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TOXIC EFFECT OF ORGANOPHOSPHORUS INSECTICIDE – METHYL PARATHION ON ALT, AST& LDH ACTIVITY IN FRESH WATER FISH *HETEROPNEUSTES FOSSILIS*

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ABSTRACT

The freshwater fish *Heteropneustes fossilis* was exposed to sublethal concentrations (0.25, 0.5, and 1.0 mg L^{-1}) of the organophosphorus insecticide methyl parathion (MP) for exposure periods of 15, 30, and 45 days. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed a concentration-dependent increase, indicating tissue damage and muscular impairment resulting from MP exposure. Similarly, elevated LDH activity suggests enhanced lysosomal mobilization and cellular necrosis. These biochemical alterations clearly demonstrate that methyl parathion exerts toxic effects even at sublethal concentrations, disrupting intermediary metabolism and indicating significant physiological stress in *Hteropneustes fossilis.*

KEYWORDS: Methyl parathion, *Heteropneustes fossilis*, marker enzymes, sub lethal exposure.

INTRODUCTION

The use of pesticides, herbicides, and fungicides in India began in the mid-1960s and has since become a common feature of Indian agriculture. Although their application has contributed to increased agricultural productivity and profitability, it has also raised several concerns, including risks to human health, mortality among farm animals, alteration of local ecosystems, and long-term societal impacts. Prolonged, low-dose exposure to these agrochemicals has been increasingly associated with adverse human health effects such as immunosuppression, endocrine disruption, reduced cognitive function, reproductive abnormalities, and carcinogenesis (Agnihotri, 1999; Gupta, 2004). Methyl parathion, an organophosphorus insecticide, is widely used in agriculture, food storage facilities, pest control programs, and fish culture systems to eliminate aquatic insect larvae that pose threats to fish fry (Aguiar et al., 2004). Fish are widely recognized as effective bioindicators for assessing the health of aquatic ecosystems and for use in toxicological

evaluations (Law, 2003). Studies have demonstrated that fish and mammals exhibit similar toxicological and adaptive responses to oxidative stress, suggesting that piscine models can serve as useful systems for understanding the mechanisms of oxidative stress and toxicity.

Freshwater aquaculture accounts for approximately one-third of India's total fish production, with Indian major carps such as Labeo rohita (rohu), Catla catla, and Cirrhinus mrigala being the predominant species. Several investigations have been conducted on the biochemical, hematological, and cellular effects of organochlorine and organophosphorus pesticides on fish (Nath & Banerjee, 1996; Das & Mukherjee, 1997, 2000a,b, 2003; Rao, 2006). However, there is limited information on the specific effects of methyl parathion on Indian major carp L. rohita. Therefore, the present study was undertaken to determine the median lethal concentration (LC_{50}) and to evaluate the biochemical and enzymatic alterations in H.fossilis exposed to sublethal concentrations of methyl parathion.

MATERIALS AND METHODS

Chemicals

Methyl parathion (50% EC; O,O-dimethyl O-4-nitrophenyl phosphorothioate; Bayer, Germany), a synthetic organophosphorus insecticide, was obtained from the local market in Cochin, India. All other analytical-grade chemicals used in the experiments were procured from Sigma (USA), Merck (Germany), and SRL (India).

Experimental Fish

Healthy specimens of Heteropneustes fossilis weighing approximately 75 ± 6 g and measuring 23 ± 5 cm in length were collected from a fish farm at Mucharla Nagaram, near Kakatiya University, Warangal, Telangana, India. The fish were transported to the laboratory and acclimatized for more than 15 days in large plastic tanks containing dechlorinated tap water under continuous aeration. Water quality parameters such as temperature and pH were maintained at nearly constant levels throughout the acclimation and experimental periods. Fish were fed daily with commercial fish feed.

Determination of Lethal Concentration (LC_{50})

The lethal range bioassay was conducted following the standard procedures of APHA, AWWA, and WPCF (1975). Groups of eight fish were exposed to sequential 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 recorded at 24, 48, 72, and 96 hours, and dead fish were promptly removed.

For the determination of the median lethal concentration (LC_{50}), fish of similar size were exposed to different 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 described by Reish and Oshida (1987). Control fish were maintained separately under identical conditions without pesticide exposure. Mortality was recorded at 24-hour intervals up to 96 hours, and the 96-hour LC_{50} value was determined to be 10.2 mg L^{-1}.

