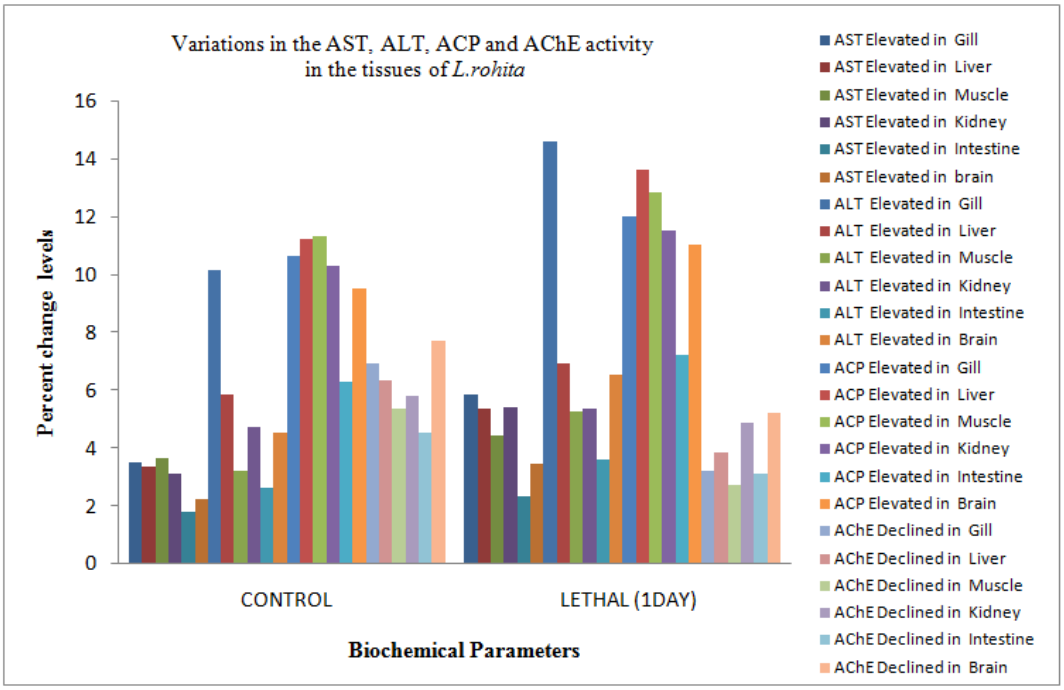

Figure 2. The % change in the specific activities of aminotransferase (AST and ALT), acid phosphatase (ACP) and acetyl cholinesterase enzyme (AChE) and % change over control in different tissues of fish *L.rohita* treated with lethal doses of phenthoate for 1 day

In the control fish group the ALT activity in different tissues is in the following order: Gill > Liver > Muscle > Brain > Kidney > Intestine.

tissues of the fish, *L. rohita* under 50% EC of Phenthoate is in the following order:

Table 3. Alterations in the specific activities of aminotransferase (AST and ALT), acid phosphatase (ACP) and acetyl cholinesterase enzyme (AChE) and % change over control in different tissues of fish *L.rohita* treated with lethal doses of Phenthoate for 1 day

