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Effect of Fluoride Concentrations on Proximate Composition and Serum Biochemistry of *Labeo rohita* Across Varying Exposure Durations

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ABSTRACT

Purpose: This study investigates the impact of fluoride exposure on the biochemical and body composition parameters of fish over 20, 40 and 60 days.

Methods: Juvenile *Labeo rohita* were subjected to different fluoride concentrations (1.6 ± 0.05, 25.4 ± 1.06, 50.7 ± 1.43, and 75.4 ± 1.68 mg/L) for 20, 40, and 60 days under controlled laboratory conditions. Water quality parameters were maintained and monitored regularly. Serum biochemical parameters and proximate body composition were analyzed using standard methodologies.

Results: Significant dose- and time-dependent changes were observed in various biomarkers. Total protein levels declined progressively with increasing fluoride concentrations (50.7 mg/L and 75.4 mg/L), significantly influenced by exposure time ($p = 0.0002$, $F = 14.47$) and fluoride concentration ($p = 0.0030$, $F = 11.35$). Albumin levels decreased markedly at higher fluoride concentrations, particularly at 75.4 mg/L by day 60, with exposure time ($p < 0.0001$, $F = 65.50$) and fluoride concentration ($p = 0.0001$, $F = 28.90$) both having significant effects. Glucose levels fluctuated, showing a hypoglycemic response followed by hyperglycemia at higher fluoride levels ($p = 0.0008$, $F = 12.57$). Serum globulin also declined significantly at 50.7 mg/L and 75.4 mg/L ($p < 0.0001$, $F = 13.21$). Elevated fluoride exposure increased liver enzyme activities, including AST ($p = 0.0003$, $F = 14.79$) and ALT ($p = 0.0004$, $F = 16.11$), indicating liver damage. Body composition analysis revealed significant reductions in protein ($p < 0.0001$, $F = 35.64$) and lipid content ($p < 0.0001$, $F = 19.27$) at higher fluoride concentrations, while moisture content increased slightly ($p = 0.0800$) and ash content remained unchanged ($p = 0.1347$).

Conclusions: These findings emphasize the harmful impact of elevated fluoride levels on fish health and overall well-being.

Key-words: fluoride exposure; toxicity; biochemical parameters; body

composition; *Labeo rohita*

INTRODUCTION

Aquatic ecosystems are increasingly subjected to anthropogenic pollutants, with fluoride being a significant contaminant due to industrial and agricultural activities [1, 2]. Fluoride, an essential trace element at low concentrations, is beneficial for the physiological and skeletal development of aquatic organisms. However, elevated levels of fluoride in water bodies have raised serious concerns about its potential toxicity [3]. Excessive fluoride exposure can disrupt normal metabolic processes by altering enzymatic activity, impairing energy metabolism, and inducing oxidative stress, leading to adverse effects on aquatic species, particularly fish, which are integral components of aquatic food chains and an important source of protein for humans [4]. Among freshwater species, *Labeo rohita* (rohu) holds a prominent place due to its high nutritional value, widespread aquaculture production, and economic importance in South Asia [5]. Studying the effects of fluoride on this species provides insights into how water quality impacts aquaculture productivity and fish health.

Fluoride contamination in aquatic environments arises from diverse sources, including industrial effluents, phosphate fertilizers, aluminum smelting, and coal combustion [6]. Additionally, natural deposits of fluoride in rocks can leach into water systems, further exacerbating the problem [7]. In some regions, fluoride concentrations in water far exceed the acceptable limits set by environmental and health organizations, posing a threat not only to aquatic organisms but also to human health through bioaccumulation and biomagnification [8]. Fish, being highly sensitive to waterborne pollutants, serve as bioindicators of environmental contamination [9]. Changes in their biochemical parameters, proximate composition, and overall physiological responses provide critical information about pollutant-induced stress and toxicity mechanisms [10, 11].