Sublethal Exposure

Based on the LC_{50} value, sublethal concentrations corresponding to 1/40, 1/20, and 1/10 of the LC_{50} (0.25, 0.5, and 1.0 mg L^{-1}, respectively) were selected 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 fed regularly with commercial feed, and water was renewed periodically to maintain oxygen levels and chemical stability.

Sample Preparation

At the end of each exposure period, fish were sacrificed by decapitation, and liver tissues were excised immediately. 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 min at 4 °C (Remi, India), and the supernatant was used for enzyme assays.

Enzyme Assays

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

Statistical Analysis

All experiments were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and were approved by the Institutional Animal Ethics Committee. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine significant differences between control and treated groups (Daniel, 1987). Statistical analyses were performed using SPSS software (version 10.0 for Windows).

RESULTS AND DISCUSSION

The lethal range-finding test conducted with methyl parathion concentrations ranging from 0.002 to 20 mg L^{-1} for 96 hours revealed no mortality up to 2 mg L^{-1}, whereas 100% mortality was observed at 20 mg L^{-1}. These results indicated that the 96-hour median lethal concentration (LC_{50}) of Heteropneustes fossilis lies between 2 and 20 mg L^{-1} of methyl parathion.

A more detailed toxicity assay revealed no mortality up to 5.4 mg L^{-1}, while exposure to 7.2 mg L^{-1} for 96 hours resulted in 10% mortality. Complete mortality (100%) was recorded within 48 hours at 16.2 mg L^{-1}. Probit analysis indicated 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 weighing approximately 75 ± 6 g.

The toxicity of xenobiotic compounds such as pesticides varies with the size, age, and physiological condition of the test organism, as well as environmental parameters such as temperature. For instance, the LC_{50} of the organophosphorus pesticide RPR-II was reported as 0.17 mg L^{-1} for Oreochromis mossambicus(Rao, 2006). Similarly, 96-hour LC_{50} values for azinphosmethyl, parathion, and carbaryl were 7.18, 6.46, and 13.86 mg L^{-1}, respectively, for goldfish (Carassius auratus) of size 2–5 g (Ferrari et al., 2004). In Labeo rohita (rohu), a 96-hour LC_{50} of 0.139 mg L^{-1} was reported for the pyrethroid pesticide cypermethrin. These findings indicate that the susceptibility of fish to pesticides is influenced by species-specific and size-dependent factors.

Effect on Liver Enzymes

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the liver of H. fossilis increased significantly ($p < 0.05$) following exposure to sublethal concentrations of methyl parathion compared to control fish (Table 1). ALT activity showed a concentration-dependent rise at all exposure levels. On the 15th day of exposure, ALT activity was markedly elevated with increasing concentrations of methyl parathion (0.25–1.0 mg L^{-1}). A

gradual decline in ALT activity was observed by the 30th and 45th days, suggesting the initiation of adaptive or repair mechanisms during prolonged exposure to the xenobiotic.

At the highest sublethal concentration (1.0 mg L^{-1}), an initial reduction in ALT activity was observed up to 30 days, followed by a pronounced increase at 45 days, indicating progressive hepatic tissue damage under chronic exposure conditions.

Similarly, AST activity exhibited a significant increase in all treated groups compared to the control. The enhanced transaminase activities reflect hepatic dysfunction and leakage of cytoplasmic enzymes into circulation, suggesting cellular membrane damage and altered metabolic activity in response to methyl parathion toxicity.

Interpretation of Enzymatic Changes

Transaminases such as ALT and AST are vital enzymes in amino acid metabolism, serving as sensitive indicators of tissue damage and stress. The observed elevation in these enzyme activities following exposure to methyl parathion indicates disruption of hepatocellular integrity and increased amino acid catabolism. These findings are consistent with earlier reports on pesticide-induced biochemical alterations in fish (Das & Mukherjee, 2003; Rao, 2006).toxicity effect of methyl parathion on protein alterationsin the fish, labeo rohita (Anandan, P., Suresh Kumar, R., And Krishnamurthy, R, 2019)

The increased enzymatic activity can be attributed to pesticide-induced oxidative stress, which enhances the permeability of hepatic cell membranes, allowing leakage of enzymes into the extracellular fluid. Prolonged exposure and higher pesticide concentrations further intensify these effects, leading to irreversible damage to liver tissues.

