

Fluoride-Induced Hepatic Dysfunction in *Channa punctatus*: Dose-Dependent Alterations in Enzymatic Biomarkers

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www.jrasb.com || Vol. 3 No. 6 (2024): December Issue

Received: 06-12-2024

Revised: 17-12-2024

Accepted: 23-12-2024

ABSTRACT

Fluoride contamination of freshwater ecosystems is an emerging environmental concern due to its persistence, bioaccumulation, and toxic effects on aquatic organisms. Fish, being continuously exposed to aquatic pollutants, serve as sensitive bioindicators of fluoride-induced toxicity. The present study evaluates hepatotoxic effects of fluoride in *Channa punctatus* using enzymatic biomarkers, including glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), and acid phosphatase (ACP). Fish were exposed to two sub-lethal concentrations of fluoride under controlled laboratory conditions for a defined period. Enzyme activities were quantified in liver tissue to assess metabolic disruption, hepatocellular damage, and lysosomal activation. Results demonstrated a significant, dose-dependent increase in all enzymes compared to the control group. Transaminase elevation reflected impaired amino acid metabolism, whereas increased phosphatase activities indicated cellular degeneration and membrane damage. Behavioral changes, such as reduced swimming activity and surfacing, were observed in fluoride-treated groups. These findings confirm the suitability of multiple enzymatic biomarkers for early detection of fluoride toxicity. The study provides mechanistic insights into fluoride-induced biochemical disturbances and highlights their ecological relevance for biomonitoring in freshwater ecosystems.

Keywords- Fluoride toxicity; Biomarkers; GOT; GPT; ALP; ACP; *Channa punctatus*; Hepatotoxicity.

I. INTRODUCTION

Fluoride contamination in freshwater ecosystems has increased significantly due to industrial effluents, phosphate fertilizers, coal combustion residues, and domestic waste (Camargo, 2022). While fluoride is essential in trace amounts, chronic exposure to elevated concentrations results in severe toxicological effects in aquatic organisms. Fish, as primary inhabitants of freshwater systems, are particularly vulnerable to fluoride accumulation via gills, skin, and dietary intake, leading to bioaccumulation in metabolically active organs such as the liver.

The liver functions as a central organ for detoxification, metabolism, and biochemical regulation. Fluoride exposure disrupts enzyme systems, membrane integrity, and lysosomal stability. Biochemical biomarkers provide early and sensitive detection of sub-

lethal toxicity, even before visible histopathological alterations occur (Singh et al., 2023). Transaminases such as GOT and GPT indicate hepatocellular injury, whereas phosphatases such as ALP and ACP reflect membrane transport dysfunction and lysosomal activity. Although several studies have investigated fluoride toxicity in fish, integrated assessments combining both transaminases and phosphatases remain limited. The present study aims to evaluate fluoride-induced hepatotoxicity in *Channa punctatus* using a comprehensive enzymatic biomarker approach to provide a sensitive tool for freshwater environmental monitoring.

II. MATERIALS AND METHODS

Healthy freshwater fish *Channa punctatus* were collected from local water bodies and transported to the

laboratory under aerated conditions. Prior to experimentation, the fish were treated with 1% potassium permanganate solution to eliminate external infections and were acclimatized in glass aquaria for a period of two weeks. During acclimatization, the fish were maintained in chlorine-free freshwater with continuous aeration.

Physicochemical characteristics of the water were analyzed following standard procedures (APHA, 1998). The recorded parameters were: pH 6.9 ± 0.02 , temperature 26 ± 2 °C, dissolved oxygen 8.8 ± 2.5 mg L⁻¹, alkalinity 90–100 mg L⁻¹, and water hardness (as CaCO₃) 118–120 mg L⁻¹. The aquarium water was renewed on alternate days, and the fish were fed once daily *ad libitum* with dry fish and goat liver pieces.

For experimental exposure, fish were randomly divided into three groups, each consisting of ten individuals. Group I served as the control, while Groups II and III were designated as experimental groups and exposed to fluoride. Group II was subjected to a low fluoride concentration (30 mg L⁻¹), whereas Group III received a higher fluoride concentration (60 mg L⁻¹). Sodium fluoride (NaF; Excel R, India Limited, Mumbai) was used as the fluoride source. The exposure experiment was conducted for a duration of 90 days, with renewal of the exposure medium on alternate days. At the end of the exposure period, both control and fluoride-treated fish were sacrificed ethically and dissected for further biochemical analysis. Samples of the liver tissue were collected and analysed for the GOT, GPT, ALP and ACP parameters.

Enzymatic Assays

Liver tissues (100 mg) were homogenized in a phosphate buffer and centrifuged. Enzyme activities were measured in the supernatant using standard methods:

- **GOT & GPT:** Reitman and Frankel (1957)
- **ALP:** Kind and King (1954)
- **ACP:** Andersch and Szczypinski (1947)

Activities were expressed in units/ml. Statistical analysis was conducted using ANOVA and significance was accepted at $p < 0.05$ (Zar, 2010).

Observations

During the experimental period, fish exposed to sub-lethal concentrations of fluoride exhibited clear behavioral and physiological changes compared to the control group. Behavioral abnormalities included reduced swimming activity, increased surfacing, sluggish response to external stimuli, and decreased feeding, indicating systemic stress. Fish in the high fluoride group showed erratic swimming, often staying near the water surface for extended periods.

Morphological changes in liver tissues were also noted. In treated groups, the liver appeared pale, slightly enlarged, and softer compared to the firm, reddish-brown liver of control fish. No mortality occurred, confirming sub-lethal exposure. These behavioral and physiological observations correlated

with biochemical alterations, indicating that fluoride toxicity affects both the metabolic and functional physiology of *Channa punctatus*. The findings highlight the value of combining behavioral observations with biochemical biomarkers for comprehensive assessment of sub-lethal toxicity.

