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Analysis of AchE and LDH in mollusc, *Lamellidens marginalis* after exposure to chlorpyrifos

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Abstract: *The enzymes Acetylcholinesterase (AchE) and Lactatedehydrogenase (LDH) are used as biological markers in the present study. Enzymes are highly sensitive and used to evaluate the biological effects of organophosphate pesticide chlorpyrifos in freshwater mussel Lamellidens marginalis. The test organisms were exposed to sub-lethal concentration (5 ppm) of chlorpyrifos for 30 days and allowed to recover for seven days. A distinct reduction of the enzyme AchE (34 ± 3.3 U l^{-1}) was found in the treated hepatopancreas. A significant increase in LDH activity in gill, hepatopancreas and muscle was observed. There was a significant recovery in AchE and LDH in the different tissues, after seven days recovery period.. Hence, the changes in the enzymes are found as the best biomarkering tool to evaluate the effect of organophosphate pesticide chlorpyrifos on the aquatic biota.*

Key words: *Biomarker, LDH, Acetylcholinesterase, Chlorpyrifos*
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Introduction

The enzyme Acetylcholinesterase (AchE) is responsible for hydrolyzing the neurotransmittors acetylcholine into choline and acetic acid (Pfeifer *et al.,* 2005). The enzyme controls ionic currents in excitable membranse and plays an essential role in nerve conduction processes at the neuromuscular junction. The inhibition on the AchE is linked directly with the mechanism of toxic action of organophosphate pesticides (Richmonds and Dutta, 1992) *Viz*., irreversible or reversible binding to the catalytic site of enzyme and potentiation of cholinergic effect as an indicator of exposure to these compounds. Acetylcholinesterase and non-specific cholinesterase activities in blood and tissues have emerged as a diagnostic tool in the biomedical area. The quantification of this enzyme has been applied in the laboratory and field studies with both vertebrates and invertebrates to assess exposure to organophosphorus and carbamate insecticides (Bocquene *et al*., 1997). The inhibitory effects of organophosphorous (op) insecticides are dependent on their binding capacity to the enzyme active site and by their rate of phosphorylation in relation to the behaviour and age (Dutta *et al*., 1995).

The elevated Lactatedehydrogenase (LDH) activity is a marker for tissue damage in fish (Ramesh *et al*., 1993), hypoxic conditions (Das *et al*., 2004) and muscular harm (Balint *et al*., 1997) and serve as a good diagnostic tool in toxicology. Marine bivalves such as oysters and mussels are widely used as bioindicators of contamination in the monitoring of pollutant effects (Havlik and Marking, 1987). As filter feeders, these species are known to be good general indicators of chemical contamination (Forester, 1980).

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Similarly, the lactatedehydrogenase (LDH) is released from the cellular organs after its cellular damage and failure due to organophosphate insecticide intoxication (Reizart and Roberts, 1999). The enzyme LDH is used as biomarker of acute hepatic damage and can serve as a diagnostic tool for assessing the liver cells (Vinodhini and Narayanan, 2008). Hence, the aim of the present study is to determine the toxicity of chlorpyrifos an organic phosphorous pesticide on the activity of the marker enzyme LDH and AchE on a benthic bivalve mollusc, *Lamellidens marginalis*.

Materials and Methods

Animal maintenance: All the reagents used in the present study were of analytical grade and used without further purification. The freshwater mussel, *Lamellidens marginalis* was collected from Cauvery river (Tamilnadu), which is relatively free from pollutants, and was brought to the laboratory in a large aerated drum. Later, they were acclimatized for 30 days in a cement tank (800 x 300 x 400 mm). The mussels were fed with dried green algae 10 g of Spirulina and transferred to a glass aquarium (60 x 30 x 30 cm) of 40 l water capacity for a further period of seven days and were fed with dried green algae for conditioning. The water in the aquarium was renewed daily and was aerated mechanically. The natural photoperiod of 13:11 L: D hours was maintained. The average values for water quality data holding in exposure tanks was temperature 26 ± 2°C, pH 7.10±0.05, dissolved oxygen 8.15±0.064 mg l^{-1}, total hardness 634.69±0.88 mg l^{-1} as $CaCO_3$, alkalinity 298.75 ± 2.06 mg l^{-1} (as $CaCO_3$), and chlorides 276.785 ± 0.92 mg l^{-1} (APHA, 1998).