Biochemical Constituents	Tissues	Control (X±SD)	Sublethal (10Days) (X±SD)	% Change	Regression
AST	Gill	3.56±0.036	6.34±0.014	78.29	Y= 2.76x +0.78
	Liver	3.34±0.02	5.44±0.013	62.72	Y= 2.1x+ 1.24
	Kidney	3.94±0.025	7.24±0.030	83.77	Y= 3.3x +0.64
	Muscle	3.19±0.041	5.44±0.044	70.38	Y= 2.25 + 0.94
	Intestine	1.66±0.036	2.45±0.026	47.53	Y= 0.79x +0.87
	brain	2.17±0.034	3.32±0.013	53.5	Y= 1.15x + 1.02
ALT	Gill	10.02±0.016	14.17 ±0.025	41.39	Y= 4.15x +5.87
	Liver	5.31 ±0.038	7.26±0.022	36.77	Y= 1.95x +3.36
	Kidney	3.17 ±0.023	7.34 ±0.022	131.74	Y= 4.17x -1
	Muscle	4.48 ±0.013	7.56 ±0.035	68.82	Y= 3.08x +1.4
	Intestine	2.63 ±0.024	4.33 ±0.024	64.89	Y= 1.7x +0.93
	brain	4.46 ±0.023	7.95 ±0.013	78.34	Y= 3.49x + 0.97
ACP	Gill	10.26±0.021	13.45±0.017	31.01	Y= 3.19x +7.07
	Liver	10.83±0.024	14.45 ± 0.025	33.42	Y=3.62x + 7.21
	Kidney	11.25±0.024	14.19±0.031	26.2	Y=2.94x +8.31
	Muscle	10.18±0.013	13.25±0.025	30.23	Y= 3.07x +7.11
	Intestine	6.05±0.030	7.78 ±0.158	28.76	Y= 1.73x +4.32
	brain	9.13±0.015	12.45±0.222	36.44	Y= 3.32x + 5.81
AChE	Gill	6.15 ±0.011	1.96 ±0.020	68.1	Y= -4.19x+10.34
	Liver	6.02 ±0.008	1.96 ±0.024	67.35	Y= -4.06x+10.08
	Kidney	5.14 ±0.016	1.54 ±0.034	70.04	Y= - 3.06x +8.47
	Muscle	5.14 ±0.011	2.35±0.026	54.28	Y= - 2.79x +7.93
	Intestine	4.15 ±0.013	2.16 ±0.024	47.81	Y= - 1.99x +6.14
	brain	7.23 ±0.018	3.78±0.021	47.68	Y= -3.45x+10.68

Values are the mean of five observations and standard deviation is indicated as (X±SD).
The values are significant at $p < 0.05$, NS – Not Significant.

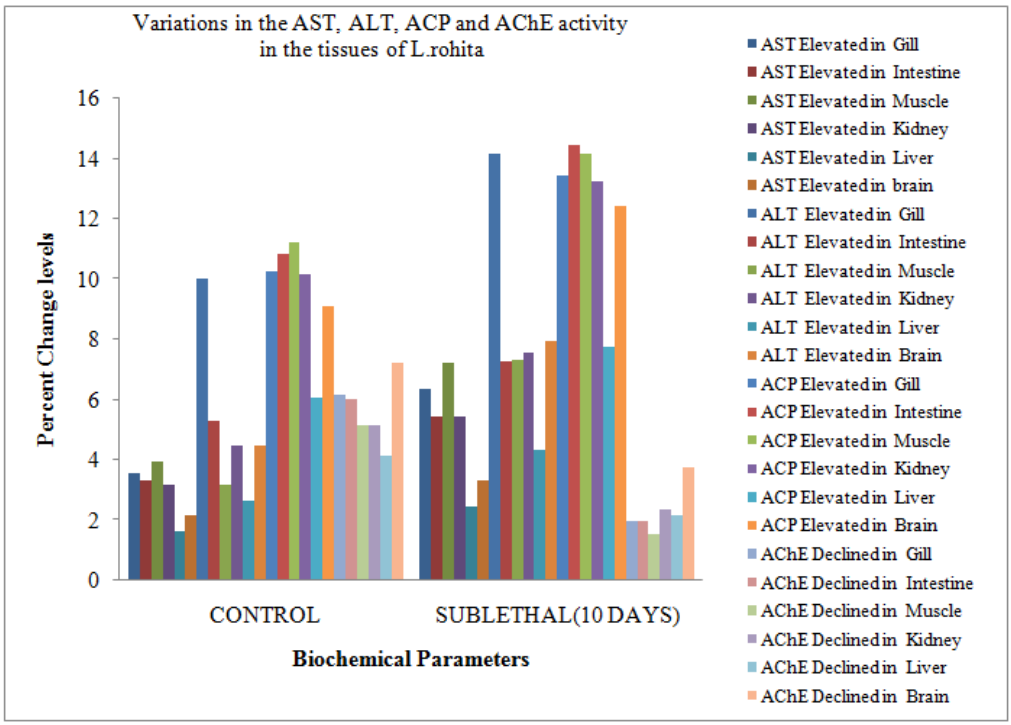

Figure 3. The % change in the specific activities of aminotransferase (AST and ALT), acid phosphatase (ACP) and acetyl cholinesterase enzyme (AChE) and % change over control in different tissues of fish *L.rohita* treated with lethal doses of phenthoate for 10 days

