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Enzymatic Responses to Pesticide Chlorpyrifos Exposures in Kidney of Fish *Gambusia affinis*

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ABSTRACT

The present study was conduct to assess the effect of Chlorpyrifos toxicity to enzymatic response to fish Gambusia affinis. Alterations in certain enzymes have been examined in the kidney of a fish, after exposure to 1/10th, 1/3rd and sub-lethal (LC_{50}) dose of chlorpyrifos. Untreated fish served as control. Enzyme estimations of ACP, ALP, LDH and ATPase activity in kidney of control as well as in test Gambusia affinis were done.

The enzyme activity ACP, ALP and LDH was increased throughout the experimental period while the ATPase activity was decline along the experimental period at three different concentrations of chlorpyrifos was observed. Enzymatic studies are good parameters which help to see the effect of pesticide on biochemical composition of vital tissue of fish. Hence attempt has been made to find out enzymatic response in tissue like kidney of fish.

Key words: *Chlorpyrifos, Gambusia affinis, Kidney, LC_{50}.*

INTRODUCTION

Environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. These pesticides even when applied in restricted areas are washed and carried away by rains and floods to large water bodies like ponds and rivers and there by alter the physicochemical properties of water,[1] this proved to the highly toxic, not only to the fishes but also to aquatic life forms and their environment[2,3]. Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish. Contamination of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national, and global levels[4, 5].

Organophosphates (OP) are one of the most preferred pesticides due to their effectiveness and low persistence in the environment. OP pesticides directly inhibit acetylcholinesterase enzyme activity in fishes and invertebrates[6-8]. Chlorpyrifos (CPF) [OO diethyl- O- (3, 5, 6, trichloro-2-pyridyl)-phosphorothiate], is one of the most wide used organophosphorous insecticide is used in both agricultural areas[9-11].

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Toxicity studies have long played an important and modify the effects of his activities on the biota. The toxicity studies are especially useful sensitive species of an ecosystem that can be used as role in mans efforts to monitor in determining the indicator species, for a particular type of pollution. The results of toxicity are generally reported in terms of median lethal concentration LC_{50} and or median tolerance. Fishes come into contact with multiple contaminants in the aquatic environment as the pollutants. These pollutants built up in the food chain are responsible for adverse effects and death in the aquatic organisms.[12] 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[13-14].

The changes in enzymatic system may alter the metabolic processes. More recently changes in enzymes concentrations are being employed in the evaluation of toxicological responses. Toxicologists have developed interest in studying the responses of individual enzymes or groups of enzymes to toxic insult. Several reports are available on the effect of insecticides on different aspects of metabolisms. Several workers have shows that the pattern of enzymatic changes during starvation may vary from tissue to tissue. Some enzymes show an increased activity while others are show a decreased one with progressive days of starvation[15].

The teleost fish *Gambusia affinis* (Mosquito fish) was selected for the present study due to its wide availability and suitability as model for toxicity testing and also due to sustainability in laboratory conditions. The fish shows a well adaptive nature with the changing environment. Hence, the present study is the impact of the chlorpyrifos pesticide on the enzymatic changes in kidney of fish *Gambusia affinis.*

MATERIAL AND METHODS

The fish, *Gambusia affinis* weight (0.5-1.0 gm), length (3.0-4.5 cm) was collected from Local pond of Jhalra, near Ajmer District. Fishes were transported to laboratory in large aerated fiber glass and maintained in aquarium tanks containing well aerated dechlorinated tap water (with physicochemical characteristics: (temperature 24±2°C, pH 7.1±0.2 at 24°C, dissolved oxygen 9.6±0.8 mg/L, carbon dioxide 6.3±0.4 mg/L, total hardness 23.4±3.4 mg as CaCO3/L, phosphate 0.39±0.002 µg/L, salinity) in different 50 L plastic tanks, for 20 days. Water was renewed every day and a 12-12 hr. photoperiod was maintained during acclimatization and test periods. The fishes were fed on commercial pelleted diet once a day, throughout the tenure of the chronic experiment.

Experimental design

Mortality of fishes was recorded in each group for 96 hr. The regression equations were established by using probit - mortality and log of concentration of pesticide and LC_{50} value was determined.