Overall, the study demonstrates that methyl parathion, even at sublethal concentrations, causes significant biochemical and enzymatic alterations in H. fossilis, highlighting its potential to disrupt intermediary metabolism and compromise fish health.

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 heteropneustes fossilis.

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

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 heteropneustes fossilis.

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

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

RESULTS AND DISCUSSION

The lethal range-finding test conducted with methyl parathion concentrations ranging from 0.002 to 20 mg L^{-1} for 96 hours revealed no mortality up to 2 mg L^{-1}, whereas 100% mortality was observed at 20 mg L^{-1}. These results indicated that the 96-hour median lethal concentration (LC_{50}) of Heteropneustes fossilis lies between 2 and 20 mg L^{-1} of methyl parathion. A more detailed toxicity assay revealed no mortality up to 5.4 mg L^{-1}, while exposure to 7.2 mg L^{-1} for 96 hours resulted in 10% mortality. Complete mortality (100%) was recorded within 48 hours at 16.2 mg L^{-1}. Probit analysis indicated 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 weighing approximately 75 ± 6 g. The toxicity of xenobiotic compounds such as pesticides varies with the size, age, and physiological condition of the test organism, as well as environmental parameters such as temperature.

For instance, the LC_{50} of the organophosphorus pesticide RPR-II was reported as 0.17 mg L^{-1} for Oreochromis mossambicus (Rao, 2006). Similarly, 96-hour LC_{50} values for azinphosmethyl, parathion, and carbaryl were 7.18, 6.46, and 13.86 mg L^{-1}, respectively, for goldfish (Carassius auratus) of size 2–5 g (Ferrari et al., 2004). In Labeo rohita (rohu), a 96-hour LC_{50} of 0.139 mg L^{-1} was reported for the pyrethroid pesticide cypermethrin. These findings indicate that the susceptibility of fish to pesticides is influenced by species-specific and size-dependent factors.

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Interpretation of Enzymatic Changes

Transaminases such as ALT and AST are vital enzymes in amino acid metabolism, serving as sensitive indicators of tissue damage and stress. The observed elevation in these enzyme activities following exposure to methyl parathion indicates disruption of hepatocellular integrity and increased amino acid catabolism. These findings are consistent with earlier reports on pesticide-induced biochemical alterations in fish (Das & Mukherjee, 2003; Rao, 2006). The increased enzymatic activity can be attributed to pesticide-induced oxidative stress, which enhances the permeability of hepatic cell membranes, allowing leakage of enzymes into the extracellular fluid. Prolonged exposure and higher pesticide concentrations further intensify these effects, leading to irreversible damage to liver tissues. Overall, the study demonstrates that methyl parathion, even at sublethal concentrations, causes significant biochemical and enzymatic alterations in H. fossilis, highlighting its potential to disrupt intermediary metabolism and compromise fish health.

CONCLUSION

The present investigation clearly demonstrates that the organophosphorus insecticide methyl parathion exerts significant toxic effects on the freshwater fish Heteropneustes fossilis, even at sublethal concentrations. The 96-hour LC_{50} value (10.2 mg L^{-1}) indicates that the species is moderately sensitive to this pesticide. Biochemical assessments revealed concentration- and time-dependent alterations in hepatic enzyme activities, particularly alanine aminotransferase (ALT) and aspartate aminotransferase (AST), reflecting cellular and metabolic disturbances. The elevated transaminase activities suggest hepatic dysfunction, membrane damage, and enhanced amino acid catabolism under pesticide-induced stress. The observed pattern of enzyme fluctuations across exposure periods indicates both initial adaptive responses and subsequent irreversible tissue damage at higher concentrations or prolonged exposure.

Overall, the findings confirm that methyl parathion disrupts intermediary metabolism and compromises liver function in H. fossilis. These results underscore the potential ecological risks associated with the indiscriminate use of organophosphorus pesticides in aquatic environments. Continuous monitoring of pesticide residues and stricter regulation of agrochemical applications are essential to safeguard aquatic biodiversity and ecosystem health.

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