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Acute and Chronic Fluoride Exposure Impairs Blood Physiology, Immunity, and Metabolic Disruptions in *Labeo rohita*

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ABSTRACT

This study aimed to evaluate the toxic effects of sodium fluoride (Na-F) on *Labeo rohita* (rohu), a freshwater species. Eighty fish (100–150 g) were acclimated for 15 days under controlled conditions (pH 7.5 ± 0.04, dissolved oxygen 8.11 ± 0.12 mg/L, temperature 22.81 ± 0.31 °C) and exposed to Na-F at 0 (control), 12.5, 15, and 17.5 mg/L for 45 days. Hematological analysis revealed severe reductions in red blood cells (RBC) (3.13 ± 0.05 × 10^6/mm^3), hemoglobin (Hb) (7.23 ± 0.33 g/dL), and hematocrit (Hct) (26.70 ± 0.7%) in the 17.5mg/ L group ($P< 0.05$). Immunological disruptions included elevated white blood cells (WBCs) (20.13 ± 0.43 × 10^3/mm^3) and neutrophils (21.32 ± 0.70%) alongside decreased lymphocytes (12.12 ± 1.51%) ($P< 0.05$). Serum biochemistry showed hypoglycemia (75 ± 2.34 mg/dL), hypercholesterolemia (167.6 ± 1.16 mg/dL), reduced albumin (1.3 ± 0.08 mg/dL), and increased serum lactate dehydrogenase (LDH) (303.3 ± 3.9 IU/L). Organ weights reduced significantly, particularly gills (1.4 ± 0.04 g, $P< 0.05$). The findings highlight Na-F's, alerting hematological, metabolic, and immune functions in *L. rohita*, necessitating stricter fluoride regulation in aquatic environments

INTRODUCTION

Fluoride contamination in freshwater ecosystems has become an increasing environmental concern due to its dual origin from natural geological weathering and anthropogenic discharges such as aluminium smelting, phosphate fertilizer production, and coal combustion **(Zuhra *et al.*, 2024; Kamruzzaman *et al.*, 2025; Kim *et al.*, 2025)**. Fluoride is an exceptionally mobile inorganic toxin that enters fish bodies through the skin

or the gills. The presence of oceanic sediments, temperature, and pH impacts bioaccumulation **(Piero *et al*., 2014)**.

Salinity concentrations alter the physical and chemical properties of water, which, in turn, reflect on the biological properties of the organisms living in the water **(Hassan *et al*., 2024)**. Elevated fluoride levels have been shown to persist in aquatic systems, accumulate in biota, and disrupt the physiological and immune functions of fish, making them effective bioindicators of environmental stress **(Dippong *et al*., 2024; Ghosh *et al*., 2024; Ikpesu *et al*., 2025; Namiq *et al*., 2025)**. Chronic exposure, even at sublethal concentrations, can impair growth, provoke oxidative stress, and increase susceptibility to infections **(Hamed *et al*., 2025; Potiris *et al*., 2025)**. As freshwater fish are vital to ecological balance and human nutrition, understanding fluoride's toxico-dynamics and developing mitigation strategies is essential for sustainable aquaculture and environmental health **(Ahmad *et al*., 2025; Rind *et al*., 2025)**.

Among cultured freshwater species, *Labeo rohita* (rohu) is particularly susceptible to fluoride toxicity due to its prolonged interaction with contaminated water and benthic substrates **(Ahuekwe *et al*., 2023; El-Bouhy, 2024)**. Fluoride exposure in this species has been linked to oxidative stress, metabolic disruption, apoptosis, and immune suppression, which collectively impair growth, behavior, and survival **(Li *et al*., 2022; Mukherjee *et al*., 2025)**. Early signs of toxicity often include erratic swimming, respiratory difficulty, and surface gasping, followed by hematological disturbances and alterations in vital organs **(Ribeiro *et al*., 2024; Sadiqa *et al*., 2024; Hassan *et al*., 2025)**. At the cellular level, fluoride induces excessive generation of reactive oxygen species (ROS), leading to oxidative damage that overwhelms antioxidant defences, promotes lipid peroxidation, and compromises DNA integrity **(Kumar *et al*., 2024; Zhang *et al*., 2024)**.

Yadav *et al*. (2014) reported that the exposure to fluoride acts as a metabolic inhibitor by competitively binding to and disrupting the activity of key enzymes involved in nutrient metabolism. In *Heteropneustes fossilis*, chronic fluoride exposure induced significant alterations in hepatic enzyme function and histoarchitectural organization. These changes included the degeneration of hepatocytes, inflammatory infiltrates, and fibrosis, indicating compromised detoxification and metabolic homeostasis **(Owais *et al.*, 2025a)**.

Fish exposed to fluoride have been shown to elicit significant hematological and immunological disruptions. A consistent decline in red blood cell counts (RBCs) and hemoglobin (Hb) concentration compromises the oxygen-carrying capacity of blood, leading to impaired physiological function **(Grădinariu *et al*., 2024; El-Houseiny *et al*., 2025)**. Simultaneously, leukopenia and increased lymphocyte apoptosis weaken immune defences, enhancing susceptibility to pathogenic invasion **(Radwan *et al*., 2023; Samah *et al*., 2023)**. Fluoride's systemic toxicity is further evidenced by hepatic and renal dysfunction, reflected in elevated serum biomarkers such as glucose, cholesterol, and lactate dehydrogenase (LDH), alongside structural damage to vital organs **(Cao *et al*.,**

2015; Saha *et al*., 2024). Chronic fluoride exposure also compromises mucosal and lymphoid immune barriers. This is substantiated by histopathological findings of cellular apoptosis in the spleen and thymus, inflammatory infiltration, and suppression of antioxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, indicative of oxidative stress and immunosuppression **(Bacou *et al*., 2021; Dezfuli *et al*., 2023)**. This research aimed to determine the organ weights, hematological, immunological, and serum biochemical parameters in *Labeo rohita* after exposing it to different concentrations of Na-F for 45-day period.

MATERIALS AND METHODS

Transport and acclimatization

Eighty rohu fish *L. roita* of a similar age group, with a mean weight of 100-150g, were captured and transferred in a plastic bag containing water and oxygen from the Bahawalpur Fishing Complex to the laboratory in the Islamia University of Bahawalpur. Fish were acclimated to laboratory conditions for fifteen days before the experiment started.

