Effects of chlorpyrifos on enzymes as biomarkers of toxicity in Fresh water field crab \textit{Barytelphusa guerini}

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ABSTRACT

The fresh water field crab, \textit{Barytelphusa guerini} is an important commercial crustacean being widely distributed along the Andhra Pradesh and some other parts of India. The crab is constantly exposed to pesticides, which are used extensively to control agricultural pests. Evaluation of toxic effect of chlorpyrifos on crab was carried out. Effect of 1/3\textsuperscript{rd} and 1/4\textsuperscript{th} LC\textsubscript{50} concentrations of chlorpyrifos was carried out in the sensitive organs such as nervous tissue, thoracic ganglia and eyestalk. Alterations in the Lactate dehydrogenase (LDH), Succinate dehydrogenase (SDH), Acid phosphatase (ACP) and alkaline phosphatase (ALP) was carried out. The enzyme LDH was elevated throughout the experimented period and the SDH, ACP and ALP activity was inhibited along the experimental period at two sub-lethal concentrations.

Key words: Crab, Organophosphates, Pesticide, Dehydrogenase, Phosphatase.

1. Introduction

Manifestation of agricultural and industrial revolution in India has added many pollutants in the environment, which are potentially hazardous, out of which some may be toxic, inflammable, explosive or corrosive. Of the various pollutants present in the hydrosphere and lithosphere, heavy metals and pesticides are toxic to livestock as well as human beings. Pesticides are commonly found in aquatic habitats, including streams, rivers, and ponds, at varying concentrations because of direct overspray, drift, atmospheric transport, agricultural and residential runoff, individual misuse, and improper disposal (Gilliom and Hamilton, 2006). Once a toxicant enters an organism, several biochemical and physiological responses occur which may be adaptive or may lead to toxicity. Thus, it is important that pollutant effects be determined and interpreted in biochemical terms, to delineate mechanisms of pollutant action, and possible ways to mitigate adverse effects.

Chlorpyrifos (CPF), O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate is a broad spectrum organophosphate (OP) pesticide used extensively for the management of domestic and agricultural pests. CPF is commercially used for more than a decade to control foliar insects (Arthropoda) that affect agricultural crops, and subterranean termites (Rao et al., 2005). CPF is the second largest selling insecticide in India and it has been found that about 4.8pb is present in soft drinks which are 47 times higher than the recommended permissible limits of Bureau of Indian Standards (CSE, 2006). Its release into the aquatic environment promotes very toxic effects to non-target aquatic species (Narra et al., 2011a).
The organophosphate insecticides are known to affect the crustaceans viz; monocrotphos affected the neuroendocrine regulation in freshwater crab, Barytelphusa guerini (Patil et al., 2008), chlorpyrifos effects survival and growth of Palaemonetes argentinensis (Montagna and Collins, 2007), and also oxygen consumption and ammonia excretion of the freshwater crab Trichodactylus borellianus (Montagna and Collins, 2008). CPF is a highly toxic pesticide for aquatic animals even at low concentrations (Narra et al., 2011a). This OP needs to be activated into its oxon metabolite by cytochrome P-450 enzyme in order to become toxic and inhibit AChE activity (Barata et al., 2004). According to earlier reports by the authors, CPF is a major pesticide residue in fishery product in Taiwan (Feei and Shan, 2008). A number of reports on the toxicity of CPF on different aquatic animal models indicated that it is a potent neurotoxic agent (Rao et al., 2005).

Stress responses occur in all animals when regulated physiological systems are extended beyond their normal range by external stressors. Failure of all or part of the integrated homeostatic response may lead to increasing physiological disturbance and ultimately death. Indicators of such stress responses are therefore useful in assessing the short-term well-being or long-term health status of an animal and such indicators have received considerable attention in commercially important crustacean species (Paterson and Spanoghe, 1997).

The fresh water field crab, Barytelphusa guerini, which is an important food source in south India, has been and is being exposed extensively to insecticide. The pesticides are having long half-life and accumulate in the food chain through consumption of infected crabs. They are not only neurotoxic but also affect other systems and have shown to have a high degree of impact on metabolism by altering the enzymes. Hence in the present study an attempt was made to study the effect of chlorpyrifos on some biomarker enzyme profiles in fresh water crab, Barytelphusa guerini.

2. Materials and method

2.1 Test chemicals

All the chemicals used in the present study of analytical grade and were used without further purification. The commercial grade 36% E.C chlorpyrifos, a light yellow liquid was selected for the present study.