The ALT activity significantly increased ($P < 0.05$) during sublethal and lethal exposures of Phenthoate when compared to the control. The percent depletion of ALT activity in test

Phenthoate Sublethal for 1 Day: Gill > Kidney > Intestine > Brain > Liver > Muscle
Phenthoate Lethal for 1 Day: Kidney > Brain > Gill > Intestine > Liver > Muscle

Phenthoate Sublethal for 10 Days: Kidney > Brain > Muscle > Intestine > Gill > Liver

In the present study, the levels of ALT activity was found to be increased in tissues of *L. rohita* on administration of sub lethal and lethal doses of Phenthoate for 1 day and 10 day when compared to control group. In the present investigation, the data on ALT activity levels elevated in both the nervous (brain) and non-nervous (gill, liver, muscle, kidney and intestine) organs of the fish *L. rohita* exposed to lethal and sublethal toxicity of organophosphate pesticides. The elevation of aminotransferases activity levels was found to be high during 10 days sub lethal followed by 1 day sublethal and 1 day lethal exposures of the pesticide Phenthoate. The elevated levels of ALT activity were highest in kidney tissues during 10 days sublethal concentration.

ACP Activity

The calculated mean values of acid phosphatase (ACP) activity along with the mean, standard deviation values and the percent change over control are represented in Table 1and 2 and comparative account of ACP activity in different tissues can be observed in Figure 1 and 2 for 1day lethal and sublethal exposure group. The 10 days sub lethal exposure group mean and standard deviation values shown in Table. 3 and ACP activity in different tissues can be observed in Figure .3 during Phenthoate exposure. In control fish of *L. rohita,* the values of ACP activity in different tissues are in the following order:

Kidney > Liver > Gill > Muscle > Brain > intestine

In the fish reared as control for 1 day and 10 days, the Acid Phosphatase activity was the highest in Kidney (11.36 and 11.25 mg pi/g of protein/h), followed by liver (11.25 and 10.83 mg pi/g of protein/h) and gill (10.65 and 10.26 mg pi/g of protein/h). Moderate values were observed in muscle (10.32 and 10.18 mg pi/g of protein/h), very low in brain (9.53 and 9.13 mg pi/g of protein/h) and least was noticed in intestine (6.33 and 6.05 mg pi/g of protein/h) respectively.

The ACP activity significantly changed ($P < 0.05$) during sublethal and lethal exposures of Phenthoate when compared to the control. The percent depletion of ACP activity in test tissues of the fish, *L. rohita* under 50% EC of Phenthoate is in the following order:

Phenthoate Sublethal for 1 Day: Liver > Muscle > Kidney > Brain > Intestine > Gill

Phenthoate Lethal for 1 Day: Liver > Brain > Intestine > Kidney > Gill > Muscle

Phenthoate Sublethal for 10 Days: Brain > liver > Gill > Muscle > Intestine > Kidney

In the present study, the levels of ACP activity was found to be increased in all fish tissues of *L. rohita* (i.e., muscle, liver, brain, gill, kidney and intestine) exposed to Phenthoate at sublethal and lethal concentration for 1day and 10 days when compared to the control fish group. The elevation in ACP level was found to be high during 10 days sublethal than 1 day lethal and 1 day sublethal exposure periods. The increased level of ACP activity was highest in brain and intestinal tissues during 10 day sublethal exposure.

AChE Activity

The calculated mean values of acetyl cholinesterase (AChE) activity along with standard deviation, mean values and the percent change over control are represented in Table 1 and 2 and a comparative account of AChE activity in different tissues can be observed in Figure .1 and 2 for 1day lethal and sublethal exposure group. The 10 day sublethal exposure group mean, standard deviation values shown in Table 3 and AChE activity in different tissues can be observed in Figure .3 during Phenthoate exposure. In control fish of *L. rohita,* the values of AChE activity in different tissues are in the following order: **Brain > Gill > Liver > Muscle > Kidney > Intestine**