Disinfection and feeding

Labeo rohita specimens were submerged with 0.1% potassium permanganate ($KMnO_4$) solution purchased from Sigma-Aldrich for 1-2 minutes to kill the pathogens **(Allan *et al*., 2000)**. During acclimatization, fish were given a basal diet (Table 1) twice over 24 hours **(Terova *et al*., 2018; Mohamed *et al*., 2020; Owais *et al*., 2024; Hassan *et al*., 2025)**.

Table 1. Ingredient composition of *L. rohita* exposed to different concentrations of sodium fluoride at 15-day intervals

Ingredient	A (0 mg/l/day)	B (12.5mg/l/day)	C (15 mg/l/day)	D (17.5mg/l/day)
Fish Meal	16	16	16	16
Corn	14	14	14	14
Soybean Meal	30	30	30	30
Barley	17	17	17	17
Wheat	20	20	20	20
Premix	2	2	2	2
Ascorbic acid	1	1	1	1

Notes: The vitamin premix was formulated to meet the essential micronutrient requirements of *L. rohita*, ensuring optimal growth, immune function, and overall health. Each kilogram of diet contained the following vitamins: Vitamin B_1 (Thiamine): 1.5 mg, Vitamin B_2 (Riboflavin): 10 mg, Vitamin B_3 (Niacin): 55 mg, Vitamin B_5 (Pantothenic Acid): 40 mg, Vitamin B_6 (Pyridoxine): 8 mg, Vitamin B_7 (Biotin): 1.8 mg, Vitamin B_9 (Folic Acid): 0.08 mg, Vitamin K (Phylloquinone): 4.5 mg, Vitamin A (Retinol): 7200 IU, Vitamin E (α-Tocopherol): 220 IU, Vitamin C (Ascorbic Acid): 90 mg. The vitamin mix was purchased from trusted suppliers, including BioNutra Pakistan (Lahore, Pakistan) and AquaVita Industries (Karachi, Pakistan).

Experimental and feeding design

Initial body weight was recorded before the experiment began, and fish were randomly distributed into eight glass aquarium tanks (dimensions: 140×35×52cm³, capacity: 100 liters) to evaluate four experimental groups, each with duplicate replicates (10 fish per tank). All groups were exposed to different Na-F concentrations purchased from the Sigma company with CAS#7681-49-4, product code SB0850.00. The first group was used as a control (A) and was not exposed to Na-F. The fish in the second group (B) were exposed to 12.5mg/ L, and in the third group (C), fish were exposed to 15mg/ L, while fish in the fourth group (D) were exposed to 17.5mg/ L for 45 days, during which daily siphoning was conducted to remove uneaten feed and waste and ensure optimal water quality.

Physicochemical parameters

Continuous aeration was provided across all experimental tanks using a combination of aquarium air pumps (Luckiness 828 model; 5w power output, 3.5L/ min airflow capacity). The compressor models included Hailea ACO-318 (45W, 70L/ min), Hailea ACO-328 (55W, 82 L/min), and Resun ACO-010 (200W, 0.135 m³/min) units. Water quality parameters were monitored daily and maintained within optimal ranges throughout the experimental period. The Mean ± SEM for parameters were: pH 7.5 ± 0.04, dissolved oxygen 8.11 ± 0.12mg/L, chloride mg/L11.10± 0.04, potassium 1.87 ± 0.30 mmol/L, and water temperature 22.81 ± 0.31°C **(Al Sulivany *et al*., 2024; Abdulrahman & Al Sulivany, 2025; Asad *et al*., 2025; Das *et al*., 2025; Omar & Al Sulivany, 2025; Owais *et al*., 2025b)**.

Clinical signs and behavioral changes

Throughout the experimental investigation, clear observations included respiratory distress (gasping, rapid operculum movement, surface breathing), neuromuscular dysfunction (jerking, convulsions, body unbalancing, fin tilting), and abnormal locomotory patterns (surface running, static positioning, faintness). These manifestations intensified with prolonged exposure, suggesting dose- and time-dependent toxicity. Respiratory anomalies indicated hypoxia or gill impairment, while erratic movements pointed to potential neurotoxicity.

Hematological and immunological parameters

All fish's blood samples were taken on the 15th, 30th, and 45th days after being collected from the caudal vein using a 3ml sterile syringe. The samples were divided into two portions: one transferred into tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation for hematological analysis, and the other into plain tubes without anticoagulants for immunological assessments.

Hematocrit levels were determined through the micro-hematocrit technique **(Gallaugher & Farrell, 1998)**. The RBCs and WBCs were quantified using a hemocytometer and the Neubauer counting chamber method, following the protocol of

Blaxhall and Daisley (1973). The Hb concentration was measured according to **Wedemeyer (1977)**. Blood indices, the MCV, MCH, and MCHC, were calculated using the following formulas:

$$MCV = \frac{10 \times PCV}{RBCs}, \qquad MCH = \frac{10 \times Hb}{RBCs}, \qquad MCHC = \frac{100 \times Hb}{PCV}$$ **(Arsalan *et al*., 2016)**.

For immunological assessments, blood smears were prepared from the samples to count leucocytes, with lymphocytes, monocytes, eosinophils, basophils, and neutrophils quantified using the Neubauer differential counting method.

Biochemical analysis

For biochemical and enzyme activity measurements, the blood was withdrawn from the fish, centrifuged at 3000 rpm for 5 minutes to get serum, and stored at -20ºC for further analysis **(Al-Turaihi *et al*., 2024)**. The blood sample was subjected to the following examinations. The biochemical indicators included glucose, albumin, cholesterol, and triglycerides. Enzyme activity indicators included Lactate dehydrogenase (LDH). Serum LDH was estimated colourimetrically by using an auto analyzer spectrophotometer (Lisa Xs-French) according to the method of **Zimmerman and Henry (1979)** and **Hamdy *et al*. (2018)**, additionally serum glucose was estimated according to the method of **Tietz (1986)**. Moreover, albumin was estimated using the method of **Doumas *et al*. (1971)**. Serum total cholesterol and triglycerides: serum total cholesterol and triglycerides were determined according to the methods of **Tietz (1995)**.

Average and relative weight of the intestine and gills

After blood collection, fish (three per tank) were caught by a dip net, killed by a sharp blow on the head, and dissected by scissors. The whole intestine and gills were carefully cut and measured by an automatic balance to determine the average and relative weight of these organs.

