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Note

Biochemical alterations in rohu, *Labeo rohita* (Hamilton, 1822) exposed to organophosphorus insecticide, methylparathion

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ABSTRACT

The effect of exposure to sublethal concentrations of methylparathion on enzyme activities in the liver of rohu, *Labeo rohita,* was studied during 96 h exposure. Alterations were observed in alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and acid phosphatase (ACP)) activities in the liver of *L. rohita*, of mean size 75 ± 6 g. ALP and ASP activity levels in methylparathion treated fishes were significantly ($p<0.05$) higher than the control fishes. The LDH activity in the liver of *L. rohita* after methylparathion exposure was two-fold higher ($p<0.05$) when compared to control. These results revealed that methylparathion affects the intermediary metabolism of *L. rohita* and that the assayed enzymes can work as good biomarkers of methylparathion contamination.

Keywords: Biochemical changes, *Labeo rohita*, Marker enzymes, Methylparathion

Organophosphorus compounds are widely used in agriculture as well as in domestic front for controlling insect pests. Owing to their rapid breakdown in water and their low environmental persistence, organophosphorus pesticides have largely replaced the use of organochlorines (Abhilash and Singh, 2009). Pesticides are included under a broad range of organic micro-pollutants that have tremendous ecological impacts. Different categories of pesticides have varying effects on living organisms and hence generalisation is difficult. Though terrestrial impacts by pesticides do occur, the principal pathway that causes ecological impacts is that of water contamination by pesticides runoff. Fish and aquatic animals are exposed to pesticides in three ways namely (i) direct absorption through the skin by swimming in pesticide contaminated waters, (ii) direct uptake of pesticides through the gills during respiration and (iii) drinking of pesticides contaminated water or feeding on pesticide contaminated prey. Exposure of fish and other aquatic animals to a pesticide depends on the biological availability, bioconcentration, biomagnifications and persistence of the chemical in the environment.

Methylparathion is a representative of the highly active insecticides, the thiophosphorus esters, developed in the 1940s by Schrader. Methylparathion is extensively used as a pesticide in agriculture, food storage shelters and also in fish culture tanks to kill the aquatic larval stages of predator insects that threaten fish larvae (Aguiar *et al*., 2004). The present study was designed to evaluate the effects of 96 h exposure to sublethal concentrations of methylparathion on biochemical alterations in liver of *L. rohita*.

Rohu (*L. rohita*) of mean body weight 75 ± 6 g and mean body length 23 ± 5 cm were procured from a fish farm located at Thiruvankulamin in Ernakulam District, Kerala, India. The fishes were brought alive to the laboratory and acclimatised for more than 15 days in plastic tanks prior to starting the experiment. Methylparathion-50% (O,O-dimethyl-O-4-nitrophenyl-Phosphorothioate-Bayer, Germany), was procured from a commercial outlet in Cochin. Six sublethal concentrations of methylparathion *viz*., 1.8, 3.6, 5.4, 7.2, 9.0 and 10.2 mg l^{-1} were selected for acute (96 h) exposure studies. Control group was also maintained without exposure to methylparathion. The sublethal doses were selected based on the lethal dose (LC 50) of methylparathion already estimated for rohu (Sivaperumal and Sankar, 2011). Fishes were fed with commercial fish feed, and the tanks were kept well aerated during the experiment. Twenty percent of the water in the tanks was replaced on a daily basis during the experimental period, the pesticide loss during this procedure being compensated by supplementing with the required quantity. On termination of the experiment, fishes were killed by decapitation and liver was analysed for enzyme activities. Liver tissues were dissected, washed in physiological saline (0.9% Nacl), and kept at –20 °C until analysis.

Liver tissue samples were homogenised for 5 min in ice-cold 0.1M Tris-HCl buffer solution (pH 7.2; 1:5 w/v) using Polytron homogeniser (Polytron Model PT3000, Kinematica-Switzerland) and centrifuged (Remi-India) at 8000 rpm for 30 min. Supernatant were used for estimation of selected enzyme activities. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed following the method of Mohun and Cook (1957). Lactate dehydrogenase (LDH) activity was estimated as per King (1965 a). Alkaline phosphatase (ALP) and acid phosphatase (ACP) were estimated by the method of King (1965 a,b). Experiments were conducted in accordance with the guidelines of the supervision of experiments on animals (CPCSEA), New Delhi, India and with the approval of the animal ethics committee of the Institute. The data were analysed using one-way analysis of variance (ANOVA) (SPSS 10.0 statistical system for windows) followed by Duncan's multiple range test to check difference between treatment pairs (Daniel, 1987).

