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Toxicity of Organophosphorus Insecticide – Methyl Parathion on Rohu *(Labeo rohita)*

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Abstract

Fresh water fish, *Labeo rohita* was exposed to sub-lethal concentrations (0.25, 0.5 and 1.0 mg l^{-1}) of the organophosphorus insecticide, methyl parathion (MP) for 15, 30 and 45 days. Aspartate and alanine amino transferase activity increased with increasing pesticide concentration, suggesting tissue damage and muscular harm due to exposure to methyl parathion. The increase in the activity of alkaline phosphatase suggests an increase in lysosomal mobilization and cell necrosis due to methyl parathion toxicity. The results clearly indicate that the organophosphorus insecticide methyl parathion is toxic at sublethal level and it affects intermediary metabolism of *Labeo rohita.*

Keywords: Methyl parathion, *Labeo rohita*, marker enzymes, sub lethal exposure

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Introduction

The use of pesticides, herbicides and fungicides was started in India during the mid-sixties and is a common feature of Indian agriculture. Though their use has led to increased production of food and increased profitability in agriculture, it has also been associated with several concerns, including the risks to human health, the death of farm animals, alteration of the local environment and also caused many long-term effects on the society. It has been observed that their long-term, low-dose exposure is increasingly linked to human health effects such as immune-suppression, hormone disruption, diminished intelligence, reproductive abnormalities, and cancer (Agnihotri, 1999; Gupta, 2004). Methylparathion is an active substance, extensively used as a pesticide in agriculture, food storage shelters, pest control programs, and fish culture tanks to kill the aquatic larval stages of predator insects that threaten fish larvae (Aguiar et al., 2004).

Fishes have been widely used as models to evaluate the health of aquatic ecosystem and in toxicological studies (Law, 2003). Research in fish has demonstrated that mammalian and piscine systems exhibit similar toxicological and adaptive responses to oxidative stress. This suggests that piscine models, may be useful for further understanding the mechanisms underlying the oxidative stress response. Freshwater aquaculture constitutes one-third of the total fish production in India, major carps being the dominant species (*Labeo rohita, Catla catla,* and *Cirrhinus mrigala*). There are number of studies on various biochemical, haematological and cellular changes due to the effect of organochlorine and organophosphorous pesticides on fish (Nath & Banerjee, 1996; Das & Mukherjee, 1997; 2000a,b; 2003; and Rao, 2006). But, no work on Indian major carp, rohu (*Labeo rohita*) has been carried out specifically on these aspects. It was decided to determine lethal concentration 50% (LC_{50}) and examine the effect of methyl parathion on biochemical and enzymatic changes in Indian major carp rohu *(L. rohita*) at sub lethal concentrations.

Materials and Methods

Methyl parathion-50% (O,O-dimethyl-O-4-nitrophenyl-phosphorothioate-Bayer, Germany) a synthetic organophosphrous insecticide was obtained from the market in Cochin. Other experimental chemicals were purchased from Sigma (USA), Merck (Germany) and SRL (India).

Rohu weighing about 75 ± 6 g and length 23 ± 5 cm were collected from a fish farm in Thiruvankulam near Ernakulam, Kerala, India. Fishes were brought to the laboratory and acclimatized for more than 15 days in plastic tanks before starting the experiment. Water pH and temperature were kept at nearly constant levels. The fish tanks were well aerated and physical and chemical parameters were kept nearly constant.

The lethal range bioassay was determined to a sequential concentrations viz., 0.002, 0.02, 0.2, 2, 20 mg l^{-1} of methyl parathion (APHA-AWWA-WPCF 1975). Eight fishes were released into a fish tank, containing 50 l of water and sequential concentrations of methyl parathion were added. The mortalities were recorded (24, 48, 72 and 96 h) and dead fishes were removed immediately.

For the determination of lethal concentration (LC_{50}) (Reish & Oshida, 1987) of the pesticide, eight fishes of approximately equal size (75 ± 6 g) were released into different fish tanks, containing different concentrations (1.8, 3.6, 5.4, 7.2, 9.0, 10.8, 12.6, 14.4, and 16.2 mg l^{-1}) of methyl parathion. The control fishes were maintained separately and mortality was recorded at 24, 48, 72 and 96 h.

The 96 h LC_{50} was found to be 10.2 mg l^{-1}. 1/10, 1/20 and 1/40 of the 96 h LC_{50}, i.e. 0.25, 0.5 and 1.0 mg l^{-1} were selected for sublethal exposure for 15, 30 and 45 days.The control was maintained in a tank containing methyl parathion free water. Fishes were fed with commercial fish feed. At the end of the experimental period fishes were killed by decapitation. The liver was homogenized for 5 min in ice-cold 0.1M Tris-HCl buffer solution pH 7.2 (1:5 w/v) using a homogenizer (Polytron Model PT3000, Kinematica-Switzerland) and centrifuged (Remi-India) at 5000 g for 30 min. Supernatants were used for determination of marker enzymes. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the method of Mohun & Cook (1957). Activities of lactate dehydrogenase (LDH), 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.