Statistical analysis

The data were analyzed using a completely randomized design ANOVA one-way repeated measure. The software statistical package for the social sciences (SPSS) v.22 was used to compare the four experimental groups. Duncan's multiple range test mean comparisons were used for post hoc comparisons. A $P< 0.05$ was used as the indicator for statistical significance.

RESULTS

Fish in the control groups exhibited no signs of behavioral or physiological distress throughout the study. In contrast, treatment groups exposed to different concentrations at Na-F; 12.5, 15, and 17.5mg/ L displayed multiple abnormalities, including irregular

respiration (gulping, surface gasping), erratic movement (jolting, sluggish or rapid swimming), loss of equilibrium (body unbalance, fin tilting), convulsions, and prolonged immobility (static posture). The severity of these symptoms increased with higher fluoride concentrations, particularly in groups C and F. During the initial 15-day period, groups A to C showed normal behavior, while group D developed slight irregularities. Between 15 and 30 days, groups A and B remained unaffected, whereas groups C and D exhibited mild to moderate symptoms.

The hematological analysis of *L. rohita* exposed to Na-F revealed dose- and time-dependent alterations, which are presented in Table (2). In the control group, the RBC count remained stable throughout the experiment (4.40 ± 0.31, 4.51 ± 0.53, and 4.53 ± 0.44 × 10^6/mm^3 at days 15, 30, and 45). However, groups exposed to Na-F showed progressive declines. At day 15, group B (12.5 mg/l/day) exhibited a slight reduction (4.31 ± 0.31 × 10^6/mm^3), while groups C (15 mg/l/day) and D (17.5 mg/l/day) displayed more pronounced decreases (4.18 ± 0.11 and 4.08 ± 0.06 × 10^6/mm^3, respectively). By day 45, RBC counts in groups C and D dropped significantly ($P< 0.05$) to 3.17 ± 0.22 and 3.13 ± 0.05 × 10^6/mm^3, respectively

The Hb levels followed a similar trend. The control group maintained stable Hb, whereas the exposed groups showed progressive declines. By day 45, Hb in group D fell to 7.23 ± 0.33 g/dL ($P < 0.05$). Hematocrit (Hct) mirrored these changes, with group D exhibiting the lowest values (26.70 ± 0.7% at day 45, $P< 0.05$). MCV and MCH also decreased significantly in the exposed groups. For instance, MCV in group D reduced from 46.25 ± 0.55 fl (day 15) to 17.05 ± 2.69 fl (day 45). Similarly, MCH in group D diminished from 45.95 ± 0.75 pg to 16.9 ± 2.93 pg.

The immunological parameters of *rohu* are presented in Table (3). In the control group (A), the WBCs remained relatively stable across days 15 (15.02 ± 0.02 × 10^3/mm^3), 30 (14.80 ± 1.12 × 10^3/mm^3), and 45 (15.22 ± 0.10 × 10^3/mm^3). However, Na-F-exposed groups exhibited progressive increases, with group D (17.5 mg/l/day) showing significant elevations ($P< 0.05$) at day 15 (16.11 ± 0.00 × 10^3/mm^3), day 30 (19.05 ± 0.25 × 10^3/mm^3), and day 45 (20.13 ± 0.43 × 10^3/mm^3). Neutrophil percentages followed a similar trend, rising from 14.60 ± 0.17% (group A, day 15) to 21.32 ± 0.70% (group D, day 45, $P < 0.05$). Conversely, lymphocyte percentages declined markedly in exposed groups, particularly in group D, decreasing from 15.95 ± 0.25% (day 15) to 12.12 ± 1.51% (day 45, $p < 0.05$). Group C (15 mg/l/day) mirrored these trends with less severity, while group B (12.5 mg/l/day) showed intermediate effects.

Table 2. Hematological parameters of *L. rohita* exposed to different concentrations of sodium fluoride at 15-day intervals

Hematologic parameter	Sample peroid	A (0 mg/l/day)	B (12.5mg/l/day)	C (15 mg/l/day)	D (17.5mg/l/day)
RBCs (10^6/mm^3)	15	4.40±0.31	4.31±0.31	4.22±0.11	4.18±0.06
	30	4.51±0.53a	4.14±0.03a	3.40±0.04b	3.29±0.14c
	45	4.53±0.44a	4.04±0.02a	3.17±0.22b	3.13±0.05b
Hb (g/dL)	15	10.15±0.70	10.09±0.5	10.02±0.41	9.95±0.22
	30	10.1±0.62a	9.98±0.5a	7.82±0.31b	7.32±0.32b
	45	10.19 ±0.7a	9.92±0.2a	7.35±0.32b	7.23±0.33b
Hct (%)	15	37.20±1.07	35.9±1.62	35.72±1.2	34.35±1.02
	30	37.65±0.8a	34.2±1.12a	31.4±.81b	27.90±0.7b
	45	37.02 ±1.5a	33.02±1.3a	28.6±1.1b	26.70±0.7b
MCV (fl)	15	57.10±0.43	54.05±1.33	50.17±0.83	46.25±0.55
	30	58.32±0.85	46.62±1.35	41.70±0.75	41.07±0.96
	45	56.9 ±0.7	33.40±1.38	26.12±1.17	17.05±2.69
MCH (pg)	15	42.22±13.9	53.97±1. 4	50.7±0.84	45.95±0.75
	30	39.55±11. 6	46.62±1.35	41.92±0.92	41.32±0.8
	45	56.87±0.7	33.40±1.38	26.15±1.18	16.9±2.93

Note:
1-RBCs; for Red Blood Cells (10^6/mm^3), Hb; for Hemoglobin (g/dL), Hct; for Hematocrit (%), MCV; Mean corpuscle cells (fl), and MCH; for Mean corpuscle Hemolobin (pg).
2-A p-value of <0.05 was considered statistically significant.

Table 3. Immunological parameters of *L. rohita* exposed to different concentrations of sodium fluoride over 45-day period at 15-day intervals

Immunological parameters	Sample peroid	A (0 mg/l/day)	B (12.5mg/l/day)	C (15 mg/l/day)	D (17.5mg/l/day)
WBCs (10^3/mm^3)	15	15.02±0.02	15.14±0.13	15.21±0.15	16.11±0.01
	30	14.80±1.12	16.32±0.3	18.93±0.23	19.05±0.25
	45	15.22 ±0.1	16.52±0.50	19.57±0.7	20.13±0.43
Neutrophils (%)	15	14.60±0.17	15.91±0.53	16.31±0.45	16.55±0.43
	30	14.50±0.22	16.05±0.5	18.52±0.29	19. 6±0.50
	45	14.28 ±0.30	17.1±0.5	19.92±0.51	21.32±0.7
Lymphocyte (%)	15	19.72±0.57	18.82±0.5	17.60±0.6	15.95±0.25
	30	19.7±0.90a	16.75±0.85a	15.2±0.43a	12.62±0.53b
	45	19.80 ±1.08a	18.±1.04a	13.35±0.96b	12.12±1.51b

Note: A *P*-value of <0.05 was considered statistically significant.

