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Research Article

Analysis of Lactate Dehydrogenase & ATPase activity in fish, *Gambusia affinis* at different period of exposureto chlorpyrifos

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ABSTRACT

The acute toxicity of an organophosphate insecticide chlorpyrifos, on biochemical parameters of fish *Gambusia affinis* was evaluated under static condition. The sub lethal concentration of the insecticide chlorpyrifos was found to be 0.284 ppm indicating the high toxicity of the insecticide. Fishes were exposed to $1/10^{th}$ of sub lethal (0.0284 ppm) and the alteration of enzyme activity in liver and kidney of fish *Gambusia affinis* were studied at 15, 30 and 45 days of exposure. A significant increase in LDH activity in both tissue Liver and Kidney and the inhibition of ATPase leads to decreased ATP breakdown and reduced the availability of free energy was observed.

Keywords: Chlorpyrifos, ATPase, LDH, Liver, Kidney and *Gambusia affinis.*

INTRODUCTION

Pesticides are occasionally used indiscriminately in largeamounts causing environmental pollution and therefore, area cause of concern. Organophosphorus insecticides (OPIs)are a major component of many pesticides with widespreaduse in both agricultural and domestic situations. However,approximately 85%-90% of applied agricultural pesticidesnever reach target organisms, but disperse through theair, soil, and water [1]. Organophosphates (OPs) have become the most widely used class of insecticides in the world replacing the persistent and problematic organochlorine compounds. Exposure of aquatic ecosystems to these insecticides is difficult to assess because of their short persistence in the water column due to low solubility and rapid degradation. However, monitoring of these insecticides is important, because they are highly toxic to aquatic organisms. They have been reported to produce a number of biochemical changes in fish, both at lethal and more often at sublethal levels.

Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridylphosphorothioate) is one of the organophosphate pesticides widely used in agricultural practices throughout world and irreversible inhibitor of cholinesterase in all animal species. Limited efforts have been made to study acute genotoxic effects of chlorpyrifos (CPF) in different tissues of fish using genotoxic biomarkers.Enzymes are one of the major targets for pesticide action. Measurement of certain patterns of cellular enzymes under different conditions of treatments with various types of toxicants could provide good evidence for thecytotoxicity and hence the impairment of cell functions[2].ATPase inhibition was found to beleading to impaired ion-transport and imbalance in homeostatic mechanism. Bindingof lipophilic chlorpyrifos is expected to be responsible for the rupturing of thelysosomal membrane. This might have led to the release ofhydrolase enzymes to thesurrounding cellular environment. The lactate dehydrogenase (LDH) is released from the cellular organs after its cellular damage and failure due to organophosphate insecticide intoxication [3]. The present study deals with the effect of different concentrations of chlorpyrifos on the enzyme activity in liver and kidney of the fish, *Gambusia affinis*.

MATERIAL AND METHODS

Gambusia affinis (Cyprinodontiform: Poeciliidae) weighing (0.5-1.0 g), length (3.0-4.5 cm) were collected from local pond in Ajmer and acclimatized to laboratory condition. Test chambers were glass aquaria of about 50 liter capacity. The aquaria were aerated with a central system for a period of 48 hours and the fish were exposed to 15, 30 and 45 days conditioning period at room temperature. The fish were fed with commercial pelleted food at least once a day during this period. Acclimatized fish were not fed 24-hr before the start of the tests. 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 sub lethal concentration of chlorpyrifos was 0.284 ppm. The concentration was calculated for experimental studies $1/10^{th}$ of sub-lethal (0.0284 ppm). The fish were exposed to this concentration for 15, 30 and 45^{th} days and a control group was maintained at an identical environment. The fish was dissected out from groups on 15, 30 and 45^{th} days for biochemical analysis. Homogenate of the fresh tissue (liver and kidney) was prepared in 0.9% ice cold saline to approximately 10% w/v and supernatant used for enzyme assay after centrifugation at 3000 rpm at 4^{o} C for 15 minutes. Estimation of Lactate dehydrogenase (LDH) by Bruns and Bergmeyer's method, 1965 [4] and ATPase estimation by Koch's method, 1970. [5]

Observation

Mortality studies showed that the sub-lethal level, LC_{50} of chlorpyrifos to *Gambusia affinis* for 96 hr. exposure was 0.284 ppm. The minimum effective doses $1/10^{th}$ of LC_{50} (0.0284 ppm) were calculated for experimentalpurposes. Biochemical estimations of Lactate dehydrogenase- by Bruns and Bergmeyer's method, 1965, ATPase estimation by Koch's method, 1970 in liver and kidney of control as well as in treated *Gambusia affinis* at

different periods (15, 30,45th days) of exposure are given in the tabular form as follows: Values are expressed mean ± SD of observations, Values are significant at $^{x}P < 0.05$, $^{y}P < 0.01$, $^{z}P < 0.001$.