The present work investigates the enzymes activities (ALP, ACP, LDH & ATPase) in liver and kidney of fish *Gambusia affinis* treated with pesticides; Chlorpyrifos. Fish were divided into four groups containing 10 individuals each, Group I: Control, Group II: Exposed to 1/10th of LC_{50} value, Group III: Exposed to 1/3rd of LC_{50} value, Group IV: Exposed to Sub lethal concentrations of 96 hrs-LC_{50} value of chlorpyrifos. Each group was exposed to 0.284 (Sub lethal), (1/10th of sub lethal) 0.028, and (1/3rd of sub lethal) 0.094 ppm of Chlorpyrifos.

The fish were exposed to this concentrations for 15, 30 and 45th days and a control group was maintained at an identical environment. The fish was dissected out from all treated groups on 15, 30 and 45th days. The kidney taken out for enzymatic studies, weighted tissue was washed in ice-cold isotonic saline. Various parameters of kidney were selected as indicators of toxicity. Saline extract was prepared by homogenizing a weighed piece of liver and kidney in measured quantity of 0.89% (ice cold) saline glass homogenizer. The homogenate was centrifuged at 4000 rpm for 15 minutes, to obtain clear supernatant. The supernatant was kept at -20°C. It was used for enzyme estimation of ALP and ACP enzyme activity was estimated by U.V. Kinetic Method[16,17] Lactate dehydrogenase activity estimated by the Bruns and Bergmeyer's

method,[18] ATPase estimated by Koch's method[19].

OBSERVATIONS:

Mortality studies showed that the sublethal level, LC_{50} of *Gambusia affinis* for 96 hr. exposure was 0.284 ppm for Chlorpyrifos. The minimum effective doses was exposed to 0.284 (Sub lethal), (1/10th of sub lethal) 0.028, and (1/3rd of sub lethal) 0.094 ppm of chlorpyrifos were calculated for experimental purposes. Enzyme estimations of ACP, ALP, LDH and ATPase activity in liver and kidney of control as well as in test *Gambusia affinis* were done. Observations are shown in below Tables:

Table 1: Changes in Acid phosphatase level in kidney of *Gambusia affinis* during control and post-treatment with three different concentration of Chlorpyrifos at different periods (15, 30 ,45th days) of exposure

Days of Exposure	Control	Exposure Concentration		
		1/10th of LC_{50}	1/3rd of LC_{50}	LC_{50}
15 Days	4.06±0.018 [x]	5.69±0.018 [x]	5.85±0.005[y]	5.97±0.005[y]
30 Days	5.33±0.019 [x]	6.14±0.011 [x]	6.72±0.005[y]	6.82±0.008[y]
45 Days	6.11±0.015 [x]	7.12±0.016 [x]	7.52±0.005[y]	7.67±0.005[y]

Value expressed in IU/L) (Mean ± SD).

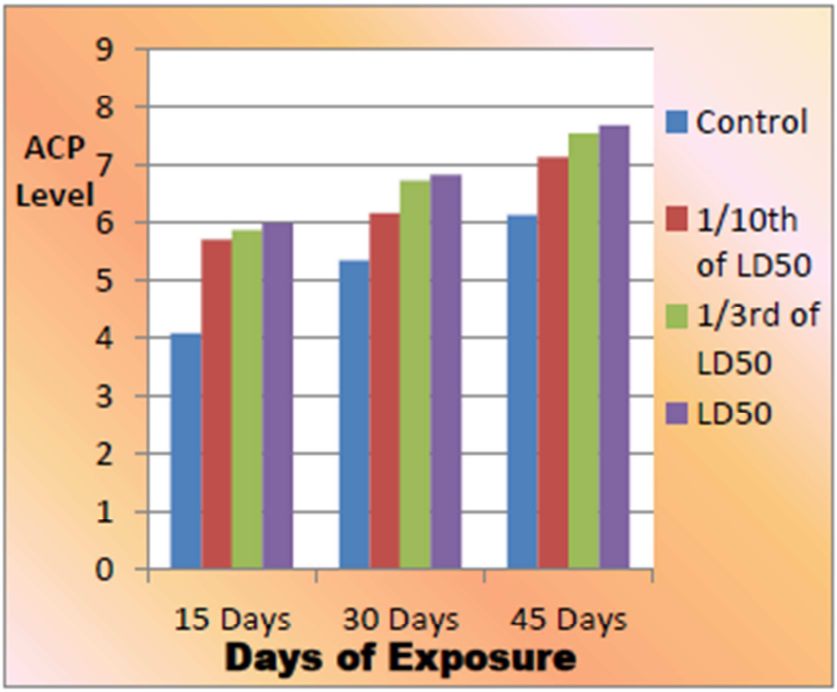

Fig. 1: Changes in Acid phosphatase level in kidney of *Gambusia affinis* during control and post-treatment with three different concentrations of chlorpyrifos at different periods (15, 30, 45th days) of exposure