The intestinal and gill weight parameters of *L. rohita* exposed to Na-F revealed significant alterations, as shown in Table (4). In group (A), the average intestine weight remained stable across days, while the relative intestine weight showed minor fluctuations but were statistically nonsignificant (3.05 ± 0.20 g to 3.40 ± 0.11 g). In contrast, Na-F-

exposed groups exhibited progressive changes, with group D (17.5 mg/l/day) displaying significant reductions ($P< 0.05$) in relative intestine weight by day 45 (2.92 ± 0.03 g). Gill weight parameters demonstrated more pronounced effects. The average gill weight in group D decreased sharply from 3.55 ± 0.11g to 1.4 ± 0.04g, $P< 0.05$), while relative gill weight declined from 3.22 ± 0.15g to 1.13 ± 0.04g over the same period. Groups B (12.5 mg/l/day) and C (15 mg/l/day) showed intermediate declines, with group C exhibiting a notable drop in average gill weight to 1.64 ± 0.01g by day 45 ($P< 0.05$).

Table 4. Average and relative weight of the intestine and gills of *L. rohita* exposed to different concentrations of sodium fluoride during 45-day period at 15-day intervals

Intestine and Gill Weight parameters	Sample Peroid	A (0 mg/l/day)	B (12.5mg/l/day)	C (15 mg/l/day)	D (17.5mg/l/day)
Intestine Average Weight (g)	15	3.40±0.14	3.04±0.05	3.35±0.1	3.05±0.2
	30	3.43±0.15	3.7±0.1	3. 6±0.05	3.7±0.05
	45	3.74 ±0.11	3.8±0.1	3.72±0.03	3.8±0.03
Intestine Relative Weight (g)	15	3.05±0.2	2.74±0.12	3.09±0.06	3.09±0.07
	30	3±0.3	3.10±0.04	3.05±0.06	3.01±0.07
	45	3.4 ±0.11a	2.94±0.4a	2.95±0.05a	2.92±0.03b
Gills Average Weight (g)	15	3.64±0.3	3.54±0.13	3.45±0.2	3.55±0.11
	30	4.03±0.40	3.7±0.2	4±0.1	4.2±0.12
	45	3.65±0.30	2.32±0.30	1.64±0.01	1.4±0.04
Gills Relative Weight (g)	15	3.14±0.14	3.2±0.2	3.2±0.20	3.22±0.15
	30	3.4±0.2	3.05±0.17	3.34±0.10	3.42±0.14
	45	3.2 ±0.34a	1.82±0.23b	1.30±0.02c	1.13±0.04c

Exposure to Na-F caused significant biochemical alterations in rohu fish that intensified with both concentration and duration (Table 4 & Fig. 1). Control fish maintained stable serum glucose levels (92.2±2.6 to 93.2±1.06 mg/dL) throughout the 45-day study, while treated groups developed progressive hypoglycemia, particularly Group D (17.5mg/ L), which showed a 17.5% reduction by day 45 ($P<0.0001$). The metabolic disturbances extended to lipid profiles, where cholesterol levels increased by high-dose groups (167.6±1.16 mg/dL vs control 136.2±0.8 mg/dL, $P<0.0001$), contrasting sharply with a 27% decrease in triglycerides (109±2.7 mg/dL vs control 151.7±1.8 mg/dL, $P<0.0001$). Protein metabolism was similarly affected, with albumin levels in Group D declining by 62% (1.3±0.08 mg/dL) compared to controls (3.4±0.17 mg/dL) on day 45 ($P<0.0001$). The most dramatic response occurred in LDH activity, where Group D exhibited elevation (303.3±3.9 IU/L) as compared with controls (255±2.8 IU/L, $P<0.0001$).

Table 5. Serum biochemical parameters of *L. rohita* exposed to different concentrations of sodium fluoride during 45-day study at 15-day intervals

Biochemical parameters	Sample Peroid	A (0 mg/l)	B (12.5mg/l)	C (15 mg/l)	D (17.5mg/l)	*P*-value
Glucose (mg/dl)	15	92.2±2.6	89.4±2.6	86.2±1.985b	84±1.703b	0.0969
	30	93.2±1.93	89.4±2.42	77±1.34a	76.2±2.28a	<0.0001
	45	93.2±1.06	90±2.75	76.6±1.28a	75±2.34a	<0.0001
Cholesterol (mg/dl)	15	132.6±3.1	133.8±3.56	140.2±1.96a	142±2.02a	0.0735
	30	135.2±2.08	135.6±2.9	158.2±6.24b	166±1.76b	<0.0001
	45	136.2±0.8	135.6±2.9	161±5.59b	167.6±1.16b	<0.0001
Triglyceride (mg/dl)	15	149.4±0.67	147.2±1.2a	126.2±2.59a	123.2±1.24a	<0.0001
	30	149.4±0.67	147.8±0.34a	123.8±3.1ab	121±3.1ab	<0.0001
	45	151.7±1.8	143.6±1.7b	115.6±3.b	109±2.7b	<0.0001
Albumin (mg/dl)	15	3.2±0.14a	3.1±0.12a	3.0±0.17a	2.7±0.14a	0.021
	30	3.4±0.17b	2.9±0.05b	2.2±0.08b	1.7±0.06b	<0.0001
	45	3.4±0.17b	11.3±8.86c	1.8±0.03c	1.3±0.08c	0.4095
LDH (IU/L)	15	241.3±1.2a	252.3±3.3a	257±1.15a	267±1.15a	0.0001
	30	249±3.0ab	261.7±4.17ab	280.3±4.37b	280.3±3.52b	0.0009
	45	255±2.8b	267.7±0.3b	292.3±1.3b	303.3±3.9c	<0.0001

Note:

1-LDH; for lactate dehydrogenase (IU/L).

2- *P*-value of <0.05 was considered statistically significant.

