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Effect of chlorpyrifos on biochemical changes in freshwater mussel *Lamellidens marginalis*

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Abstract
The freshwater mussel *Lamellidens marginalis* was exposed to sub-lethal concentration (5ppm) of an organophosphorus insecticide, chlorpyrifos for 30 days and allowed to recover for seven days. Alanine aminotransferase (ALAT), aspartate aminotransferase (AAT), and acetylcholinesterase (AChE), were assayed in plasma and different tissues at regular intervals of day and after recovery period of seven days. The ALAT and AAT activities were increased in plasma and muscle, where as hepatopancreas and gill showed decrease. AChE activity was observed in gill, muscle and hepatopancreas, and reduction of 73% was observed in hepatopancreas. There was a significant recovery in all the above biochemical parameters studied in plasma and different tissues, after seven days recovery period. These results revealed that chlorpyrifos affects the intermediary metabolism of *Lamellidens marginalis* and that the assayed enzymes can work as good biomarkers of organophosphorus contamination.

Keywords: Chlorpyrifos, *Lamellidens marginalis*, Marker enzymes, AChE

1. Introduction
Responses to OP insecticides by aquatic organisms are broad ranged depending on the compound, exposure time, water quality and the species [5, 9]. Acetylcholinesterase (AChE, E.C: 3.1.1.7) enzyme is widely used for rapid detection to predict early warning of pesticide toxicity [4]. The enzyme acetylcholinesterase (AchE) is responsible for hydrolyzing the neurotransmitters acetylcholine into choline and acetic acid [10]. The enzyme control ionic currents in excitable membrance and plays an essential role in nerve conduction processes at the neuromuscular junction. The inhibition on the AchE is liked directly with the mechanism of toxic action of organophosphate pesticides. 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 emerged as a diagnostic tool in the biomedical area. The quantification of this enzyme has been applied to laboratory and field studies with both vertebrates and invertebrates to assess exposure to organophosphorus and carbamate insecticides.
The inhibitory effects of OP 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 [12]. The alanine aminotransferase (ALAT) and aspartate aminotransferase (AAT) are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histophathalogic changes and can be assessed within a shorter time [2]. The increase in ALAT and AAT indicate the tissue damages in liver, kidney and gill [8, 11]. In toxicological studies of sub acute exposure, the alterations in the enzymatic activities directly reflect the metabolic disturbances and cell damage in specific organs [3]. Hence, the present study is designed to study the sub-lethal effects of chlorpyrifos on biochemical parameters of plasma, gill, hepatopancreaes and muscle tissues of freshwater mussel, *Lamellidens marginalis*.

2. Materials and methods
2.1. Animal maintenance
All the reagents used in the present study were of analytical grade and were used without further purification. The freshwater mussel, *Lamellidens marginalis* were collected from Cauvery River (Tamilnadu), which is relatively free from pollutants, and were brought to the laboratory in a large aerated drum.

Later, they were acclimatized for 30 days in a cement tank (800mm length, 300mm width, and 400mm height) were set up each containing 50 mussels. The mussels were fed with dried green algae (Spirulina), weighing 10 g were 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 ºC, pH 7.10 ± 0.05, dissolved oxygen 8.15 ± 0.064 mg l^{-1}, total hardness 634.69 ±0.88 mg l^{-1} asCaCO3, alkalinity 298.75 ± 2.06 mg l^{-1} (as CaCO3), and chlorides 276.785 ±0.92 mg l^{-1}[1].

2.2. Sub-lethal studies

During sub-acute studies a total of 45 mussels (15 mussels per aquarium) were exposed to 5ppm (1/10 of the LC_{50}) for a period of 30 days. The required concentration was maintained by adding the toxicant directly in 40 l of water and renewed daily with out aeration. The control experiments were also performed without toxicant. The biochemical changes in the freshwater mussel, plasma, gill, hepatopancreas and muscle 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.

2.3. Biochemical studies

AAT (E.C. 2.6.1.1) and ALAT (E.C. 2.6.1.2) activity in plasma, gill, hepatopancreas and muscle were estimated according to the standard procedure of [13]. The enzyme activity was expressed as µmol of pyruvate formed/mg protein/h. AchE (E.C. 3.1.1.7) activity (µmol /mg protein/min) was determined by the method of [14].

2.4. Statistical analysis

The experiments were repeated on three different occasions in triplicate and that 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.

3. Results and discussion

In the present study significant changes of AAT, ALAT and AChE was observed and were time dependant and are presented in Table 1 & 2. Mussel exhibited higher AAT and ALAT activities in plasma and muscle and significant reduction was observed in gill and hepatopancreas tissues. Plasma activity concentrations of AAT and ALAT are the most commonly used biochemical markers of hepatocellular necrosis [6, 7]. The AChE activity in the gill, muscle and hepatopancreas showed a continuous decrease as the exposure progressed. The maximum reduction in AChE activity of the gill, muscle and hepatopancreas of exposed mussels were 65%, 66% and 73% respectively by the end of exposure. At the end of recovery period hepatopancreas AChE value was still 13% below controls whereas the gill and muscle AChE were 9% and 32% below control.

4. Conclusions

The present biochemical alterations in the freshwater mussel, *Lamellidens marginalis*, sub-lethal intoxicated with chlorpyrifos suggests that the treated mussels faced a serious metabolic crisis. The results revealed that chlorpyrifos affects the intermediary metabolism of *Lamellidens marginalis* and that the assayed enzymes can work as good biomarkers of organophosphorus contamination. Fortunately, most of the metabolic disorders did not persist when the freshwater mussels were allowed to recover in clean water for less than a week.