Table 2: Changes in Alkaline phosphatase level in kidney of *Gambusia affinis* during control and post-treatment with three different concentration of Chlorpyrifos at different periods (15, 30 ,45th days) of exposure

Days of Exposure	Control	Exposure Concentration		
		1/10th of LC_{50}	1/3rd of LC_{50}	LC_{50}
15 Days	5.62±0.011 ^{x}	6.35±0.015 ^{x}	6.55±0.005^{y}	6.72±0.008^{y}
30 Days	6.75±0.013 ^{x}	7.23±0.011 ^{x}	7.65±0.005^{y}	7.80±0.005^{y}
45 Days	7.28±0.013 ^{x}	8.53±0.012 ^{x}	8.71±0.008^{y}	8.97±0.005^{y}

Value expressed in (IU/L) (Mean ± SD).

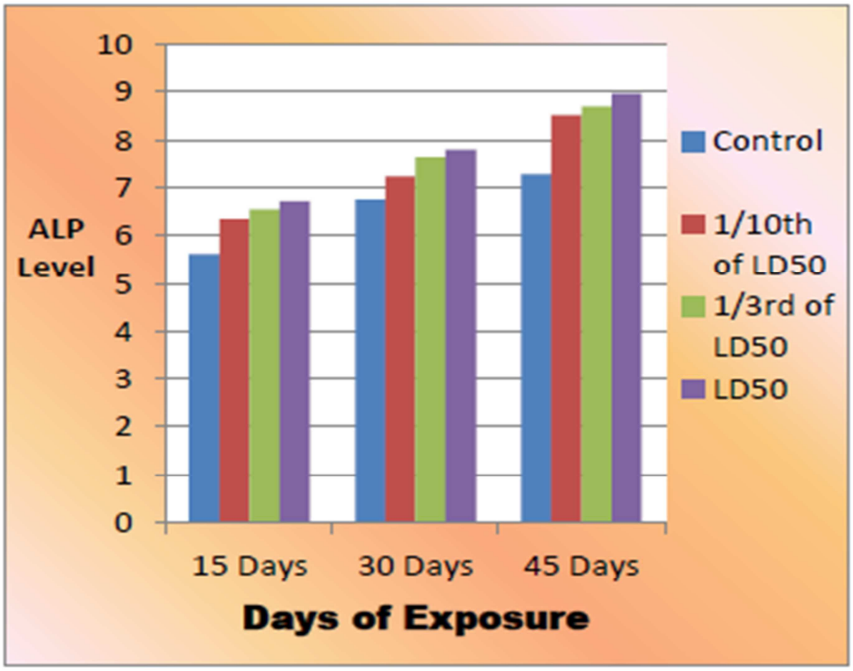

Fig. 2: Changes in Alkaline phosphatase level in kidney of *Gambusia affinis* during control and post-treatment with three different concentrations of chlorpyrifos at different periods (15, 30, 45th days) of exposure

Table 3: Changes in Lactate Dehydrogenase activity in kidney of *Gambusia affinis* during control and post-treatment with three different concentration of Chlorpyrifos at different periods (15, 30 ,45th days) of exposure

Days of Exposure	Control	Exposure Concentration		
		1/10th of LC_{50}	1/3rd of LC_{50}	LC_{50}
15 Days	1.16±0.008 ^{y}	1.35±0.008 ^{y}	1.42±0.008 ^{y}	1.52±0.008 ^{y}
30 Days	1.39±0.005 ^{y}	1.47±0.01^{x}	1.58±0.005 ^{y}	1.63±0.005 ^{y}
45 Days	1.53±0.008 ^{y}	1.67±0.008 ^{y}	1.73±0.008 ^{y}	1.87±0.008 ^{y}

Value expressed in (IU/L) (Mean ± SD).