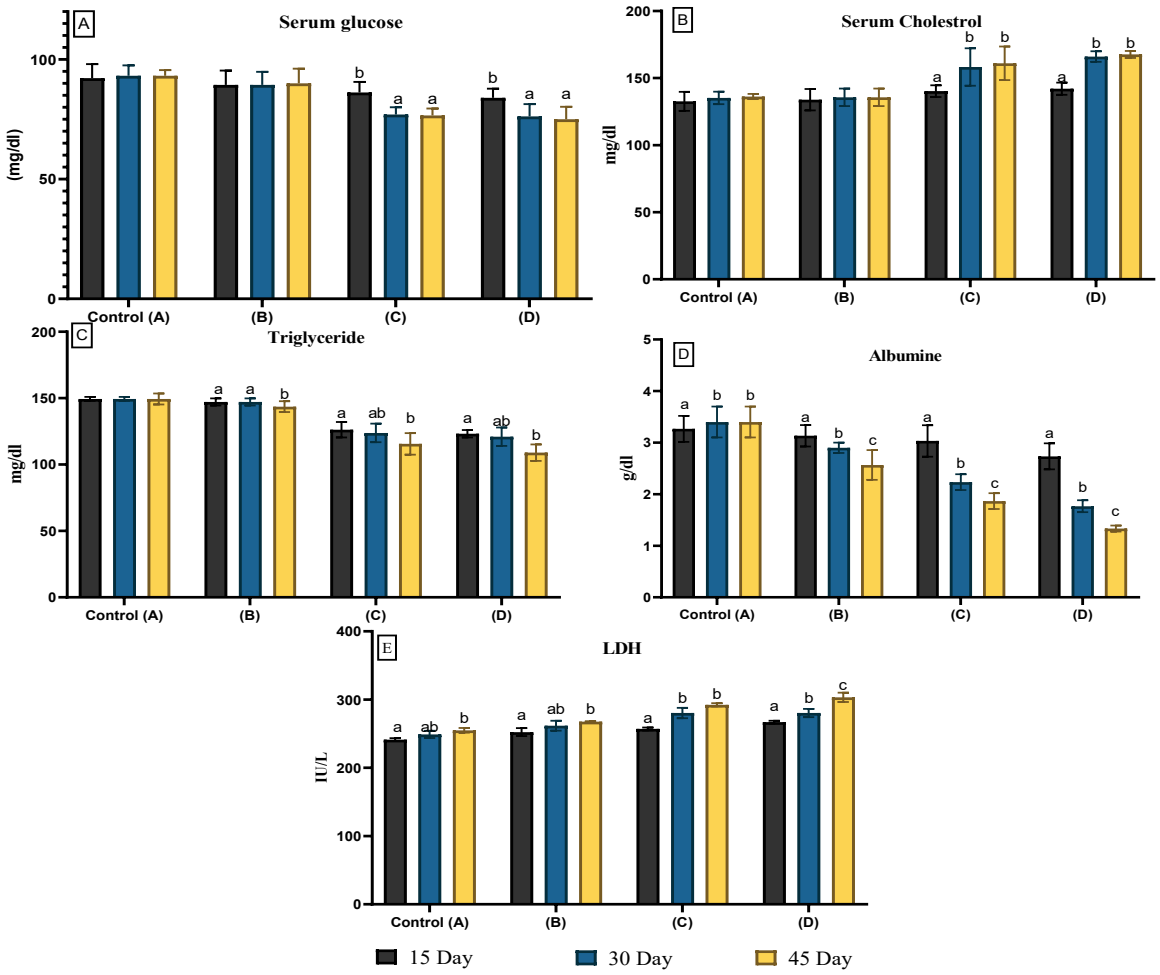

Fig. 1. Serum biochemical parameters (A: Glucose, B: cholesterol, C: triglyceride, D: albumin, and E: lactate dehydrogenase) of *L. rohita* exposed to different concentrations of sodium fluoride during 45-day experiment

DISCUSSION

When rohu fish were exposed to Na-F, they showed behavioral abnormalities, demonstrating characteristic effects of neurotoxicity caused by fluoride in aquatic species. As fluoride levels increased, the concentration-dependent progression started with labored respiratory distress (gulping, gasping at the surface) and progressed to severe neurological issues like convulsions and loss of balance in Group D (17.5mg/ L). This finding agrees with those of **Kaur *et al.* (2017)** study on *Catla catla*, where fluoride above 15mg/ L interfered with gill and neuronal function. Groups C and D showed delayed symptoms, appearing only after 15-30 days of exposure. This points to a buildup of fluoride over time,

which aligns with previous research showing that fluoride accumulates in nerve tissues **(Perera *et al*., 2018)**. The fish exposed to fluoride showed irregular, jerky movements and seizures, similar to what Canzian and colleagues observed in adult zebrafish in their 2021 study. This neurological damage probably occurs because fluoride interferes with acetylcholinesterase, an enzyme crucial for proper nerve function. **Canzian *et al.* (2021)** found that this enzyme's activity dropped by 62% at 20mg/ L fluoride concentrations. This would seriously disrupt normal neurotransmitter imbalance, suggesting species-specific sensitivity potentially linked to differences in gill morphology and fluoride uptake rates. We observed symptoms progressing from respiratory symptoms to neurological impairment, matching the two-stage toxicity pattern described by **Balde *et al.* (2024)**. A study was conducted to determine the lethal concentration (LC) of CuSO4 and to evaluate its toxicity to the gills and central nervous system (CNS) (brain and spinal cord) of *Cyprinus carpio*. Fish were exposed to 0, 2.5, 5, and 10mg/ L for 24 hours. Mortality was 100% at 10mg/ L, which represents the LC, while the median LC50 was determined by the Tryphan method and was 5mg/ L. Fish with LC100 concentrations above 10mg/ L exhibited abnormal respiration with gasping, swimming, and neurological signs with ups and downs, remained in the tank, and died within 2–3 hours **(Adeeb *et al.,* 2022)**. Their model explains how fluoride first disrupts osmoregulation before affecting the nervous system. This is particularly concerning for bottom-dwelling fish like *L. rohita*, as they will likely face extended exposure to fluoride-contaminated sediments in polluted waterways.