RESULT AND DISCUSSION

LDH is an anaerobic enzyme involved in the conversion of pyruvate to lactate in glycolysis. The activity of Lactate dehydrogenase (LDH) at the exposure of 0.0284 ppm (1/10th of LD50) concentration of chlorpyrifos in liver of experimental fish *Gambusia affinis* showed continuous increased from 1.55 to 2.00 IU/L on 15 days, 1.78 to 2.11 IU/L on 30 days and 1.81 to 2.54 IU/L on 45 days.

The activity of Lactate dehydrogenase (LDH) in kidney at the exposure of 0.0284 ppm (1/10th of LD50) concentration of chlorpyrifos showed continuous increased from 1.16 to 1.35 IU/L on 15 days, 1.39 to 1.47 IU/L on 30 days and 1.53 to 1.67 IU/L on 45 days.

LDH activity increased might be due to a shift from aerobic respiration to anaerobic respiration promoting the glycolytic rate and the conversion of pyruvic acid to lactic acid, under insecticide-induced stress. The tissues might have tried to compensate the incapability of oxygen consumption under stress condition.Increased activity of LDH may be attributed to a repressor effect in their synthesis or to the direct action of pesticides on the enzymes.

Table 1 Changes in Lactate Dehydrogenase activity in liver and kidney of *Gambusia affinis* during control and post-treatment with 0.0284 ppm concentration of Chlorpyrifos at different periods (15, 30,45th days) of exposure

Tissue	Days of Exposure					
	15 Days		30 Days		45 Days	
	Control	Experimental	Control	Experimental	Control	Experimental
Liver	1.55 ± 0.01^{x}	2.00 ± 0.01^{x}	1.78 ± 0.01^{x}	2.11 ± 0.01^{x}	1.81 ± 0.01^{x}	2.54 ± 0.01^{x}
Kidney	1.16 ± 0.008^{y}	1.35 ± 0.008^{y}	1.39 ± 0.005^{y}	1.47 ± 0.01^{x}	1.53 ± 0.008^{y}	1.67 ± 0.008^{y}

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

As a result the energy for the osmoregulation has to be obtained through glycolysis, which is up regulated and LDH activity increased. Our data are inconcordance with those of who noticed that during exposure to a low concentration of 0.5 µg Cu^{2+} /l, the energy supply of adaptation process in carp is performed by the aerobic pathway, whereas at moderate concentrations, aerobic processes are inhibited in fish tissues and glycolysis is activated [6]. Such an increase in LDH activity may be attributed to the prevalence of anoxia. Osmaninvestigate the sub- lethal effect of cypermethrin on lactate dehydrogenase activity in different organ tissues of *Channa striatus*[7]. The activity of glycolytic enzyme LDH activity significantly increased in different tissues such as Gills, Kidney, Intestine, Brain and Liver of the fish. *Channa striatus* was exposed to sub-lethal doses of cypermethrin except 24 hrs. exposure period. The increase in lactate dehydrogenase (LDH) activity has been reported by Ghosh,in the fish *Channa punctatus* treated with the pesticides, quinalphos, dichlorvos and suquin [8]. The elevation of lactate dehydrogenase activity in the muscle and gill of the freshwater fish, *Heteropneustes fossilis* was due to the pesticide rogor exposure[9].The ATPase activity at the exposure of 0.0284 ppm (1/10th of LD_{50}) concentration of chlorpyrifos in liver of experimental fish *Gambusia affinis* showed continuous decreased from 1.85 to 1.23 nmol Pi/min/mg protein on 15 days, 1.42 to 1.11 nmol Pi/min/mg protein on 30 days and 1.33 to 0.88 nmol Pi/min/mg protein on 45 days.