The proximate composition of fish is a direct measure of their nutritional quality and health status [5]. Fluoride exposure has been reported to alter these parameters by disrupting metabolic pathways, leading to reduced growth, poor body condition, and compromised market value [12]. Similarly, serum biochemical parameters are reliable indicators of physiological and metabolic changes in fish [13]. Elevated or suppressed levels of these parameters can signal stress, organ dysfunction, and overall health deterioration caused by fluoride toxicity [14]. Numerous studies worldwide have investigated the effects of microplastics, pesticides and other contaminants on human and aquatic organisms [15, 16]; however, there is a lack of comprehensive research focusing on the effects of different fluoride concentrations and exposure periods on fish health. Thus this study evaluates the effects of different fluoride concentrations on the proximate composition and serum biochemical parameters of juvenile *L. rohita* at various exposure durations.

MATERIAL AND METHODS

Experimental design

In this study, we obtained 80 health juvenile *L. rohita* of length (11.76 ± 0.25 cm) and weight (18.0 ± 0.32 g) from local fish farm. They were kept in glass aquaria in the laboratory for 14 days to acclimate to laboratory conditions prior to exposure. They were fed twice a day (morning and evening) with commercially available food (4% of their body weight) during acclimation and exposure period. After the acclimation period, they were divided into groups and exposed to different concentration of fluorides for 60 days such as 1.6 ± 0.05 mg/L (control), 25.4 ± 1.06 mg/L, 50.7 ± 1.43 mg/L and 75.4 ± 1.68 mg/L. During this period, the water quality parameters were measured after every three days such as temperature (24.6 °C), pH (6.92), hardness ($CaCO_3$: 18.89 mg/L), dissolved oxygen (5.2 mg/L). Water was renewed weekly, and aquaria were thoroughly cleaned. The fluoride concentration in each tank was monitored daily,

using the fluoride ion selective electrode method. Measured fluoride concentrations remained stable between weekly water renewals. After 20 days, 40 days, and 60 days of exposure, 15 juvenile fish were randomly selected from each group to determine the body chemical composition. Fish blood samples were collected from the caudal vein using a syringe. After clotting at 48 °C, the blood was centrifuged at 2800 g for 5 min at room temperature, and the collected serum was stored at 20.0 °C for later analysis [17].

Determination of proximate body composition

The body composition of juvenile *L. rohita* were determined after collection of 15 fish from each treatment and control, then they were slaughtered. Protein, lipid, moisture and ash were determined by following the method of AOAC [18].

Determination of biochemical parameters

Blood sampling were done from both the control and treated fish at different exposure periods (After 20 days, 40 days, and 60 days). The fish were fasted for at least 24 hours before sampling. To ensure the fish were unconscious and minimize stress during handling, MS-222 (dose 40 mg/l) was used. Blood was swiftly collected from the caudal vein using a sterile syringe, shortly after the fish were rendered unconscious. serum total protein, albumin, glucose, globulin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using commercially available kits, including those from Thermo Fisher Scientific.

Data analysis

The statistical analysis of the study was conducted using a two-way analysis of variance (ANOVA) to assess the effects of exposure time and fluoride concentration on various biochemical parameters and body composition in fish. Further line graphs were used to assess the trend of the parameters under the influence of fluoride. All statistical analysis was carried out using GraphPad Prism (version: 10.4.1).

RESULTS

Table 1 shows findings for *L. rohita* serum biochemical analysis, it was found that protein, albumin and globulin followed the similar pattern and results revealed the exposure time (protein: $p=0.0002$, $F = 14.47$; albumin: $p < 0.0001$, $F = 65.50$; globulin: $p = 0.0003$, $F = 11.66$), fluoride concentration (protein: $p=0.003$, $F=11.35$; albumin: $p = 0.0001$, $F = 28.90$; globulin: $p < 0.0001$, $F = 13.21$) and the interaction between exposure time and fluoride concentration (protein: $p=0.0077$, $F=3.419$; albumin $p < 0.0001$, $F = 11.01$; globulin: $p = 0.0470$, $F = 2.220$) were significant. Further Figure 1 shows that at 1.6 mg/L, total protein, albumin and globulin remained stable throughout the study, indicating no significant effect. However, at 50.7 mg/L and 75.4 mg/L, a progressive decline was observed, with the most pronounced reduction at 75.4 mg/L after 60 days. For serum glucose exposure time ($p = 0.0008$, $F = 12.57$) and interaction between exposure and concentration ($p = 0.0001$, $F = 5.66$) was again a significant factor. Although the effect of fluoride concentration was not statistically significant ($p = 0.1$). Interestingly, at higher concentrations, glucose levels showed a marked decrease at 20 and 40 days, followed by a sharp increase at 60 days, particularly at 75.4 mg/L.