2.2 Animal maintenance and sub-lethal study

Crabs of similar size and weight were collected from local supplier and kept in good aeration for 3 weeks to eliminate any prior pesticide residues. The natural photoperiod of 12:12 (light: dark) was maintained and balanced fish meal was provided. The average mean values of water quality during investigation are temperature 25 ± 3˚C, pH 7.4 ± 0.4, dissolved oxygen 8.24 ± 0.2 mg L⁻¹, total hardness 415 ± 1.2 mgL⁻¹ as CaCO₃, alkalinity 348 ± 1.6 mgL⁻¹ as CaCO₃, and chlorides 245.6 ± 1.44mg L⁻¹.

The LC₅₀ value of chlorpyrifos was determined in the laboratory using the probit analysis (Fenny, 1971) and found to be 21.14 ± 0.05 mg/l. In the experiment 48 crabs were used in each group i.e., control and exposed to 1/4 th and 1/3 rd (5.28 and 7.047 mg/l) of LC₅₀ concentration for a period of 28 days. The required concentration was maintained by adding the toxicant directly in the experimented tank water and renewed daily. Feeding was withdrawn 24 hours prior to the experimentation to avoid the metabolic differences, if any.
due to differential feeding. The enzymatic alterations in nervous tissue, thoracic ganglia, and eyestalk were studied at regular intervals on day 7, 14, 21 and 28 days.

2.3 Enzyme assays

The ALP (E.C.3.13.1) enzyme activity was estimated by the method of Moss et al. (1971), ACP (E.C.3.13.2) enzyme activity was estimated by the method of Jabeen, (1984) and expressed as mg $p$-nitrophenol g $^{-1}$ protein h $^{-1}$. SDH activity was estimated by the method of Nachlas et al. (1960) and LDH activity was estimated by the method of Srikanthan and Krishnamurthy, (1955) and expressed as µ moles of formazan formed/mg protein/hr. The detail protocols are given in detail Narra et al. (2011b).

2.4 Statistical analysis

The experiments were repeated thrice and data was analyzed by the Student ‘‘t’’ test. Significant differences from exposure values p>5% was accepted as levels of significance.

3. Results and discussion

Toxicants cause a disturbance in the physiological state of the animal which affects enzyme activity. Toxicants bring about distortions in the cell organelles, which may bring about elevation or inhibition in the activity of various enzymes. The alterations in dehydrogenase enzymes might be due to the severe cellular damage leading to release of these enzymes and impaired carbohydrate and protein metabolism as suggested by Sivakumari et al. (1997).

![Figure 1: LDH activity in the tissue of Barytelphusa guerini, exposed to two sub-lethal concentrations of chlorpyrifos for 28 days. Each value is mean ± standard error (n = 6). *Not significant over control (p < 0.05).](image-url)
The lactate dehydrogenase activity was increase in both concentrations and found to be tissue specific and time dependent. The highest increase observed in nervous tissue (110%) followed by eyestalk and thoracic ganglia at the end of 28 days period (Figure 1). The activity of lactate dehydrogenase, which is a cytoplasmic enzyme, shows a marked elevation in activity in the nervous tissue, thoracic ganglia and eyestalk. Lactate dehydrogenase activity is generally associated with cellular metabolic activity which acts as a pivotal enzyme between the glycolysis and citric acid cycle. Thus, the elevation of LDH may suggest a bias towards the anaerobic glycolytic pathway.

The increased LDH activity and decreased succinate dehydrogenase activity was reported in Oziotelphusa senex in response to OP pesticide sumithion (Reddy et al., 1983). Chandravathy and Reddy, (1995) studied the effect of lead on Anabas scandens and found that there was increase in the activity of LDH and decrease in the SDH activity. LDH activity increased in the hepatopancreas in the fielder crab, Uca pugilator in response to cadmium (Devi et al., 1993). Similar reports observed in fresh water crab, Spiralothelephusa hydrodroma treated with the pesticides, cypermethrin by Sreenivasan et al. (2011). The results of the present study are well in accordance with that of previous investigations in the increased activity of LDH in chlorpyrifos treated crabs.

The succinate dehydrogenase activity was decrease in both concentrations and found to be tissue specific and time dependent. The highest decrease observed in nervous tissue (49%) followed by thoracic ganglia and eyestalk (Figure 2). The decrease in the activity of respiratory oxidative enzyme, the succinate dehydrogenase in nervous tissue, thoracic ganglia and eyestalk indicates decline in enzyme synthesis, since chlorpyrifos disrupt the membrane bound enzyme. Mitochondrial damage leads to decreased respiration and partial coupling of oxidative phosphorylation (Teras and Khan, 1966). Suppression of SDH activity disrupts mitochondria in anoxic or hypoxic conditions when exposed to toxicants. SDH plays an important role in regulating osmoregulation and any change in its activity would disrupt the osmoregulatory mechanism (Bashamohideen and Rao, 1979).