In the fish reared as control for 1 day and 10 days, the AChE activity was the highest in brain(7.75 and 7.23 µ moles of acetylthiocholine iodine hydrolysed/g of tissue/min), followed by gill (6.94and 6.15 µ moles of acetylthiocholine iodine hydrolysed/g of tissue/min) and liver (6.37 and 6.02 µ moles of acetylthiocholine iodine hydrolysed/g of tissue/min). Moderate values were observed in muscle (5.83 and 5.14 µ moles of acetylthiocholine iodine hydrolysed/g of tissue/min), very low in kidney (5.36 and 5.14 µ moles of acetylthiocholine iodine hydrolysed/g of tissue/min) and least was noticed in intestine (4.54 and 4.14 µ moles of acetylthiocholine iodine hydrolysed/g of tissue/min) respectively.

The AChE activity significantly decreased ($P < 0.05$) during sublethal and lethal exposures of Phenthoate when compared with control fish group. The percent depletion of AChE activity in test tissues of the fish, *L. rohita* under 50% EC of Phenthoate is in the following order:

Phenthoate Sublethal for 1 Day: Kidney > Gill > Liver >Intestine > Brain > Muscle

Phenthoate Lethal for 1 Day: Gill > Kidney > Liver > Brain > Intestine > Muscle

Phenthoate Sublethal for 10 Days: Kidney > Gill > Liver > Muscle > Intestine > B rain

In the present investigation the data on AChE activity level revealed a decrease in the enzyme activity in both the nervous (brain) and non-nervous (gill, liver, muscle, kidney and intestine) organs of the fish *L. rohita* exposed to acute and chronic toxicity of Phenthoate sublethal and lethal toxicity exposure. The decrement in AChE level was found to be more in 10 days sublethal than 1 day sublethal and lethal exposure period.

DISCUSSION

Aminotransferases are widely accepted for their significance in protein metabolism by virtue of their ability to control both

synthesis and degradation of amino acids. 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 evaluation of AST and ALT enzyme are the best indicators of organophosphate pollution. Alterations in AST and ALT enzyme activity in fish have been used frequently as potential stress biomarker in contamination of aquatic ecosystem (Kim *et al*., 2008; Hedayati *et al*., 2010). The activities of the aspartate and alanine amino transferases serve as strategic links between protein and carbohydrate metabolism under several physiological and pathological conditions (Shivakumar *et al*., 2005).

Amino transferases play a key role in the utilization of amino acids for the oxidation or gluconeogenesis process (Kumar *et al*., 2011). AST and ALT are the enzymes, which are frequently used for the identification of damage caused by various toxicants in different tissues of fish organs such as liver, muscle and gill (De la Torre *et al*., 1999). The elevation of AST and ALT activity provides the oxaloacetate required for the gluconeogenesis pathway to meet the additional supply of glucose for the production of energy under reduced phase of oxidative metabolism (Venkata Chandrudu and Radha krishnaiah., 2013; Velumurugan *et al*., 2007). AST and ALT are also an enzymatic stress biomarker and its change detects damages in several tissues and organs of fish. Elevation in the levels of AST and ALT activity in different tissues of brain, liver, muscle gill and kidney of the fish *L. rohita* can be considered as a response to the toxic stress. Significant elevation in the activities of AST and ALT in different tissues on exposure to the organophosphate pesticide 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 favour gluconeogenesis or energy production (Prasanth and Neelagund, 2008). Similar elevation in activity was observed in gill, liver and muscle of *Cyprinus carpio* a freshwater fish, when it was exposed to lethal and sublethal concentration for different durations due to cypermethrin stress (Khalid Abdullah *et al* ., 2014) and Thiamethoxam stress in *Channa punctatus* (Anil Kumar *et al*., 2010). The increased activities of both AST and ALT as observed in the present study may also be due to the mitochondrial disruption and damage as a result of Phenthoate induced stress and also the elevation of aminotransferase activity in different tissues of treated fishes suggest either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or fatty acids during Phenthoate intoxication. AST and ALT are non-plasma specific enzymes that are localized in tissue cells of liver, heart, gills, kidneys, muscles and other organs and their presence in the plasma may give specific information about organ dysfunction (Gabriel and George 2005). The enhanced activity of AST and ALT was observed in the fish tissues under toxic stressful condition of enhanced gluconeogenesis. Many of the scientists have reported the alterations in AST and ALT activity on exposure to different pesticides (Begum, 2005; Neelima *et al*., 2013; Gabriel *et al*., 2012). The elevation trend in ALT activity levels were noticed when various toxicants worked on *L. rohita* as well as *C. punctatus* (Tilak *et al*., 2009; Saravanan *et al*., 2010). Moreover the AST and ALT is liver specific enzyme and is sensitive measures of hepato toxicity. According to Gabriel and George (2005), transamination is one principle pathway for synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuating energy demands of the organism under various adaptive conditions. Yildirim *et al*., (2006) have reported an increase in AST and ALT enzyme activities in gills, liver and kidney and have proposed that elevated enzyme activity is with the intension to increase the role of proteins for energy production during stress. Similar elevation in aminotransferases also have been reported by peer researchers (Arshad *et al*., 2007; Velmurugan *et al*., 2008; Gabriel *et al*., 2011 and 2012), which is due to the increased utilization of amino acids for energy synthesis, as a consequence of this, fish suffers from toxic stress and energy crisis.