According to the Table 1, for AST and ALT exposure time ($p = 0.0003$, $F = 14.79$; $p = 0.0004$, $F=16.11$) and fluoride concentration ($p = 0.0111$, $F = 7.315$; $p=0.0007$, $F = 17.88$) both had significant effects. The interaction effect in both cases was also significant (AST: $p=0.0110$, $F = 3.196$; ALT: $p = 0.0105$, $F = 3.223$). Figure 1e and 1f shows that at 1.6 mg/L, enzyme levels remained stable, indicating no apparent liver stress. However, at 50.7 mg/L and 75.4 mg/L, both AST and ALT levels increased progressively over time, with the highest values observed at 75.4 mg/L after 60 days.

Detail results of the fish body composition is given in the Table 2. According to the results effect of exposure time, concentration of fluoride and interaction between both protein and lipids content is highly significant, with a p-value (0.0001), Protein and lipids levels decline significantly over time at higher fluoride concentrations (50.7 mg/L and 75.4 mg/L) (Figure 2), with the decline being more pronounced at 75.4 mg/L. At lower concentrations (1.6 mg/L and 25.4 mg/L), protein content stabilizes or exhibits minimal decline over the exposure period. Moisture and ash content

showed no significant changes, though they increased slightly over time, with higher fluoride concentrations (50.7 mg/L and 75.4 mg/L) causing a more pronounced rise.

Table 1. Two-way ANOVA results showing the effects of exposure time, fluoride concentration, and their interaction on biomarkers in juvenile *Labeo rohita*. Statistically significant P-values (≤ 0.05) are highlighted for each factor and biomarker.

	Total Protein		**Albumin**		**Glucose**		**Globulin**		**AST**		**ALT**	
Fixed effects (type III)	P value	F (DFn, DFd)	P value	F (DFn, DFd)	P value	F (DFn, DFd)	P value	F (DFn, DFd)	P value	F (DFn, DFd)	P value	F (DFn, DFd)
Exposure Time	0.0002	F (2.160, 17.28) = 14.47	<0.0001	F (1.862, 14.90) = 65.50	0.0008	F (1.594, 17.00) = 12.57	0.003	F (2.031, 21.66) = 11.66	0.0003	F (1.870, 14.96) = 14.79	0.0004	F (1.694, 13.55) = 16.11
Fluoride concentration	0.003	F (3, 8) = 11.35	0.001	F (3, 8) = 28.90	0.1141	F (3, 32) = 2.144	<0.0001	F (3, 32) = 13.21	0.0111	F (3, 8) = 7.315	0.007	F (3, 8) = 17.88
Exposure Time x Fluoride concentration	0.007	F (9, 24) = 3.419	<0.0001	F (9, 24) = 11.01	0.0001	F (9, 32) = 5.669	0.0470	F (9, 32) = 2.220	0.0110	F (9, 24) = 3.196	0.0105	F (9, 24) = 3.223

Table 2. Two-way ANOVA results showing the effects of exposure time, fluoride concentration, and their interaction on the body composition of juvenile *Labeo rohita*. Statistically significant P-values (≤ 0.05) are highlighted for each factor and nutritional parameter.

	Crude protein		**Crude lipid**		**Moisture**		**Ash**	
Fixed effects (type III)	P value	F (DFn, DFd)	P value	F (DFn, DFd)	P value	F (DFn, DFd)	P value	F (DFn, DFd)
Exposure Time	<0.0001	F (2.178, 23.23) = 35.64	<0.0001	F (2.332, 24.88) = 19.27	0.0800	F (1.690, 18.02) = 3.037	0.1347	F (1.991, 21.24) = 2.208
Fluoride concentration	<0.0001	F (3, 32) = 19.48	<0.0001	F (3, 32) = 22.70	<0.0001	F (3, 32) = 25.50	0.3091	F (3, 32) = 1.247
Exposure Time x Fluoride concentration	0.0047	F (9, 32) = 3.426	0.0010	F (9, 32) = 4.298	0.5923	F (9, 32) = 0.8319	0.9982	F (9, 32) = 0.1357