The exposure to Na-F triggered alarming changes in the blood parameters of *L. rohita*, aligning with emerging evidence of fluoride's hematopoietic toxicity in fish, though with notable interspecies variations. Like *Cyprinus carpio* in **Sanker *et al.* (2025)** study, rohu also developed microcytic hypochromic anemia under fluoride exposure, appearing at much lower contaminant levels. These findings reveal rohu's particular susceptibility, meaning we may need to enforce more stringent fluoride limits in waters where this ecologically important species lives. This differential susceptibility may stem from variations in fluoride bioaccumulation patterns or antioxidant defense mechanisms between cyprinids. The underlying pathology involves three synergistic mechanisms: fluoride-induced oxidative damage to erythrocyte membranes through lipid peroxidation cascade **(Zhou *et al*., 2015)**. Disruption of heme biosynthesis occurs through the inhibition of δ-aminolevulinic acid dehydratase, along with impaired iron utilization resulting from fluoride–iron complex formation in the gastrointestinal tract **(Ruliffson *et al.,* 1963; Chauhan *et al.,* 1997; Umbuzeiro & Collier, 2019)**. Interestingly, while **Rai *et al.* (2022)** reported reversible hematological changes in *Channa striatus* after fluoride withdrawal, the progressive deterioration observed in our study suggests potential irreversible damage in rohu at concentrations above 15mg/ L. The observed aberrant behaviors in the fish may be attributed to hematological disruptions caused by fluoride exposure. When fluoride impairs erythrocyte function, it compromises oxygen transport, leading to systemic hypoxia, effectively inducing internal suffocation **(Kishore *et al*., 2022)**. This hypoxic state

initiates a cascade of physiological stress responses, exacerbating cellular dysfunction **(Angwa *et al*., 2022)**.

The immunomodulatory effects demonstrate a complex dysregulation of immune function characterized by leukocytosis, neutrophilia, and lymphocytopenia, consistent with chronic inflammatory responses observed in other fluoride-exposed aquatic species. The elevation in leukocyte counts is similar to the findings by **Das *et al*. (2006)** in *Catla catla*. However, the neutrophilic response in rohu suggests a more robust innate immune activation compared to other cyprinids. This pattern is probably caused by fluoride-related tissue injury, which activates the recruitment of neutrophils through interleukin-8 signalling, combined with direct fluoride stimulation of myeloid progenitor cells **(Farley *et al*., 1983; Refsnes *et al*., 2008)**. The concurrent lymphocytopenia contrasts with observations in fluoride-exposed *Clarias gariepinus* by **Singh *et al*. (2017)**, who reported maintained lymphocyte counts, possibly indicating species-specific differences in glucocorticoid-mediated stress responses or fluoride's differential effects on lymphoid tissue architecture. The immunological disturbances may reflect compromised disease resistance, as demonstrated by **Asha *et al*. (2024)**, who found increased susceptibility to bacterial infections in fluoride-exposed carp with similar hematological profiles.

Tissue damage was observed in *L. rohita* when exposed to different concentrations of Na-F, particularly in gill structures. These alterations result from fluoride's disruptive effects on calcium metabolism and oxidative stress pathways, as demonstrated in similar studies on carp species **(Cao *et al*., 2015)**. The increased sensitivity of the gills compared to the intestinal tissue is consistent with observations in other freshwater fish species **(Wood *et al.,* 2002)**, suggesting a common target organ for fluoride toxicity across species.

The biochemical disturbances reveal significant metabolic dysfunction, particularly in carbohydrate and lipid metabolism. The progressive hypoglycemia results from fluoride-induced inhibition of gluconeogenic enzymes and impaired hepatic glycogenolysis, as previously reported in *Cyprinus carpio* by **Sankar *et al*. (2025)**. The paradoxical combination of hypercholesterolemia and hypotriglyceridemia suggests fluoride disrupts lipid homeostasis through multiple pathways, including enhanced cholesterol biosynthesis via β-Hydroxy β-methylglutaryl-CoA **(**HMG-CoA) reductase activation and inhibition of lipoprotein lipase activity (**Guth *et al*., 2020**). The marked reduction in albumin levels indicates hepatic synthetic dysfunction, consistent with histopathological observations of fluoride-induced hepatocyte damage in *Clarias gariepinus* **(Singh *et al*., 2017)**. The elevated LDH activity reflects cellular injury and compromised membrane integrity, supporting findings by **Muchtar *et al*. (2017)** in fluoride-exposed tilapia. These metabolic alterations demonstrate concentration-dependent effects, with the most severe manifestations occurring at 17.5mg/ L, suggesting a threshold for metabolic compensation in this species.

CONCLUSION

The experimental results demonstrate that NaF exposure poses significant health risks to *L. rohita*, particularly affecting blood composition and metabolic functions. Toxicological analysis revealed concerning alterations in blood cell counts, including reduced erythrocyte production and elevated WBC activity. Metabolic disturbances were evident through shifts in serum components. These physiological changes are important biomarkers for assessing fish health and water quality. The findings highlight the urgent need to prevent chemical contaminants from entering aquatic ecosystems, as pollution threatens wildlife and food safety. Future monitoring programs should incorporate these biological indicators to detect environmental contamination at early stages.

REFERENCES

Abdulrahman, P. and Al Sulivany, B. (2025). Dietary *Quercus infectoria* Mitigates Lead Nitrate Toxicity in Common Carp (*Cyprinus carpio*): Impacts on Growth Performance, Condition Factors, Weight Length Relationship, Hematological Responses, and Detoxification Potential During 60-Day Exposure. *Egyptian Journal of Aquatic Biology and Fisheries*, 29(2), 383-405.

Adeeb F. S.; Shahbaa K. Al-Taee and Nidhal T. Al-Taee. (2022). Morphohistopathological alteration in the gills and central nervous system in *Cyprinus carpio* exposed to lethal concentration of copper sulfate. Iraqi Journal of Veterinary Sciences, 36(4), 981-989.

Ahmad, S.; Shakeel, A.; Ahmed, F. and Badar, M. S. (2025). Assessment of Drinking and Irrigation Water Quality Indices Using Geospatial Techniques in the Coal Mining Region of Ramgarh, Jharkhand. Water, Air, and Soil Pollution, 236(7), 1-29.

Ahuekwe, E. F.; Isibor, P. O.; Akinhanmi, F.; Ajiboye, I.; Salami, A. O.; Adekeye, B. T. and Obembe, O. O. (2023). Utilization of nanochitosan in the sterilization of ponds and water treatment for aquaculture. In Next generation nanochitosan (pp. 301-338). Academic Press.

Al Sulivany, B. S. A.; Hassan. N. E. and Mhammad, H. A. (2024). Influence of Dietary Protein Content on Growth Performance, Feed Efficiency, Condition Factor, and Length-Weight Relationship in *Cyprinus Carpio* During the Summer Season. *Egyptian Journal of Aquatic Biology and Fisheries*, 28(2), 505-521.

