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Research paper

Methyl Parathion effect on ALT, AST and LDH in Freshwater fish, *Heteropneustes fossilis*

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ABSTRACT

Heteropneustes fossilis, a freshwater fish, was subjected to sub-lethal doses (0.25, 0.5, and 1.0 mg L^{-1}) of the organophosphorus insecticide methyl parathion (MP) over periods of 15, 30, and 45 days. The activities of aspartate amino-transferase (AST) and alanine amino-transferase (ALT) increased in a concentration-dependent manner, signifying tissue damage and muscle impairment due to MP exposure. Likewise, the rise in LDH activity indicates increased lysosomal mobilization and cellular necrosis. These biochemical changes clearly show that methyl parathion has toxic effects even at sub-lethal levels, disrupting intermediary metabolism and causing significant physiological stress in the fish.

1. Introduction

In India, the introduction of pesticides, herbicides, and fungicides dates back to the mid-1960s, and these substances have since become integral to the country's agricultural practices. While their use has boosted agricultural yields and profitability, it has also sparked numerous concerns, such as potential health risks to humans, increased mortality in farm animals, disruption of local ecosystems, and long-term societal effects. Continuous exposure to low doses of these agrochemicals has been linked to negative health outcomes in humans, including immune system suppression, endocrine system interference, cognitive decline, reproductive issues, and cancer development (Agnihotri, 1999; Gupta, 2004). Methyl parathion, an organophosphorus insecticide, is extensively employed in agriculture, food storage, pest control, and fish farming to eradicate aquatic insect larvae that threaten fish fry (Aguiar et al., 2004). Fish are widely acknowledged as reliable bioindicators for evaluating the health of aquatic environments and conducting toxicological assessments (Law, 2003). Research has shown that fish and mammals respond similarly to oxidative stress, indicating that fish models can be valuable for studying oxidative stress mechanisms and toxicity.

Freshwater aquaculture contributes to about one-third of India's total fish production, with Indian major carps like Labeo rohita (rohu), Catla catla, and Cirrhinus mrigala being the most common species. Numerous studies have explored the biochemical,

hematological, and cellular impacts of organochlorine and organophosphorus pesticides on fish (Nath & Banerjee, 1996; Das & Mukherjee, 1997, 2000 a, b, 2003; Rao, 2006). However, there is a scarcity of data on the specific effects of methyl parathion on the Indian major carp L. rohita. Consequently, this study was conducted to ascertain the median lethal concentration (LC_{50}) and to assess the biochemical and enzymatic changes in H. fossilis when exposed to sublethal doses of methyl parathion.

2. Materials and Methods

Methyl parathion (50% EC), a man-made organophosphorus insecticide, was sourced from the local market in Saran, Bihar. Healthy *Heteropneustes fossilis* specimens, each weighing around 75±6g and measuring 23±5cm in length, were gathered from nearby ponds. These fish were then transported to the laboratory, where they were acclimated for over 15 days in large plastic tanks filled with dechlorinated tap water and continuously aerated. The water quality parameters were kept nearly constant during both the acclimation and experimental phases. The fish were fed daily with commercial fish feed.

The lethal range bioassay was carried out according to the standard methods of APHA, AWWA, and WPCF (1975). Groups of eight fish were subjected to increasing concentrations of methyl parathion (0.002, 0.02, 0.2, 2.0, and 20.0 mg L^{-1}) in 50 L of water. Mortality was observed at 24, 48, 72, and 96 hours, with deceased fish being promptly removed. To determine the median lethal concentration (LC_{50}), fish of similar size were exposed to various concentrations of methyl parathion (1.8, 3.6, 5.4, 7.2, 9.0, 10.8, 12.6, 14.4, and 16.2 mg L^{-1}) as outlined by Reish and Oshida (1987). Control fish were kept separately under the same conditions without pesticide exposure. Mortality was recorded every 24 hours up to 96 hours, and the 96-hour LC_{50} was found to be 10.2 mg L^{-1}.

Based on the LC_{50} value, sub-lethal concentrations equivalent to 1/40, 1/20, and 1/10 of the LC_{50} (0.25, 0.5, and 1.0 mg L^{-1}, respectively) were chosen for chronic exposure. Fish were exposed to these concentrations for 15, 30, and 45 days, while a control group was maintained in pesticide-free water. Throughout the experiment, fish were regularly fed with commercial feed, and water was periodically renewed to maintain oxygen levels and chemical stability.

At the end of each exposure period, fish were euthanized by decapitation, and liver tissues were immediately excised. The tissues were homogenized (1:5 w/v) in ice-cold 0.1 M Tris–HCl buffer (pH 7.2) using a Polytron homogenizer (Model PT3000, Kinematica, Switzerland). The homogenate was centrifuged at 5000 × g for 30 minutes at 4 °C (Remi, India), and the supernatant was used for enzyme assays.

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured following the method of Mohun and Cook (1957). Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities were determined using the procedures described by King (1965a,b).

All experiments were conducted in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and received approval from the Institutional Animal Ethics Committee. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to identify significant differences between control and treated groups (Daniel, 1987). Statistical analyses were performed using SPSS software.

3. Results and Observations

The lethal range-finding test with methyl parathion concentrations from 0.002 to 20 mg L^{-1} over 96 hours showed no deaths up to 2 mg L^{-1}, while 100% mortality occurred at 20 mg L^{-1}. This suggests that the 96-hour median lethal concentration (LC_{50}) for *Heteropneustes fossilis* is between 2 and 20 mg L^{-1} of methyl parathion. A more comprehensive toxicity test found no mortality at concentrations up to 5.4 mg L^{-1}, but 10% mortality was observed after 96 hours at 7.2 mg L^{-1}. At 16.2 mg L^{-1}, complete mortality was noted within 48 hours. Probit analysis determined that the LC_{50} values for 24, 48, 72, and 96 hours were 15.5, 12.3, 11.4, and 10.2 mg L^{-1}, respectively, for H. fossilis with an average weight of 75 ± 6 g.