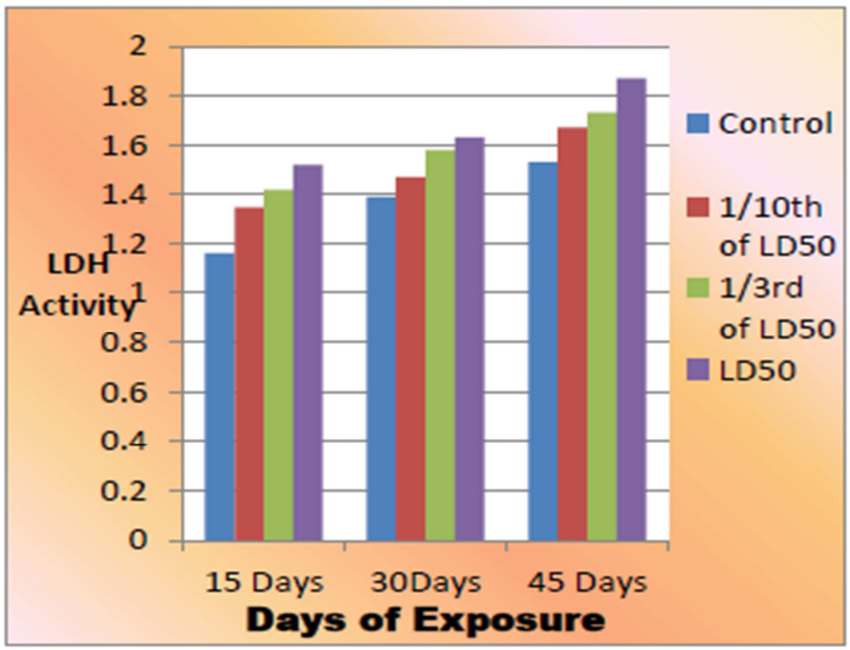

Fig. 3: Changes in Lactate dehydrogenase activity in kidney of *Gambusia affinis* during control and post-treatment with three different concentrations of chlorpyrifos at different periods (15, 30, 45th days) of exposure

Table 4: Changes in ATPase activity in kidney of *Gambusia affinis* during control and post-treatment with three different concentration of Chlorpyrifos at different periods (15, 30 ,45th days) of exposure

Days of Exposure	Control	Exposure Concentration		
		1/10th of LC_{50}	1/3rd of LC_{50}	LC_{50}
15 Days	0.96±0.005 ^{y}	0.91±0.005 ^{y}	0.85±0.008 ^{y}	0.78±0.005 ^{y}
30 Days	0.92±0.005^{y}	0.77±0.005 ^{y}	0.71±0.005 ^{y}	0.68±0.005 ^{y}
45 Days	0.89±0.005 ^{y}	0.52±0.005 ^{y}	0.49±0.005 ^{y}	0.43±0.005 ^{y}

Value expressed in (nmol Pi/min/mg protein) (Mean ± SD).

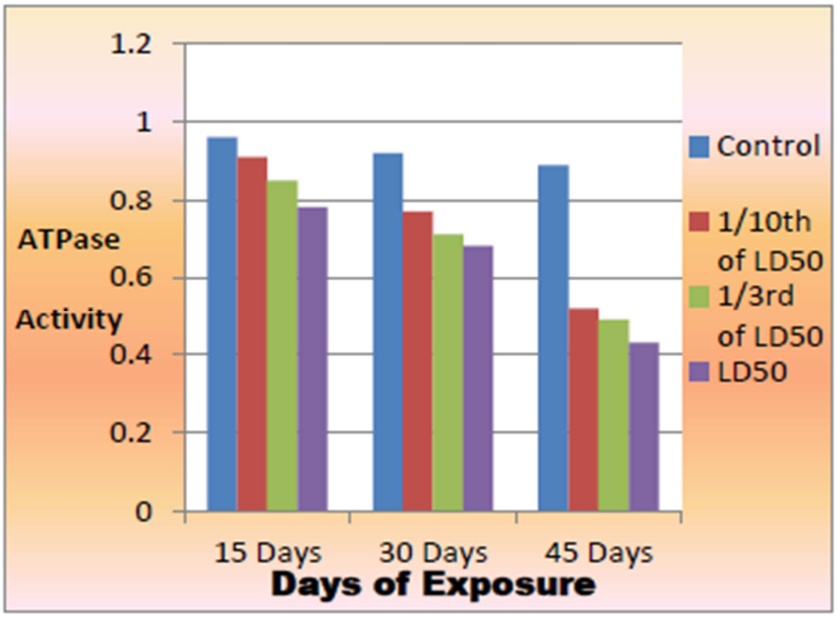

Fig. 4: Changes in ATPase activity in kidney of *Gambusia affinis* during control and post-treatment with three different concentrations of chlorpyrifos at different periods (15, 30, 45th days) of exposure

Statistical analysis: The experiments were repeated thrice and data was analyzed by the Student ''t'' test. Values are expressed mean ± SE of observations Values are significant at x $P<0.05$, y $P<0.01$, z $P<0.001$.