During sub-lethal studies, a total of 45 mussels (15 mussels per aquarium) were exposed to 5 ppm (1/10 of the LC_{50}) for a period of 30 days. The required concentration was maintained by

Table - 1: Acetylcholinesterase (AchE) activity in different tissues of mollusc treated with chlorpyrifos for 30 days and recovery period of 7 days

Day	Tissue					
	Gill (U l^{-1})		Hepatopancreas (U l^{-1})		Muscle (U l^{-1})	
	AchE	LDH	AchE	LDH	AchE	LDH
Control 0 day	140 ± 2.2	77 ± 2.4	100 ± 2.4	15 ± 1.2	70 ± 3.7	17 ± 1.6
$_5$ th	110 ± 3.2^c	85 ± 3.2^a	60 ± 1.6^c	44 ± 4.1^c	50 ± 2.3^c	41 ± 4.3^c
15th	70 ± 1.7^c	90 ± 2.4^c	40 ± 3.3^c	93 ± 3.3^c	30 ± 3.3^c	75 ± 4.1^c
30th	50 ± 1.4^c	97 ± 2.3^c	34 ± 3.3^c	146 ± 1.4^c	47 ± 3.7^c	166 ± 2.8^c
Recovery after 7 days	130 ± 3.3	84 ± 4.2	90 ± 4.4	80 ± 2.2	50 ± 3.2	149 ± 2.4

Significant differences from control values ($p<0.05$)^a ($p<0.01$)^b and ($p<0.001$)^c. Each value is the mean ± SD of three individual observations

adding the toxicant directly in 40 l of water and renewed daily without aeration. The control experiments were also performed without toxicant. The enzyme changes in the gill, hepatopancreas and muscle of *L. marginalis* were studied at regular intervals *i.e*., on day, 5, 15, 30 and the recovery levels of various enzymes were studied after the day 7, of the post exposure period.

Enzyme analysis: The experimental and control fresh water mussels were sacrificed by decapitation and the tissues rapidly excised. The tissues were rinsed, blotted and homogenized in a motordriven, all glass homogenizer with two volumes of chilled saline (0.7% NaCl). Homogenates were centrifuged (Model 101, M13 corporation, Bombay) at 10,000 rpm for 15 min. The supernatant fractions were diluted with ten volumes of chilled saline and used as the enzyme source. The activities of lactatedehydrogenase and acetylcholinesterase were determined according to the methods described by Pilz (1965).

Statistical analysis: The experiments were repeated on three different occasions in triplicate and the data were analyzed by Student's t-test. Statistical comparisons were done between control and exposure data from the same species. Significant differences from control values $p<0.05$, $p<0.01$ and $p<0.001$ were accepted as levels of statistical significance.

Results and Discussion

The enzyme, AchE activity in gill, muscle and hepatopancreas has decreased in all the treated bivalve molluscs. AchE in the gill was found higher (140±2.2 U l^{-1}) than the hepatopancreas (100±2.4 U l^{-1}) and muscle (70±3.7 U l^{-1}). The enzyme activity was inhibited in treated molluscs with sublethal concentration (5 ppm) of chlorpurifos at all the time interval *i.e.* 5, 15 and 30 d. Acetylcholinesterase is an important enzyme for conducting nerve impulse in the animal system (Nahid Akhtor *et al*., 2009). The level of acetylcholinesterase was highly inhibited in *L. marginalis* after exposure of chlorpyrifos. As this enzyme is involved in the maintenance of the structural and functional integrity of cellular membrane (Najimi *et al*., 1997), it may be inferred that chlorpyrifos causes disturbance in the normal cellular functions and a large decrease in the acetylcholinesterase activity (Dange and Masyrekar, 1987). The present study shows that at 5 ppm chlropyrifos could cause neuronal disturbances in the bivalve as evidenced by low level of AchE.

Changes in the LDH activity may indicate the facility with which mussel can shift to anaerobic metabolism under adverse conditions. LDH is also involved in the conversion of pyruvate to lactate in the animal system (Nicholson and Lam, 2005). In the present investigation the LDH level has increased in the muscle (166±2.8 U l^{-1}) gill (97±23 u l^{-1}) and hepatopancreas (146±1.4 U l^{-1}) of treated animals than in control.

The LDH activity was not suppressed upto certain level of the toxicant. Lagana *et al.* (2007) reported that LDH and its homotetramer LDH-B4 were not affected upto 4 m Urea treatment in the fan-shell associated shrimp *Pontonia pinnophydax*. However, the increased concentration of the biopesticides (azadiractin) suppressed the acivity of LDH in the gut regio of the leaffolder observed by Senthi Nathan *et al.* (2006). Anitha and Kumar (1997) observed decreased carbohydrate activity in the secondary gill lamellae of the fresh water teleost (*Channa punctatus,* under the exposure to the polluted water.

Nicholson and Lam (2005) observed changes in activity of glucose-6 phosphate dehydrogenease (G6PDH) and LDH in the adductor muscles of *P. viridis* (mytilid muscles) used as biomarkers to monitor the marine pollution in south east Asia.

The bivalve molluscs are generally viewed as reliable indicators of pollution levels because they remain sedentary for easy and repeated observation and withstand high rate of contaminants (Forester, 1980; Havlik and Marking, 1987). Tilak *et al*. (2005) have studied about the impact of chlorpyrifos on the gill, liver and brain and kidney in a freshwater fish, *Cirrhinus mrigala*.

The present investigation reveals the possible changes in the enzymes AchE and LDH in the gill, muscle and hepatopancreas of the *L. marginalis* and can be used as the best biological tool to monitor the toxicity of chlorpyrifos.

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