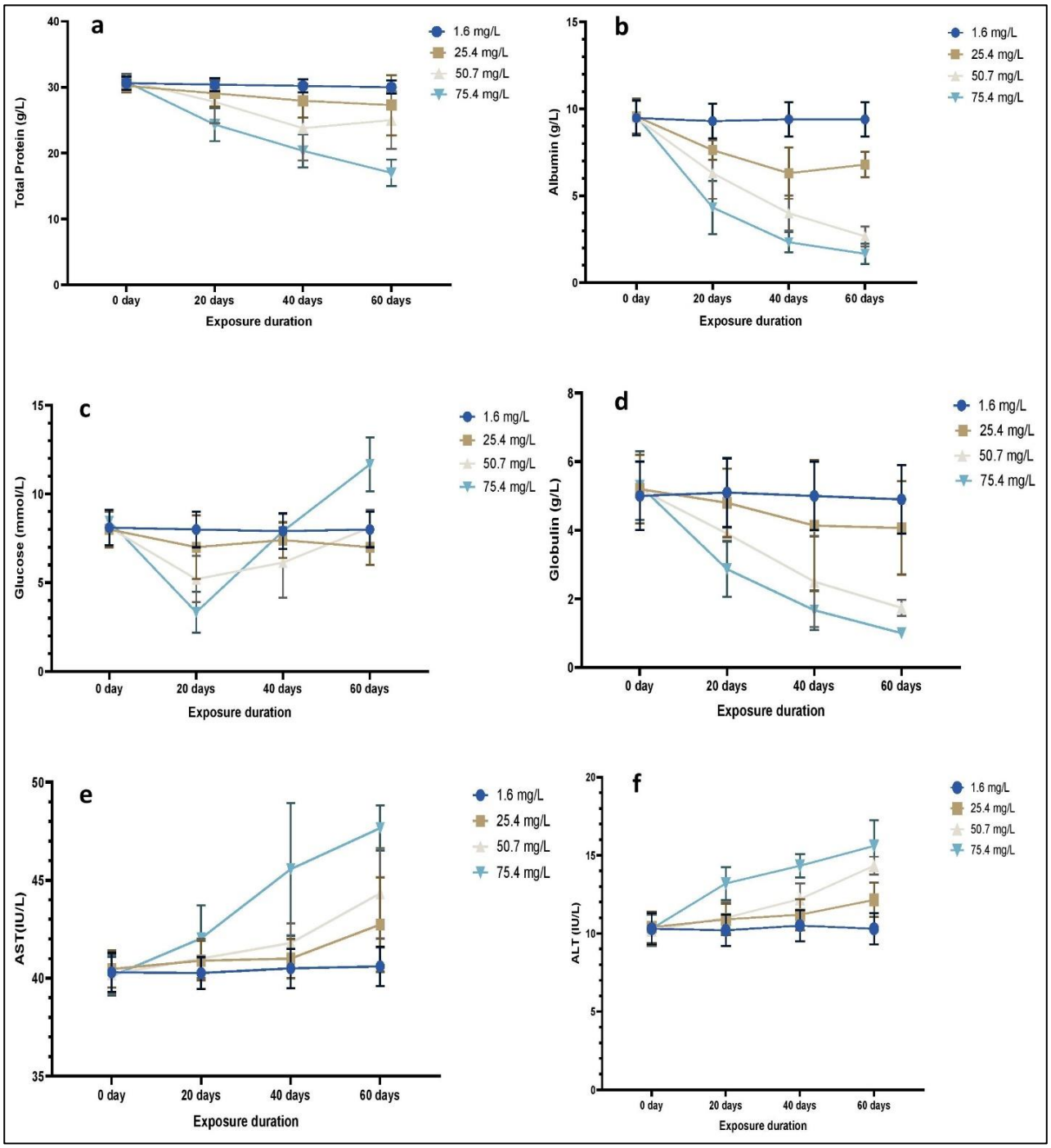

Figure 1. Temporal changes in various biomarkers across different fluoride concentrations over time: (a) Total protein levels, (b) Albumin levels, (c) Glucose levels, (d) Globulin levels, (e) AST (aspartate aminotransferase) activity, and (f) ALT (alanine aminotransferase) activity. The results illustrate the time- and concentration-dependent effects of fluoride exposure on these biomarkers.