III. RESULTS

Chronic exposure to fluoride for 90 days induced significant alterations in hepatic enzymatic biomarkers of *Channa punctatus*. A **dose-dependent response** was observed in all parameters when compared with the control group.

Glutamic Oxaloacetic Transaminase (GOT)

GOT activity showed a significant increase in fluoride-treated groups. Fish exposed to 30 mg L⁻¹ fluoride exhibited a moderate but significant elevation ($p < 0.05$), whereas those exposed to 60 mg L⁻¹ showed a highly significant increase ($p < 0.01$) compared to control. The elevation of GOT activity indicates hepatic cellular damage and increased membrane permeability.

Glutamic Pyruvic Transaminase (GPT)

GPT activity followed a similar trend to GOT. A significant rise was recorded in both fluoride-exposed groups, with maximum elevation observed in the high-dose group (60 mg L⁻¹). The increased GPT activity reflects hepatocellular dysfunction and leakage of intracellular enzymes into the circulation.

Alkaline Phosphatase (ALP)

ALP activity showed a pronounced increase in fluoride-exposed fish. The increase was dose-dependent and statistically significant ($p < 0.01$) in the high-dose group. Elevated ALP activity suggests impairment of membrane transport processes and possible damage to bile canaliculi and liver architecture.

Acid Phosphatase (ACP)

ACP activity also increased significantly in fluoride-treated groups. The rise was more marked in the high-dose exposure group, indicating lysosomal membrane destabilization and enhanced catabolic activity in hepatic tissues.

Table 1. Effect of Fluoride on Hepatic Enzymatic Biomarkers in *Channa punctatus*

Enzyme	Concentration Of Fluoride		
	Control	Low Dose(30mg/L)	High Dose(60mg/L)
GOT (U/ml)	0.37 ± 0.008	0.46 ± 0.02	0.69 ± 0.03
GPT (U/ml)	0.35 ± 0.01	0.41 ± 0.02	0.57 ± 0.01
ALP (U/ml)	2.18 ± 0.05	2.74 ± 0.08	3.46 ± 0.10
ACP (U/ml)	1.64 ± 0.04	2.12 ± 0.06	2.89 ± 0.09

Values are mean ± SD; statistically significant at $p < 0.05$ – 0.001 .



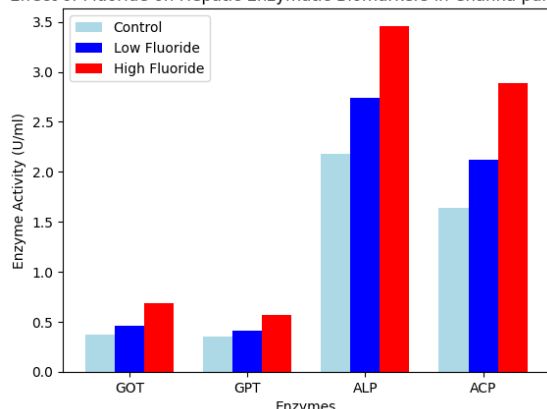
Effect of Fluoride on Hepatic Enzymatic Biomarkers in *Channa punctatus*

Figure 1

The bar graph illustrates dose-dependent increases in GOT, GPT, ALP, and ACP activities across control, low fluoride (30mg/L), and high fluoride groups (60mg/L). Maximum enzymatic activity was observed in the high fluoride group, confirming hepatotoxicity and dose-response relationships.

IV. DISCUSSION

The present study demonstrates that chronic fluoride exposure causes significant alterations in hepatic enzymatic biomarkers in *Channa punctatus*, indicating liver dysfunction. GOT and GPT are key transaminases involved in amino acid metabolism, and their elevated activities are widely recognized indicators of hepatocellular injury. Similar increases in transaminase activity following fluoride exposure have been reported in freshwater fish and mammals, suggesting membrane damage and enzyme leakage due to fluoride-induced toxicity (Chattopadhyay et al. 2011; Sharma et al. 2017).

The significant elevation of ALP observed in fluoride-exposed fish reflects disruption of membrane-bound enzyme systems and altered phosphate metabolism. Fluoride is known to interfere with enzymatic activity by forming complexes with metal cofactors, thereby affecting cellular integrity (Ghosh et al. 2002). Increased ALP activity has been associated with liver tissue degeneration and impaired biliary function under toxic stress.

ACP, a lysosomal enzyme, showed marked elevation in treated groups, particularly at higher fluoride concentration. Increased ACP activity suggests lysosomal destabilization and enhanced autophagic or degradative processes in hepatic cells. Similar observations have been reported in fish exposed to fluoride and other environmental pollutants (Singh and Tripathi 2015; Reddy et al. 2019).

Overall, the dose-dependent increase in GOT, GPT, ALP, and ACP activities confirms the hepatotoxic

potential of fluoride and highlights these enzymes as reliable biomarkers for assessing fluoride-induced stress in aquatic organisms.

V. CONCLUSION

The study demonstrates that sub-lethal fluoride exposure causes significant biochemical and physiological disturbances in *Channa punctatus*. Elevated GOT and GPT activities indicate hepatocellular injury, while increased ALP and ACP activities reflect membrane disruption and lysosomal activation. Behavioral changes, including reduced swimming activity and increased surfacing, corroborate enzymatic evidence of stress. The dose-dependent response observed in all biomarkers confirms their sensitivity for early detection of fluoride toxicity. Integrating enzymatic biomarkers with behavioral observations provides a comprehensive tool for assessing sub-lethal pollutant impacts in freshwater ecosystems. These findings support the application of biomarker-based monitoring in ecological risk assessment and provide a framework for protecting aquatic biodiversity from fluoride contamination.

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