**Figure 2:** SDH activity in the tissue of Barytelphusa guerini, exposed to two sub-lethal concentrations of chlorpyrifos for 28 days. Each value is mean ± standard error (n = 6).
*Not significant over control (p < 0.05).*

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Decrease or increase in the enzyme activity represents the metabolic stress in any organism. The effects of metal mixtures in the fish, *Oreochromis mossambicus* and the suppression of SDH activity indicated anoxic hypoxic conditions when the fish was exposed to toxicant and it was possibly due to the mitochondrial disruption, leading to decrease in the activities of oxidative enzymes and an increase in the glycolic enzymes reported by Dubale and Awasthi, (1982). Narra et al. (2011b) reported decreased SDH activity in different tissues of food fish *Clarias batrachus* exposed to chlorpyrifos. The decrease in SDH activity has been reported in the fresh water crab, *Spiralothelphusa hydrodroma* treated with the pesticides, cypermethrin by Sreenivasan et al. (2011). The results of the present study are also in conformity with those of the earlier observations.

In the present study the alterations in dehydrogenases activity of the crab *Barytelphusa guerini* treated with chlorpyrifos might have been increased depending on anaerobic carbohydrate metabolism, cumulative effect or possibly to meet the increased energy demands under sustained and prolonged toxic stress of chlorpyrifos.

**Figure 3:** ACP activity in the tissue of *Barytelphusa guerini*, exposed to two sub-lethal concentrations of chlorpyrifos for 28 days. Each value is mean ± standard error (n = 6).

*Not significant over control (p < 0.05).*

The acid phosphatase activity was increased in all tissues throughout exposure period in both concentrations and is time dependent. The highest decrease observed in eyestalk (59%) followed by nervous tissue and thoracic ganglia (Figure 3). Acid phosphatase is a lysosomal enzyme that hydrolyses the phospho-esters in acidic medium. The intracellular distribution patterns of enzymes in the rat liver tissue and reported that generally the decreased activity of acid phosphatase (ACP) activity attributed to the activation of enzyme, which was kept in latent state inside the membrane of lysosomes reported by Deduve et al. (1955). O'Connor and Gilbert, (1968) reported increase in acid phosphatase activity due to accumulation of mercury in the lysosome and blockage in the release of enzymes and carbohydrate forms the major reserve of many crustaceans accumulated in the hepatopancreas. In edible crab *scylla seratta* were of the opinion that degradation and necrosis induced by toxicants in hepatopancreas causes release of acid phosphatase (Ahmed et al., 1997).
Narra et al. (2011b) reported increased ACP activity in different tissues of food fish *Clarias batrachus* exposed to chlorpyrifos. Similar reports observed in fresh water crab, *Spiralothelphusa hydrodroma* treated with the pesticides, cypermethrin (Sreenivasan et al., 2011). In the present study, the increased ACP activity was observed in both the lower and higher sub-lethal concentrations of chlorpyrifos in both 7 to 28 days of treatment. The increase in ACP activity was high in the crab treated with higher sub-lethal concentration in a period of 28 days. The increase in ACP activity may be inferred as a response to altered metabolism due to chlorpyrifos stress.

The alkaline phosphatase activity was decrease in all the tissues throughout exposure period in both concentrations. The highest decrease observed in eyestalk (61%) followed by thoracic ganglia and nervous tissue (Figure 4). Goldfisher, (1964) studied the effect of the pollutants in aquatic animals and stated that alkaline phosphatase is a brush border enzyme, which splits various phosphorous esters at an alkaline pH and mediated transport. ALP is involved in carbohydrate metabolism, growth and differentiation, protein synthesis, synthesis of certain enzymes, secretion activity, and transport to phosphorylated intermediates across the cell membranes (Vijayavel and Balasubramanian, 2006). Thus, any alteration in the activity of ALP affects the organisms.

Ahmed et al. (1997) reported the effect of copper on oxygen consumption and phosphatase in *scylla serrata* and concluded that there was decrease in alkaline phosphatase activity in muscle, hepatopancreas and haemolymph. The decrease in ALP activity has been reported in the fresh water crab, *Spiralothelphusa hydrodroma* treated with the pesticides, cypermethrin by Sreenivasan et al. (2011). Similar observations were noted in the *scylla serrata* crab in response to naphthalene (Elumalai et al., 1997). In the present investigation, the activity of ALP was found to decrease in the tissues of the test crabs when compared to the control crabs.
The maximum decrease was seen in the crabs exposed to higher sub-lethal concentration of chlorpyrifos for 28 days.

4. Conclusion

The present results offer information about the deleterious effects of an organophosphate insecticide, chlorpyrifos on fresh field crab *Barytelphusa guerini*. From the results it was clear that the effects were dose and time dependent. This kind of information could be beneficial to take preventive measure to protect the aquatic animals from the polluted areas.

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5. References

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