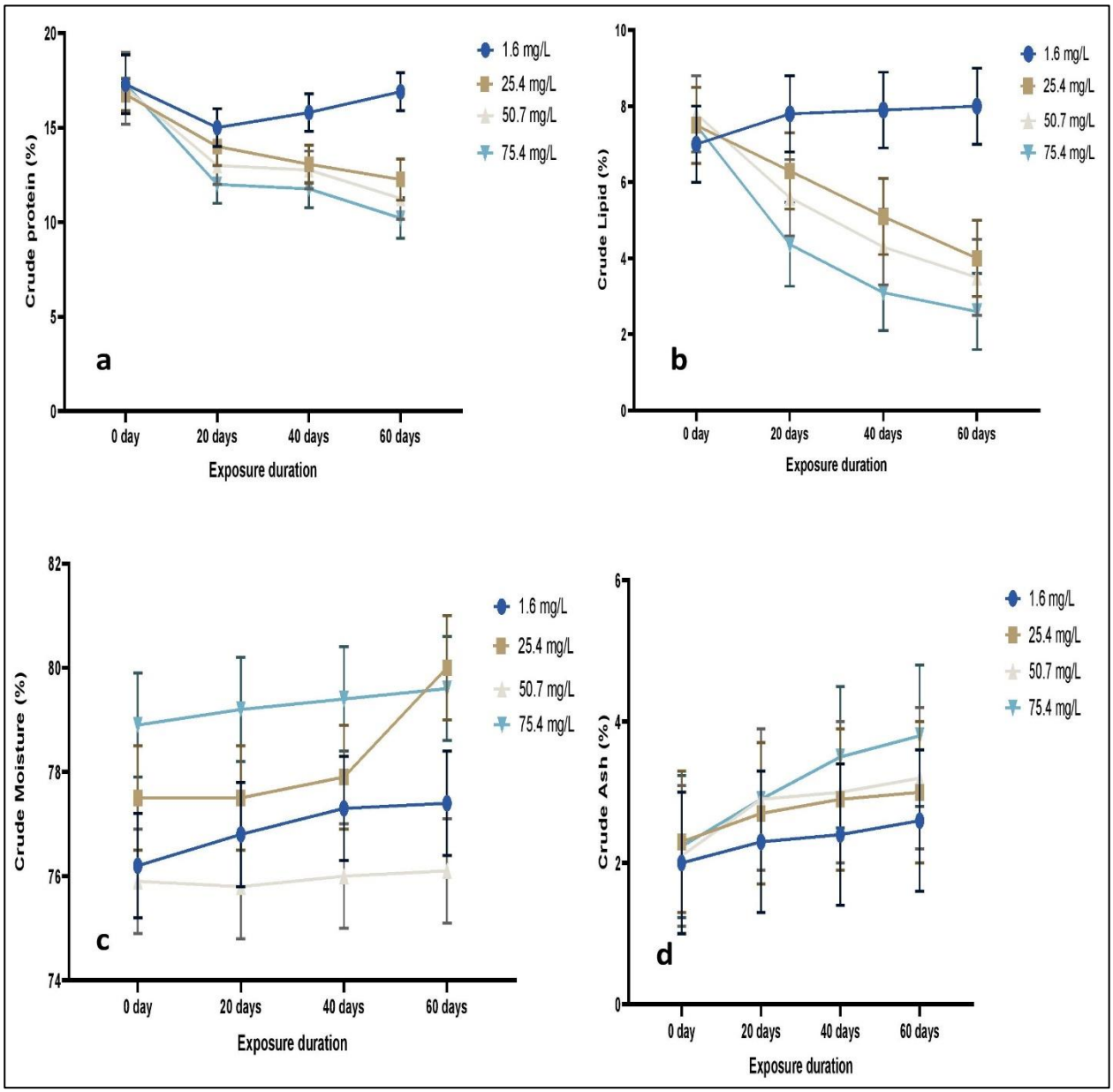

Figure 2. Changes in the body composition of *Labeo rohita* across different exposure durations and fluoride concentrations: (a) Crude protein, (b) Crude lipid, (c) Moisture level, and (d) Ash content. The results highlight the effects of exposure duration and fluoride concentration on the nutritional composition over time.