Table 2 Changes in ATPase activity in liver and kidney of *Gambusia affinis* during control and post-treatment with 0.0284 ppm concentration of Chlorpyrifos at different periods (15, 30,45th days) of exposure

Tissue	Days of Exposure					
	15 Days		30 Days		45 Days	
	Control	Experimental	Control	Experimental	Control	Experimental
Liver	1.85 ± 0.01^{x}	1.23 ± 0.01^{x}	1.42 ± 0.01^{x}	1.11 ± 0.01^{x}	1.33 ± 0.005^{y}	0.88 ± 0.005^{y}
Kidney	0.96 ± 0.005^{y}	0.91 ± 0.005^{y}	0.92 ± 0.005^{y}	0.77 ± 0.005^{y}	0.89 ± 0.005^{y}	0.52 ± 0.005^{y}

The ATPase activity at the exposure of 0.0284 ppm (1/10th of LD_{50}) concentration of chlorpyrifos in kidney of experimental fish *Gambusia affinis* showed continuously decreased level from 0.96 to 0.91 nmol Pi/min/mg protein on 15 days, 0.92 to 0.77 nmol Pi/min/mg protein on 30 days and 0.89 to 0.52 nmol Pi/min/mg protein on 45 days**.**

In the present study, it has been found that increasing dose exposure of chlorpyrifos caused decreased activity of ATPase in liver. This could be due to pesticide induced effect on cell membrane because of their strong affinity for interaction with member lipids. ATPase responses in the osmoregulatory tissues, due to the type of stress factors, tissues and exposure durations providing a valuable data for bio-monitoring the chlorpyrifos toxicity on fish metabolism, especially in freshwater with increased salinities. Inhibition of ATPase activity by phenolic compounds may reduce ATPproduction as this enzyme has been reported to be involved in oxidative phosphorylation[10]. The reduction in the Ca^{2+}ATPase activity indicated the interaction of phenolic compounds with the microsomal and basolateral Ca^{2+} transporting ATPase[11]. Several reports studied inhibition in the activity of ATPase in several tissues of a teleost, fishes.

CONCLUSION

It is found that Chlorpyrifos disturb the chemical constituents of the fish which leads to cell damages and finally death of fishes. The changes in the enzymes are found as the best biomarkering tool to evaluate the effect of organophosphate pesticide chlorpyrifos on the aquatic biota. The Lactate dehydrogenase (LDH) is released from the cellular organs after its cellular damage and failure due

to organophosphate insecticide intoxication. The elevation of LDH was highest in liver and kidney. The activity of ATPase was, in decreasing order, in liver and kidney, and was significant in chlorpyrifos concentration at all treatment periods.

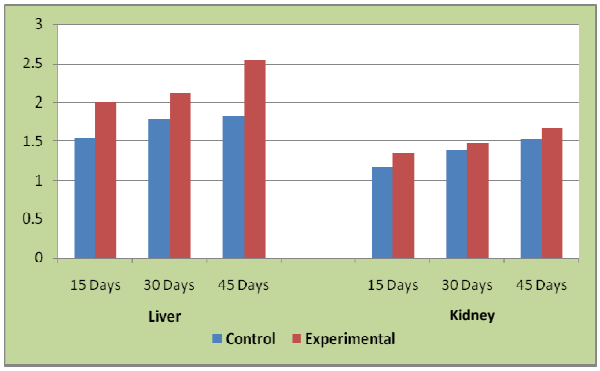

Figure 1 Lactate Dehydrogenase (LDH) activity in liver and kidney of *Gambusia affinis* during control and post-treatment with 0.0284 ppm concentration of Chlorpyrifos at different periods (15, 30,45th days) of exposure.

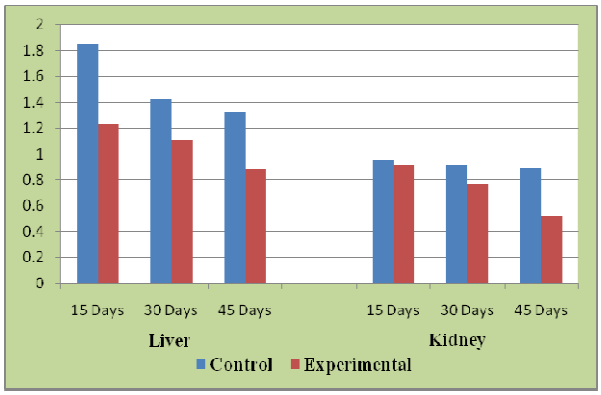

Figure 2 ATPase activity in liver and kidney of *Gambusia affinis* during control and post-treatment with 0.0284 ppm concentration of Chlorpyrifos at different periods (15, 30,45th days) of exposure

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