The increase in ALT and AAT activity levels indicated that the tissue damages in liver, kidney and gill tissues in toxicant exposed fish group. The similar results were observed in catfish *Clarias albopunctatus* when exposed to ambient urea and heavy metals by the (Oluah, 1999). During the experiment, it was observed that the reduction of proteins under the stressful condition due to Phenthoate toxicity observed in different tissues of fish *L. rohita* indicates proteolysis, prompting the suggestion that the proteins were utilized to meet the excess energy demands imposed by the toxic stress. Thus, the changes in the activity levels of aminotransferases induced by the pesticide in experiment clearly indicated that the stress brings about the metabolic reorientation in the tissues by raising energy resources through transaminase systems. The statistical analysis shows significant increase ($P < 0.05$) in the AST and ALT activity levels in all the tissues except intestinal tissues of fish *L. rohita* under organophosphate pesticide Phenthoate toxicity. 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. It is difficult however 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 ACP and ALP activity in fishes (Tejendra *et al*., 1990). Increased ACP enzyme activity at all the concentrations of Phenthoate 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 kidney, liver and gill of *L. rohita* 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 Phenthoate. 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 dysfunctions of organs. The elevation in ACP activity proposes an increase in the lysosomal mobilization and cell necrosis due to the pesticide toxicity (Venkateswara Rao, 2006). The enzyme ACP activity elevation in brain tissue was described in stress-exposed *C. punctatus* (Abdul Naveed *et al*., 2011) and *Anabas testudineus* (Santhakumar and Balaji, 2000). The sub-acute exposure pesticide chlorpyrifos revealed increased activity of ACP content in the liver and kidney tissues of *Gumbusia affinis*, and ACP activity is a conventional indicator of liver damage in the fish (Sabiha Khan and Neelam Sharma, 2012). Dose dependent and significant increase in the activity of acid phosphatase may be attributed to the hepatic and renal damage (Sreenivasan *et al*., 2010). The increased ACP activity appears as a result from improved enzyme turn over under pesticide stress. It plays an important role in carbohydrate metabolism. This enzyme can be found inside the membrane of lysosomes (Sabiha Khan, 2014). So, any damage to the membrane of lysosomes can cause the release of this enzyme into muscle and increase its levels. 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 (Rao, 2006; Nchumbeni *et al*. 2007; Nte *et al*., 2011). 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 (Neelam Sharma, 2014; Umamaheswari and Senthilnathan, 2014a). 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 gill and intestinal tissues during 1day sublethal exposure. The AChE activity was estimated in different tissues like gill, liver, kidney, brain, muscle and intestine of the fish *L. rohita* exposed to sublethal and lethal concentrations of Phenthoate for 1 day and 10 days. The acetyl cholinesterase activity is a central neurotoxic parameter used to assess the pesticide toxicity in fish species (Candida Toni *et al*., 2011). The enzyme AChE is widely used for rapid detection to predict early warning of pesticide toxicity (Dutta and Arends, 2003a). The variation in activity levels of acetyl cholinesterase in different tissues of fish suggests the variations in neural activities of those particular organs. During the sublethal and lethal concentrations of organophosphate Phenthoate, the AChE activity levels were found to be declined in all the tested tissue of fish for 1day and 10 days. The decreased trend of AChE activity was highest in brain followed by gill, liver, muscle, kidney and intestine of fish tissues exposed to the toxicant. Decline of AChE enzyme activity in brain leads to accumulation of acetylcholine in the brain tissue, interfering with energy metabolism in the nervous system and preventing transmission of nerve impulses, thereby causing behavioral changes (Rao *et al*., 2005a). In the present experiment brain, gill and liver AChE activity level was susceptible of being inhibited by pesticides and therefore it may be responsible for some of the locomotor indications of muscular dysfunction. However Sarma *et al*., (2010) have explained that brain AChE inhibition caused by exposure to high concentrations of endosulfan for 4 days in spotted murrel (*C. punctatus*). So the AChE activity level was deeply reduced in brain tissue of *L. rohita* due to the involvement of the enzyme in the neurotoxic effects of fish exposed to Phenthoate. The inhibition of AChE results in buildup of acetylcholine within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission. The toxicant in the present experiment reduced instinctive behavioral responses and affected morphological features by depression of AChE activity. The pesticides inhibit AChE activity due to the effects of their active toxicokinetics of poison in the fish. The ratio between the toxification/detoxification reactions determines the degree of enzyme inhibition and can be used to evaluate metabolism processes (Tim chalk *et al*., 2002).