DISCUSSION

Investigating fluoride toxicity in fish is crucial to understanding the ecological and health impacts of environmental fluoride contamination [3]. Serum biochemical analysis provides valuable insights into the physiological and metabolic disturbances caused by fluoride exposure, including alterations in enzyme activity, electrolyte imbalances, and fluctuations in biomarkers indicative of organ function [19, 20]. Simultaneously, body composition analysis identifies alterations in growth, nutrient reserves, and overall health, providing a comprehensive assessment of fluoride's toxic effects [21]. This study aims to evaluate the effects of fluoride toxicity on juvenile *L. rohita* by analyzing serum biochemical parameters and body composition. In this study, significant decline in total protein observed over time and at higher fluoride concentrations reflects disruptions in protein metabolism. High fluoride levels may impair protein synthesis or induce protein degradation, potentially due to liver dysfunction or oxidative stress [22]. Proteins play critical roles in maintaining osmotic balance, blood circulation, and metabolic stability, making them sensitive indicators of fluoride-induced physiological stress [21, 23]. Studies, such

as those by Raposo et al. [24] and Fishta et al. [25], demonstrate how exposure time and fluoride concentration interact to exacerbate protein loss. Similar findings in other studies like Ibrahim et al. [26] and Al-Asgah et al. [27] linked heavy metal exposure to reduced plasma proteins, primarily due to oxidative stress and impaired hepatic function. Albumin, a key liver-synthesized protein, also declines significantly under stress, highlighting its sensitivity to liver dysfunction. The interplay of fluoride concentration and exposure duration further exacerbates albumin reduction. Research by Javed and Usmani [28] and Firat and Kargin [29] illustrates how heavy metal exposure can disrupt protein turnover and synthesis, emphasizing albumin as a reliable biomarker for assessing environmental stress in fish. Glucose levels show dynamic fluctuations under fluoride exposure, with significant effects from time and interaction with concentration. Initial hypoglycemia may result from stress-induced energy utilization, followed by hyperglycemia as a compensatory mechanism, reflecting metabolic dysregulation [30]. Study by Wijaya et al. [31], confirm that fluoride disrupts glucose homeostasis by inducing oxidative stress, impairing glycogenolysis, and affecting hormonal regulation. Globulin levels, indicative of immune function, also decline under chronic fluoride exposure. Reduced globulin levels in this study suggest weakened immune responses and disruptions in protein metabolism, aligning with findings from Cao et al. [32]. The observed decline points to cumulative stress and compromised disease resistance in fluoride-exposed fish.

Results of this study revealed that the fluoride exposure significantly decreases protein and lipid content in fish tissues, particularly at higher concentrations. Lipid reductions result from oxidative stress-induced lipid peroxidation, energy redistribution during stress, and disruptions in lipid synthesis pathways. Studies by Johnston and Strobel [33] and Chen et al. [34] corroborate these findings, highlighting the broad metabolic impacts of fluoride and heavy metal exposure. While ash and moisture content in *L. rohita* remain relatively stable, both exhibits increasing trends over time and with higher fluoride concentrations. Ash content reflects fluoride accumulation in calcified tissues, such as bones and scales, while moisture content increases due to impaired cellular integrity and water retention [35].

CONCLUSIONS

The study highlights the toxic effects of elevated fluoride levels on fish, with dose- and time-dependent impacts on biochemical parameters, liver function, and body composition. Significant reductions in protein, lipid content, and immune function were observed at higher concentrations, indicating metabolic and physiological disruptions. These findings underscore the need for stricter regulation of fluoride levels in freshwater environments to protect aquatic life, maintain ecosystem balance, and inform sustainable aquaculture practices and environmental management strategies.

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CONFLICT OF INTERESTS

None

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