RESULT AND DISSCUSION

The kidney, specifically the trunk (posterior) kidney, is one of the more important excretory organs of teleost fish. The kidneys are varied function in fish species. One would assume that the renal tissues would be at major toxicological risk since they receive large volumes of blood flow from both the renal portal venous system and the renal arteries.

The increase in acid phosphatase activity in kidney may be inferred as a response to altered metabolism due to chlorpyrifos stress. Acid phosphatases belong to class of enzymes called hydrolases and they are characterized by their ability to hydrolyse a large variety of organic phosphates esters with the formation of an alcohol and a phosphate ion [20]. Acid phosphatase (ACP) activity in the liver and kidney of fish exposed to heavy metals provides a measurement of the hydrolase latency and lysosomal membrane stability and furnish information on mechanisms involving molecular alterations in the lysosomal membranes which undoubtedly contribute to disturbances of the integration of cellular function.[21]

The increase in alkaline phosphatase in the kidney it may be possible that there is hypersynthesis of ALP to facilitate transport and excretion of phosphate ions resulting in the noted increase in the ALP activity. Inhibition of ALP reflects alteration in protein synthesis and uncoupling of oxidative phosphorylation.

Baby Shakila *et al* inferred that severe acidosis may be responsible for inhibition of alkaline phosphatase. This in turn could be adoptive for fish to meet the energy demand via anaerobic breakdown of glycogen.[22]

Increased LDH activity in tissue under various toxic conditions, LDH is an important glycolytic enzyme in biological system and is inducible by oxygen stress. Therefore, the activity of several regulatory enzymes may be altered in order to meet the required energy demands under toxic stress. Increased activity of LDH may be attributed to a repressor effect in their synthesis or to the direct action of pesticides on the enzymes.

LDH interconverts lactate and pyruvate and has very important role in carbohydrate metabolism. LDH acts as a pivotal enzyme between glycolytic pathway and TCA cycle. It catalyses the conversion of pyruvate into lactate, under anaerobic conditions.[23] A fish under stress prefentially meets its energy requirements through anaerobic oxidation.[24] LDH activity depends on its five 1soenzymes and the activity changes under pathological conditions.[25]

ATPase responses in the osmoregulatory tissues, due to the type of stress factors, tissues and exposure durations providing a valuable data for biomonitoring the chlorpyrifos toxicity on fish metabolism, especially in freshwater with increased salinities. ATPase activity decreased generally following salinity alone exposure of chlorpyrifos. Decrease of this enzyme may result from the breakdown of the active transport mechanism and the pesticide inhibits enzymes involved in osmoregulatory ion transport particularly ATPases. Thaker *et al*. indicated that inhibition of Ca2+ ATPase activity in the gill, kidney and intestine of *Periophthalmus dipes* exposed to Cr6+ was related to the blocked active transport system by Cr6+ and thus decreased enzyme activity was observed due to the affected osmoregulatory mechanism.[26] Inhibition of ATPase activity by pheolic compounds may reduce ATP production as this enzyme has been reported to be involved in oxidative phosphorylation.[27]

The inhibition of ATPases leads to decreased ATP breakdown and reduced the availability of free energy. The reduced energy supply may affect several metabolic processes.[28] Hence, results of the present investigation conclude that organophosphate chlorpyrifos has inhibitory and acceleratory effect on the ACP, ALP, LDH and ATPase activities in kidney of an experimental fish *Gambusia affinis.*

CONCLUSION

The present study has showed the manner of response and the induction of toxicological effects in fish *Gambusia affinis* after exposure of (chlorpyrifos) at different doses at the different

period of exposure. It is of importance to note here that the fish were exposed to these doses for a period to disturb the kidney functions. The increase in renal acid phosphatase activity in intoxicated animals as observed in the present investigation may be due to the destruction of the lysosomal membrane which resulted in the release of the enzyme. It is found that chlorpyrifos disturb the chemical constituents of the fish which leads to cell damages and finally death of fishes.

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