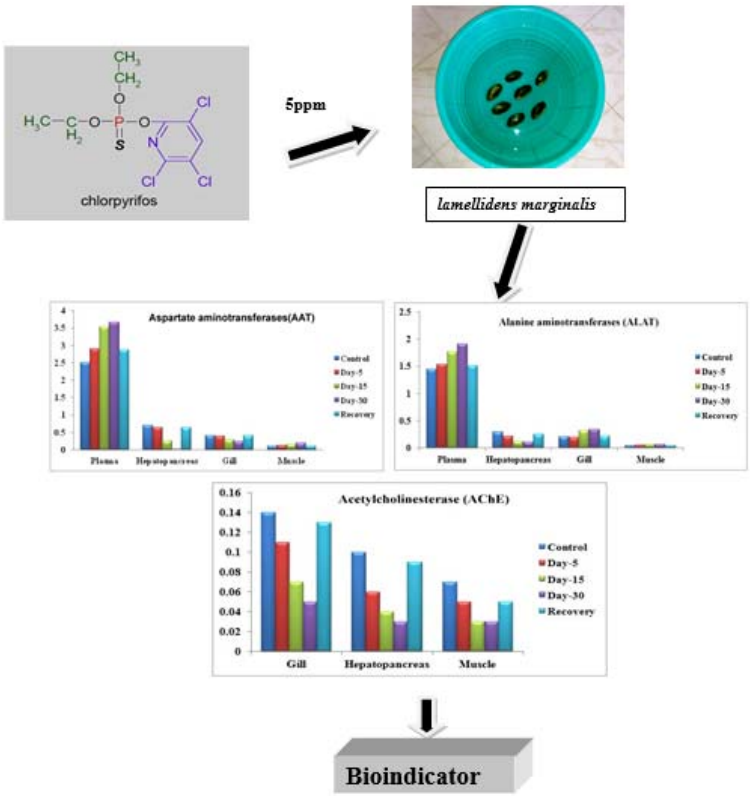

Table 1: Changes in AAT[A] and ALAT[A] levels in different organs of *Lamellidens marginalis* after treatment with Chorpyrifos

Days	Plasma	Hepatopancreas	Gill	Muscle
Aspartate aminotransferases (AAT)				
Control	2.51 ± 0.06	0.697 ± 0.031	0.402 ± 0.003	0.101 ± 0.005
Day-5	2.91 ± 0.03[c] (16)	0.637 ± 0.054 (–8)	0.381 ± 0.004[b] (–5)	0.122 ± 0.003[b] (21)
Day-15	3.54 ± 0.08[c] (41)	0.253 ± 0.052[c] (–64)	0.289 ± 0.003[c] (–28)	0.164 ± 0.006[c] (62)
Day-30	3.66 ± 0.07[c] (46)	0.015 ± 0.006[c] (–77)	0.241 ± 0.002[c] (–40)	0.185 ± 0.004[c] (83)
Recovery	2.88 ± 0.05[b] (15)	0.644 ± 0.047 (–8)	0.397 ± 0.003 (–1)	0.109 ± 0.003 (8)
Alanine aminotransferases (ALAT)				
Control	1.45 ± 0.03	0.292 ± 0.002	0.209 ± 0.004	0.038 ± 0.002
Day-5	1.53 ± 0.03[a] (6)	0.211 ± 0.004[c] (–28)	0.193 ± 0.003 (–8)	0.047 ± 0.003[a] (24)
Day-15	1.77 ± 0.02[c] (22)	0.127 ± 0.002[c] (–57)	0.320 ± 0.005[a] (53)	0.061 ± 0.001[c] (61)
Day-30	1.91 ± 0.03[c] (32)	0.101 ± 0.002[c] (–65)	0.345 ± 0.004[a] (65)	0.065 ± 0.001[c] (71)
Recovery	1.51 ± 0.04 (4)	0.258 ± 0.003[c] (–12)	0.213 ± 0.002 (1)	0.042 ± 0.002 (11)

Significant differences from control values [a]($P < 0.05$), [b]($P < 0.01$) and [c]($P < 0.001$).
Values in the parenthesis indicated as percent induction or percent reduction (indicated as negative).
[A] µmol pyruvate formed/mg protein/h. Each value is the mean ± SE of three individual observations.

Table 2: Effect of chlorpyrifos on acetylcholinesterase (AChE)[A] activity in different tissues of *Lamellidens marginalis*

Days	Gill	Hepatopancreas	Muscle
Control	0.14 ± 0.002	0.10 ± 0.002	0.07 ± 0.003
Day-3	0.11 ± 0.003[c]	(24) 0.06 ± 0.001[b] (45)	0.05 ± 0.002[c] (27)
Day-15	0.07 ± 0.001[c] (59)	0.04 ± 0.003 (63)	0.03 ± 0.003[c] (65)
Day-30	0.05 ± 0.001[c] (65)	0.03 ± 0.003[a] (73)	0.03 ± 0.002[c] (66)
Recovery	0.13 ± 0.003[a] (13)	0.09 ± 0.004 (9)	0.05 ± 0.003[c] (32)

Significant differences from control values [a]($P < 0.05$), [b]($P < 0.01$) and [c]($P < 0.001$).
Values in the parenthesis indicate percent reduction.
[A] lmol acetylcholine hydrolysed/min/mg protein. Each value is the mean ± SE of three individual observations.

Highlights

- Effect of chlorpyrifos on biochemical changes in freshwater mussel *lamellidens marginalis.*
- Sublethal concentration creates alteration, but not physiology or lethality.
- Self regulating mechanisms might have prevented from Lethality.
- Biochemical altered with increasing dose levels of chlorpyrifos

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