Significant increases ($p < 0.05$) in the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed in the liver of H. fossilis exposed to sublethal methyl parathion concentrations compared to the control group (Table 1).

Table 1 Effect of sub-lethal concentrations of methylparathion on the liver ALT and AST (µ mol pyruate librated h-1 l-1) in *Heteropneustes fossilis* as mean±SD (n = 3)

Control/Dose	15DaysALT	15DaysAST	30DaysALT	30DaysAST	45DaysALT	45DaysAST
Control	271.3± 16	498.4± 67	250.6± 25	499.4± 38	221.1± 45	488.9± 40
0.25	277.8± 16	529.8± 12	241.3± 14	504.1± 17	235.9± 17	492.9± 85
0.50	288.1± 17	617.3± 33	264.6± 15	675.4± 30	254.6± 11	766.1± 32
1.00	332.1± 19	712.8± 11	289.6± 18	810.5± 60	304.9± 06	892.1± 62

ALT activity increased in a concentration-dependent manner across all exposure levels. By the 15th day of exposure, ALT activity significantly rose with higher concentrations of methyl parathion (0.25–1.0 mg L^{-1}). However, by the 30th and 45th days, there was a gradual decrease in ALT activity, indicating the possible onset of adaptive or repair mechanisms during extended exposure to the xenobiotic. At the highest sublethal concentration (1.0 mg L^{-1}), ALT activity initially decreased up to 30 days, then sharply increased by 45 days, suggesting ongoing hepatic tissue damage under chronic exposure conditions. Likewise, AST activity significantly increased in all treated groups compared to the control.

Table 2 Effect of sub-lethal concentrations of methyl parathion on the liver specific activity of LDH (µmol pyruate librated h-1 l-1) in heteropneustes fossilis as mean±SD (n = 3)

Control/Dose	15DaysLDH	30DaysLDH	45DaysLDH
Control	212.3± 07	238.7± 11	261.8± 10
0.25	203.7± 10	465.5± 16	344.1± 30
0.50	307.2± 04	961.0± 37	509.2± 40
1.00	470.1± 14	962.9± 18	952.3± 19

The increased transaminase activities indicate hepatic dysfunction and the leakage of cytoplasmic enzymes into the bloodstream, pointing to cellular membrane damage and altered metabolic activity due to methyl parathion toxicity (Table 2).

4. Discussion

The toxicity of xenobiotic substances like pesticides is influenced by factors such as the size, age, and physiological state of the test organism, along with environmental conditions like temperature. For example, the LC_{50} for the organophosphorus pesticide RPR-II was found to be 0.17 mg L^{-1} for *Oreochromis mossambicus* (Rao, 2006). Likewise, the 96-hour LC_{50} values for azinphosmethyl, parathion, and carbaryl were determined to be 7.18, 6.46, and 13.86 mg L^{-1}, respectively, for goldfish (*Carassius auratus*) weighing between 2 and 5 grams (Ferrari et al., 2004). In the case of *Labeo rohita* (rohu), a 96-hour LC_{50} of 0.139 mg L^{-1} was recorded for the pyrethroid pesticide cypermethrin. These results suggest that fish susceptibility to pesticides is affected by species-specific and size-related factors.

Enzymes like ALT and AST, which are crucial in amino acid metabolism, act as sensitive markers for tissue damage and stress. The increase in these enzyme activities after exposure to methyl parathion suggests a compromise in hepatocellular integrity and heightened amino acid breakdown. These observations align with previous studies on pesticide-induced biochemical changes in fish (Das & Mukherjee, 2003; Rao, 2006). The impact of methyl parathion on protein changes in *Labeo rohita* is documented by (Anandan, Suresh Kumar, and Krishnamurthy, 2019).

The rise in enzymatic activity is likely due to oxidative stress caused by pesticides, which increases the permeability of liver cell membranes, leading to enzyme leakage into the extracellular space. Extended exposure and higher pesticide levels exacerbate these effects, resulting in irreversible liver damage. Overall, the research indicates that even sublethal doses of methyl parathion can cause notable biochemical and enzymatic changes in H. fossilis, underscoring its potential to disrupt intermediary metabolism and affect fish health.

5. Conclusion

The current study clearly shows that the organophosphorus insecticide methyl parathion has notable toxic effects on the freshwater fish *Heteropneustes fossilis*, even at concentrations that are not immediately lethal. The 96-hour LC_{50} value of 10.2 mg L^{-1} suggests that this species has a moderate sensitivity to the pesticide. Biochemical evaluations indicated that hepatic enzyme activities, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were altered in a manner dependent on both concentration and time, indicating cellular and metabolic disruptions. The increased activities of these transaminases point to liver dysfunction, membrane damage, and increased amino acid breakdown due to stress from the pesticide. The pattern of enzyme changes observed over the exposure periods suggests initial adaptive responses followed by irreversible tissue damage at higher concentrations or with extended exposure.

In summary, the results confirm that methyl parathion interferes with intermediary metabolism and impairs liver function in *H. fossilis*. These findings highlight the potential ecological dangers posed by the unregulated use of organophosphorus pesticides in aquatic settings. It is crucial to continuously monitor pesticide residues and enforce stricter regulations on agrochemical use to protect aquatic biodiversity and ecosystem health.

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