Several reports on AChE inhibitory effects due to various pesticides in different fish species are in corroboration with the present findings (Elif and Demet, 2007; Khalid *et al*., 2008; Vineet *et al*., 2008). 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 (Uncer *et al*., 2006). Inhibition of AChE impairs cholinergic nerve impulses and may result in death of organisms (Salles *et al*., 2006). 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 acetyl cholinesterase, 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 (Silva *et al*., 2004; Madhusudhan Reddy *et al*., 2011a). Recently, Marigoudar *et al*. (2009) revealed that the cypermethrin inhibits AChE activity at sublethal concentration in functionally different organs of *L. rohita*. It has been reported that similar observations were noticed in *L. rohita* when it treated with the sublethal concentration of Endosulfan pesticide (Kumari *et al*., 2010a; Kumari and Sinha, 2006). In the present study AChE activity levels in brain was more inhibited by the exposed pesticide. Similar trend was observed in various fish species exposed to different metals observed by Rajkumar and Milton (2011), de Lima *et al*. (2013) also supports the present work. In the present study brain AChE activity was the most inhibited of 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 Phenthoate in brain tissues were maximum, where the inhibition of activity was also maximum, as the exposed fish is continuously swimming in the pesticide medium throughout the exposure period.

However *L. rohita* is more sensitive to the toxicity of both the pesticides 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. The statistical analysis revealed that, the alterations in the AChE activity levels showed a non significant ($P > 0.05$) change in the muscle tissue. But in the remaining all the tested tissues (liver, kidney, brain, gill and intestine) showed significant change ($P < 0.05$) in the present study.

Conclusions

An increase in the aminotransferases, acid phosphatase activity and decrease in acetyle cholinesterase in all tissues significantly change in the present study may be due to the stressful condition of pesticide toxicity and also enhanced gluconeogenesis. The alterations in the AST, ACP, ALT activity levels induced by the organophosphate pesticides clearly designated that the stress brings about the metabolic reorientation in the tissues by elevate energy resources through transaminase systems. Pesticide poisoning altered the activity of AST, ACP and ALT. Acid phosphatase activity of *Labeo rohita* increased in all the three doses increase in protease activity may be due to the damage caused to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytosol. The enzyme AChE activity level was susceptible of being inhibited by pesticide toxicity and therefore it may be responsible for some of the locomotor indications of muscular dysfunction. Decrease in AChE enzyme activity indicates an accumulation of acetylcholine in the brain tissue, interfering with energy metabolism of the nervous system, arrest transmission of nervous impulses, and thereby causing behavioral changes. Activity of several enzymes was damage by acute exposure to sub-lethal concentrations of Phenthoate toxicity in the experimentation.

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