Allan, G. L.; Parkinson, S.; Booth, M. A.; Stone, D. A. J.; Rowland, S. J.; Frances, J. and Warner-Smith, R. (2000). Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture*, *186*(3–4), 293–310.

Al-Turaihi, Z. M. R.; Al-Niaeem, K. S. and Ahmed, A. R. (2024). Assessment of growth performance, blood biochemical parameters, and intestinal microbiota in common carp (*Cyprinus carpio*) following dietary Tinospora cordifolia root and stem powder

supplementation: *Veterinary Integrative Sciences*, *23*(3), 1–11.

Angwa, L. M.; Jiang, Y.; Pei, J. and Sun, D. (2022). Antioxidant Phytochemicals for the Prevention of Fluoride-Induced Oxidative Stress and Apoptosis: a Review. *Biological trace element research*, *200*(3), 1418–1441.

Arsalan, M. Z. H.; Hussain, S. M.; Asrar, M.; Anwar, H.; Rehan, M. M. H.; Shahzad, M. M.; Riaz, D.; Ahmad, N. and Wahab, N. (2016). Effects of Moringa oleifera leaf meal (MOLM) based diets on carcass composition and hematology of *Labeo rohita* fingerlings. *Journal of Biodiversity and Environmental Sciences*, *9*(1), 214–223.

Asad, F.; Nadeem, A.; Naseer, S.; Ashraf, A.; Sulivany, B. and Jamal, R. (2025). Toxic and synergistic effects of micro-nanoplastics with radioactive contaminants on aquaculture: Their occurrence, distribution, role as vectors, detection and removal strategies. *International Aquatic Research*.

Asha, A. A.; Haque, M. M.; Hossain, M. K.; Hasan, M. M.; Bashar, A.; Hasan, M. Z.; Shohan, M. H.; Farin, N. N.; Schneider, P. and Bablee, A. L. (2024). Effects of Commercial Probiotics on the Growth Performance, Intestinal Microbiota and Intestinal Histomorphology of Nile Tilapia (*Oreochromis niloticus*) Reared in Biofloc Technology (BFT). *Biology*, *13*(5), 299.

Bacou, E.; Walk, C.; Rider, S.; Litta, G. and Perez-Calvo, E. (2021). Dietary oxidative distress: A review of nutritional challenges as models for poultry, swine and fish. Antioxidants, 10(4), 525.

Balde, A.; Ramya, C. S. and Nazeer, R. A. (2024). A review on current advancements in zebrafish models to study chronic inflammatory diseases and their therapeutic targets. *Heliyon*, *10*(11), e31862.

Blaxhall, P. C. and Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, *5*(6), 771–781.

Canzian, J.; Franscescon, F.; Müller, T. E.; Stefanello, F. V.; Souza, T. P.; Rosa, L. V. and Rosemberg, D. B. (2021). Stress increases susceptibility to pentylenetetrazole-induced seizures in adult zebrafish. *Epilepsy and behavior: E and B*, *114*(Pt A), 107557.

Cao, J.; Chen, J.; Xie, L.; Wang, J.; Feng, C. and Song, J. (2015). Protective properties of sesamin against fluoride-induced oxidative stress and apoptosis in the kidney of carp (*Cyprinus carpio*) via JNK signalling pathway. *Aquatic toxicology (Amsterdam, Netherlands)*, *167*,180–190.

Chauhan, S.; Titus, D. E. and O'Brien, M. R. (1997). Metals control activity and expression of the heme biosynthesis enzyme delta-aminolevulinic acid dehydratase in *Bradyrhizobium japonicum*. *Journal of Bacteriology*, *179* (17), 5516–5520. https://doi.org/10.1128/jb.179.17.5516-5520.1997

Das, P. C.; Ayyappan, S. and Jena, J. K. (2006). Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus*

mrigala (Hamilton) exposed to acidic and alkaline water pH, Aquaculture, 256;1–4; 80-87. https://doi.org/10.1016/j.aquaculture.2006.02.019

Das, P. S.; Rohani, M. F.; Al Sulivany, B. S. A.; Nibir, S. S.; Juthi, R. A.; Abdus Satter.; Hossain, M. S. and Ismael, S. S. (2025). Dietary Silica Nanoparticle Ameliorates The Growth Performance And Muscle Composition Of Stinging Catfish, Heteropneustes Fossilis. *Science Journal of University of Zakho*, *13*(1), 33–39.

Del Piero, S. T.; Masiero, L. and Casellato, S. (2014). Toxicity and bioaccumulation of fluoride ion on *Branchiura sowerbyi,* Beddard (Oligochaeta, Tubificidae). Zoosymposia, 9, 44-50.

Dezfuli, S. B.; Lorenzoni, M.; Carosi, A.; Giari, L. and Bosi, G. (2023). Teleost innate immunity, an intricate game between immune cells and parasites of fish organs: who wins, who loses. Frontiers in Immunology, 14, 1250835.

Dippong, T.; Resz, M. A.; Tănăselia, C. and Cadar, O. (2024). Assessing microbiological and heavy metal pollution in surface waters associated with potential human health risk assessment at fish ingestion exposure. Journal of Hazardous Materials, 476, 135187.

Doumas, B.; Watson, W. and Biggs, H. (1971): Albumin standard and the measurement of serum albumin with bromo cresol green. ClinicaChimicaActa, 31: 87-96.

El-Bouhy, Z.; Reda, R. M.; Mohamed, F. A.; Elashhab, M. W. and Abdel Rahman, A. N. (2024). Polycyclic Aromatic Aydrocarbons (PAHs) Pollution Approaches in Aquatic Ecosystems: Perils and Remedies Using Green Technologies. Zagazig Veterinary Journal, 52(1), 49-87.

El-Houseiny, W.; Basher, A. W.; Mahmoud, Y. K.; Bayoumi, Y.; Abdel-Warith, A. W. A.; Younis, E. M. and Assayed, M. E. M. (2025). Mitigation of sodium fluoride-induced growth inhibition, immunosuppression, hepatorenal damage, and dysregulation of oxidative stress, apoptosis, and inflammation-related genes by dietary artichoke (*Cynara scolymus*) leaf extract in *Oreochromis niloticus*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 277, 111068.