One-way analysis of variance (ANOVA) was run using the SPSS 10.0 statistical system for windows. ANOVA was employed followed by Duncan's test to calculate the significant difference between control and pesticide treated fish (Daniel, 1987).

Results and Discussion

The lethal range finding test carried out for concentration of methyl parathion between 0.002 mg l^{-1} to 20 mg l^{-1} for a period of 96 h showed no mortality up to concentration of 2 mg l^{-1} while at 20 mg l^{-1} methyl parathion concentration, 100% mortality was observed. Therefore it was concluded that the lethal concentration 50% (LC_{50}) of *Labeo rohita* is between 2 and 20 mg l^{-1} of methyl parathion.

The study shows that no mortality was noticed up to a concentration of 5.4 mg l^{-1}. The exposure of fish to a concentration of 7.2 mg l^{-1} of methyl parathion for 96 h, showed 10% mortality, while exposure to concentration of 16.2 mg $l^{-1}$for 48 h caused 100% mortality. The probit analysis shows that the lethal concentration for 50% mortality (LC_{50}) of the fishes at 24, 48, 72 and 96 h were 15.5, 12.3, 11.4 and 10.2 mg l^{-1} respectively, for *Labeo rohita* of size 75 ± 6 g. The toxicity of xenobiotic in living organisms depends upon the size of fish and temperature of exposure. The LC_{50} of the organophosphorus pesticide RPR-II was found to be 0.17 mg l^{-1} for *Oreochormis mossambicus* of size 5 ± 1 g (Rao, 2006). The 96 h LC_{50} of azinphosmethyl, parathion and carbaryl were 7.18, 6.46 and 13.86 mg l^{-1} respectively for goldfish (*Carrassius auratus*) of size 2-5 g (Ferrari et al., 2004). For pyrethroid pesticide cypermethrin, the 96 h LC_{50} was reported to be 0.139 mg l^{-1} for rohu *(Labeo rohita)* of size 8.52 ± 2.54 g. These results suggest that the changes in LC_{50} of pesticide depend on size of the fish.

Alanine aminotransferase (ALT) activity in the liver of *Labeo rohita* after methyl parathion exposure at sub lethal level was higher when compared to control (Table 1). There was a significant ($p<0.05$) increase in ALT activity with increase in the concentration of methyl parathion. The result showed a clear alteration in relation to methyl parathion concentration and exposure periods for 0.25 and 0.5 mg l^{-1} concentration. On 15th day of exposure ALT activity increased with increase in the concentration of methyl parathion. On further exposure to 30 and 45 days the activity showed gradual decline. At 30th day, ALT activity showed a decline indicating the repair mechanism in the initial periods of exposure to the xenobiotics. However, at higher concentration of methylparathion

(1.0 mg l^{-1}) after initial decrease of AST activity up to 30 days, the activity increased by 45th day indicating extensive damage to hepatic tissues at higher concentration.

The aspartate aminotransferase (AST) activity in the liver of *Labeo rohita* after methyl parathion exposure at sub-lethal level was higher compared to control (Table 1). There was significant ($p<0.05$) increase in AST activity with increase in the concentration of methyl parathion for varying duration. A 6% increase was noticed at the lowest concentration of 0.25 mg l^{-1}, which shot up by 24 and 48% respectively, for 0.5 and 1.0 mg $l^{-1}$of methyl parathion for the same duration of exposure. During exposure to 30 and 40 days, at the lowest concentration (0.25 mg l^{-1}) a marginal decrease in AST level was noticed but at higher concentration, a significant ($p<0.05$) increase was noticed. This clearly indicates the extent of damage to hepatic cells as a result of methyl parathion intoxication. At 1.0 mg l^{-1}concentration for 45 days about 82% increase was noticed. At 0.25 mg l^{-1}concentration, the AST activity marginally decreased as a result of the cells getting adjusted to mild concentration of methyl parathion. The increase in ALT and AST activities noticed in the study suggests tissue damage (Oluah, 1998; 1999). The increase in ALT and AST activities in our study supports earlier findings and serves as indicators of tissue damage (Rao, 2006). The increase in ALT and AST activities in liver after exposure of methyl parathion may be due to cell necrosis caused by methyl parathion toxicity.

Lactate dehydrogenase (LDH) activity in the liver of *Labeo rohita* after methyl parathion exposure to sub lethal level was higher when compared to control (Table 2). There was a significant ($p<0.05$) increase in LDH activity with increase in the concentration of methyl parathion. LDH activity on 15th day showed a decreasing trend at a concentration of 0.25 mg l^{-1}, which increased with the increase in pesticide concentration. After 30 days of exposure, LDH activity increased ($p<0.05$) several folds at all concentrations of methyl parathion. However, LDH activity showed decrease on 45 days of exposure. Decrease in LDH activities of liver and muscles reflects a possible decrease in the biosynthetic activities and anaerobic capacity, which indicate that glycolysis in tissues was decreased (Tripathi & Verma, 2004). LDH is present in numerous tissues, and is considered as a marker of tissue damage. Its increased level is reported in liver necrosis (Ramesh et al., 1993; Yousef et al., 2002).