On acute exposure to sublethal concentrations of methylparathion, the fishes exhibited symptoms of dullness, loss of equilibrium, loss of feeding and erratic swimming. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) are liver specific enzymes and their activity levels are sensitive measures of hepatotoxicity (Balint *et al*., 1997). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities recorded in the liver of *L. rohita* after methylparathion exposure was found to be higher than control fishes (Table1). There was significant ($p<0.05$) increase in ALT activity with increasing concentration of methylparathion, and more than 2 fold increase was noticed at a pesticide concentration of 10.2 mg l^{-1} compared to control. Significant ($p<0.05$) increase was also recorded in AST activity with increase in concentration of methylparathion and a threefold increase was noticed at concentration of 10.2 mg l^{-1} when compared to control group. The increase in ALT and AST activities in liver after exposure of methylparathion indicates cell necrosis due to methylparathion toxicity. The increase in ALT and AST activites observed in the present study supports earlier findings and these enzymes serve as indicators of tissue damage (Das *et al*., 2003; Venkateswara Rao, 2006). ALT and AST are more sensitive measures of hepatotoxicity and can be assessed within a short time (Balint *et al*., 1997). Increase in ALT and AST indicate tissue damage in liver, kidney or gill (Rajyasree and Neeraja, 1989; Oluah, 1999).

The lactate dehydrogenase (LDH) activity in the liver of *L. rohita* after methylparathion exposure was also higher when compared to control (Table 1). There was significant ($p<0.05$) increase in LDH activity with an increase in the concentration of methylparathion, and a two-fold increase was noticed at 10.2 mg l^{-1}. LDH is one of the important metabolic requirements for tissue and involved in energy production. It mediates the inter-conversion of lactate and pyruvate, depending up on the availability of NAD. LDH is present in numerous tissues, and is a marker of tissue damage. Increased levels of LDH is reported in liver necrosis (Lamaire *et al*., 1991). Injured cells, organs and tissues often release LDH into the blood, which raises the level of this enzyme in blood and it can be used as an indicator of cellular damage and cytotoxicity of toxic agents (Ramesh *et al*., 1993).

The alkaline phosphatase (ALP) activity in the liver of *L. rohita*, after methylparathion exposure was also higher when compared to control (Table 1). There was significant ($p<0.05$) increase in ALP activity with an increase in the concentration of methylparathion. ALP is a broad term associated with non-specific phosphomonoesterases with activity optima at alkaline pH. ALP is mainly localised at the cell membrane and any damage to hepatic cells may result in the alternation of ALP activity. The acid phosphatase (ACP) activity in the liver tissue of *L. rohita* after methylparathion exposure was also found to be higher when compared to that in control fishes (Table1). Significant ($p<0.05$) increase in ACP activity was noticed with increase in concentration of methylparathion. Acid phosphatase (ACP) is known to be localised in lysosomes. The changes in ACP, suggest an increase in lysosomal

Table 1. Effect of sublethal concentrations of methylparathion on the liver specific enzyme activity in *Labeo rohita*

Concentration (mg l^{-1})	ALT	AST	LDH	ALP	ACP
Control	099.5 ± 4.7^{a}	240.6 ± 09^{a}	593.9 ± 10^{a}	110.9 ± 10^{a}	209.3 ± 05^{a}
1.8	102.6 ± 11^{a}	267.6 ± 17^{b}	839.7 ± 14^{b}	227.3 ± 09^{b}	235.6 ± 19^{b}
3.6	124.8 ± 5.9^{b}	399.3 ± 17^{c}	862.8 ± 18^{b}	250.3 ± 15^{b}	242.7 ± 13^{bc}
5.4	107.5 ± 5.6^{a}	576.1 ± 26^{d}	927.7 ± 15^{c}	185.2 ± 09^{c}	251.5 ± 09^{bcd}
7.2	127.5 ± 7.5^{b}	645.5 ± 03^{e}	1003 ± 14^{d}	240.1 ± 06^{b}	262.7 ± 16^{cd}
9.0	149.8 ± 6.9^{c}	657.5 ± 14^{e}	1105 ± 12^{e}	296.7 ± 18^{d}	272.7 ± 12^{de}
10.2	227.4 ± 8.8^{d}	707.5 ± 07^{f}	1191 ± 27^{f}	357.7 ± 17^{e}	287.5 ± 03^{e}

Results are given as mean±SD (n = 3). Values bearing different superscripts (a,b,c,d,e,f) differ significantly ($p<0.05$)
Units: ALT, AST, LDH – μ mol pyruate librated $h^{-1}l^{-1}$; ALP, ACP – μ mol phenol librated $h^{-1}l^{-1}$

mobilisation and cell necrosis due to methylparathion toxicity. Ram and Singh (1988) reported elevation in ALP and ACP activity in the liver of carbofuran-treated C*hanna punctatus*. Alterations in ALP and ACP activities in tissues and serum have been reported in fish (Jyothi and Narayan, 2000).

The results indicated that, in response to methylparathion exposure, the activities of the liver enzymes *viz*., ALT, AST, ACP and ALP increased, which can lead to disruption of normal liver function. There are reports indicating that increase in ALP could be a result of damage of liver cells and progressive liver necrosis (Shakoori *et al*., 1994, Tietz, 1976). Acute toxicity due to deltamethrine has been reported to increase ALT and AST in Nile tilapia (Velisck *et al*., 2006; Velisck *et al*., 2007)

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