Hepatic specific activity of alkaline phosphatase (ALP) and acid phosphatase (ACP) for experimental fish increased with increase in the ($p<0.05$) concentration of methyl parathion exposure (Table 3). For a particular concentration, the activity increased with increase in the duration of exposure. The elevation in alkaline phosphatase suggests an increase in the lysosomal mobilization and cell necrosis due to methyl parathion toxicity. Elevation of ACP activity in brain was reported earlier in stress-exposed *Channa punctatus* (Sastry & Sharma, 1981) and in *Labeo rohita* (Das & Mukherjee, 2003). There are also reports indicating increase in the activities of these enzymes in serum as a result of impairment of hepatic tissue and liberation of these enzymes into circulation from the damaged tissues (Oruc & Uner, 1999). Acid phosphatase (ACP) is known to be localized in lysosomes, and surrounded by a lipoprotein membrane. Increase in

Table 1. Effect of sub-lethal concentrations of methylparathion on the liver specific activity of ALT and AST (µ mol pyruate librated h^{-1} l^{-1}) in *Labeo rohita.*

Conc (mg l^{-1})	Duration					
	15 days		30 days		45 days	
	ALT	AST	ALT	AST	ALT	AST
Control	271.3 ± 16^{a}	498.4 ± 67^{a}	250.6 ± 25^{a}	499.4 ± 38^{a}	221.1 ± 45^{a}	488.9 ± 40^{a}
0.25	277.8 ± 16^{ab}	529.8 ± 12^{a}	241.3 ± 14^{a}	504.1 ± 17^{a}	235.9 ± 17^{ab}	492.9 ± 85^{a}
0.50	288.1 ± 17^{ab}	617.3 ± 33^{b}	264.6 ± 15^{ab}	675.4 ± 30^{b}	254.6 ± 11^{b}	766.1 ± 32^{b}
1.00	332.1 ± 19^{b}	712.8 ± 11^{c}	289.6 ± 18^{b}	810.5 ± 60^{c}	304.9 ± 06^{c}	892.1 ± 62^{c}

Results are given as mean ± SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly ($p<0.05$) Duncan's multiple range test).

Table 2. Effect of sub-lethal concentrations of methylparathion on the liver specific activity of LDH (µ mol pyruate librated h^{-1} l^{-1}) in *Labeo rohita.*

Conc (mg l^{-1})	Duration		
	15 days	30 days	45 days
Control	212.3 ± 07[a]	238.7 ± 11[a]	261.8 ± 10[a]
0.25	203.7 ± 10[a]	465.5 ± 16[b]	344.1 ± 30[b]
0.50	307.2 ± 04[b]	961.0 ± 37[c]	509.2 ± 40[c]
1.00	470.1 ± 14[c]	962.9 ± 18[c]	952.3 ± 19[d]

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c,d) differ significantly (p<0.05Duncan's multiple range test).

Table 3: Effect of sub-lethal concentrations of methylparathion on the liver specific activity of ALP and ACP (µ mol pyruate librated h^{-1} l^{-1}) in *Labeo rohita.*

Conc (mg l^{-1})	Duration					
	15 days		30 days		45 days	
	ALP	ACP	ALP	ACP	ALP	ACP
Control	136.6 ± 14[a]	194.9 ± 33[a]	131.1 ± 38[a]	216.1 ± 10[a]	143.7 ± 14[a]	211.7 ± 22[a]
0.25	147.2 ± 10[a]	204.7 ± 02[a]	148.5 ± 07[a]	224.5 ± 56[a]	159.7 ± 05[a]	235.1 ± 31[a]
0.50	221.8 ± 42[b]	241.4 ± 41[a]	234.0 ± 21[b]	248.6 ± 31[a]	231.0 ± 28[b]	244.7 ± 14[ab]
1.00	237.8 ± 14[b]	250.1 ± 16[a]	241.9 ± 10[b]	270.7 ± 17[a]	250.4 ± 10[b]	286.7 ± 19[b]

Results are given as mean±SD (n = 3). Values that have a different superscripts (a,b,c) differ significantly (p<0.05) Duncan's multiple range test).

ALT and AST indicates tissue damage in liver, kidney and gill (Oluah, 1999).

The results clearly indicate that *Labeo rohita* exposed to higher concentration (1 mg l^{-1}) of sub lethal dose of methyl parathion had significant effect on marker enzymes although the fish eventually developed tolerance against methyl parathion toxicity. The results also showed that there was an increase in LDH activity in the liver of fish exposed to higher concentration of the insecticide, for 15, 30 and 45 days. This could be due to increased energy demand that occurs when aerobic oxidation is reduced under the hypoxic conditions caused by methyl parathion. The activities of ALT, AST, LDH, ACP and ALP can be used as a disease diagnostic biomarkers in fish to evaluate poisoning due to methyl parathion.

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