Farley, J. R.; Wergedal, J. E. and Baylink, D. J. (1983). Fluoride directly stimulates proliferation and alkaline phosphatase activity of bone-forming cells. *Science (New York, N.Y.)*, *222*(4621), 330–332.

Gallaugher, P. and Farrell, A. P. (1998). Hematocrit and blood oxygen-carrying capacity. In *Fish physiology* (Vol. 17, pp. 185–227). Elsevier.

Ghosh, S.; Sadhu, A.; Mandal, A. H.; Biswas, J. K.; Sarkar, D. and Saha, S. (2024). Copper oxide nanoparticles as an emergent threat to aquatic invertebrates and photosynthetic organisms: a synthesis of the known and exploration of the unknown. Current Pollution Reports, 11(1):6.

Grădinariu, L.; Dediu, L.; Crețu, M.; Grecu, I. R.; Docan, A.; Istrati, D. I. and

Vizireanu, C. (2024). The Antioxidant and Hepatoprotective Potential of Berberine and Silymarin on Acetaminophen Induced Toxicity in *Cyprinus carpio* L. Animals, 14(3), 373.

Guth, S.; Hüser, S.; Roth, A.; Degen, G.; Diel, P.; Edlund, K.; Eisenbrand, G.; Engel, K. H.; Epe, B.; Grune, T.; Heinz, V.; Henle, T.; Humpf, H. U.; Jäger, H.; Joost, H. G.; Kulling, S. E.; Lampen, A.; Mally, A.; Marchan, R.; Marko, D. and Hengstler, J. G. (2020). Toxicity of fluoride: critical evaluation of evidence for human developmental neurotoxicity in epidemiological studies, animal experiments and *in vitro* analyses. *Archives of Toxicology*, *94*(5); 1375–1415.

Hamdy, S. M. **H.; Zaghloul, K.; S.Gad, N. and M. A. Elramady, M.** (2018). Impact of water quality on biochemical and physiological parameters of *Oreochromis niloticus* in different aquatic habitats. *Biochemistry Letters*, *14*(1), 94-108. doi: 10.21608/blj.2018.47588.

Hamed, M.; Abdel-Tawab, H. S.; Abdel-Maksoud, M. A.; Malik, A.; Fatima, S. and Martyniuk, C. J. (2025). Impacts of Voliam Flexi on Antioxidant Responses, Oxidative Stress, and Histopathological Changes in Juvenile African Catfish (*Clarias gariepinus*). Aquaculture Research, 2025(1), 8608065.

Hassan, H.U.; Ali, A. and Al Sulivany, B.S.A. *et al.* (2025) Effects of *Tribulus terrestris* extract and 17 α-methyl testosterones on masculinization, growth, economic efficiency and health assessment of Nile tilapia (*Oreochromis niloticus*). *Aquacult Int* 33, 156 (2025). https://doi.org/10.1007/s10499-024-01817-5

Hassan, S.M.; Albassam, N.H.; Madlul, N.S. and Mahmood, A.S. (2024). Influence of different salinity concentrations on water quality and subsequent effects on hatchability rate of Artemia salina eggs. Aip Conference Proceedings Open source preview, (2024), 3079(1), 020007.

Hassan, S.M.; Rashid, M.S.; Albassam, N.H.; Muhaimeed, A.R. and Sulaiman, M.A. (2025). Using the Diverse Vegetables as a Filtration plants in Aquaculture Intensive System. Tikrit Journal for Agricultural Sciences, 2025, 25 (1); pp. 1–16.

Ikpesu, T. and Odagwe, A. A. (2025). Correlation of oxidative stress enzymes and histological alteration in the gastrointestinal tract of *Clarias gariepinus* induced with alpha olefin sulfonate. Journal of Water and Health, 23(3): 277-287.

Kamruzzaman, M.; Khan, M. S. U.; Ritu, S. A.; Khanom, S.; Hossain, M.; Islam, M. R. and Uddin, S. (2025). Diving Deep: Exploring Fluoride in Groundwater—Causes, Implications, and Mitigation. In Fluorides in Drinking Water: Source, Issue, and Mitigation Strategies (pp. 189-221). Cham: Springer Nature Switzerland

Kaur, R.; Saxena, A. and Batra, M. P. (2017). A Review Study on Fluoride Toxicity in Water and Fishes: Current Status, Toxicology and Remedial Measures. *International Journal of Environment, Agriculture and Biotechnology (ISSN: 2456-1878)*.2(1), 456-466.10.22161/ijeab/2.1.58.

Kim, J.; Kim, J.; Kaown, D. and Joun, W. T. (2025). Natural and anthropogenic factors

controlling hydrogeochemical processes in a fractured granite bedrock aquifer, Korea. Environmental Monitoring and Assessment, 197(5), 1-26.

Kishore, D. Shubhajit, S. and Chukwuka, A.V. (2022). Behavioural toxicity and respiratory distress in early life and adult stage of walking catfish *Clarias batrachus* (Linnaeus) under acute fluoride exposures. *Toxicol. Environ. Health Sci.* 14, 33–46.

Kumar, S.; Shenoy, S.; Swamy, R. S.; Ravichandiran, V. and Kumar, N. (2024). Fluoride-induced mitochondrial dysfunction and approaches for its intervention. Biological Trace Element Research, 202(3), 835-849.

Li, Y.; Wang, R.; Li, Y.; Sun, G. and Mo, H. (2022). Protective effects of tree peony seed protein hydrolysate on Cd-induced oxidative damage, inflammation and apoptosis in zebrafish embryos. Fish and Shellfish Immunology, 126, 292-302.

Mohamed, A. M.; Shamjhi, I. A. J. and Sadiq, N. H. (2020). Detection of bacterial contamination in imported and local red and white meat in TIkrit City, Iraq. *Ann. Trop. Med. Public Health*, *23*(4).

Muchtar, E.; Dispenzieri, A.; Lacy, M. Q.; Buadi, F. K.; Kapoor, P.;Hayman, S. R.; Gonsalves, W.; Warsame, R.; Kourelis, T. V.; Chakraborty, R.; Russell, S.; Lust, J. A.; Lin, Y.; Go, R. S.; Zeldenrust, S.; Dingli, D.; Leung, N.; Rajkumar, S.V.; Kyle, R.A.; Kumar, S.K. and Gertz, M.A. (2017). Elevation of serum lactate dehydrogenase in AL amyloidosis reflects tissue damage and is an adverse prognostic marker in patients not eligible for stem cell transplantation. *British journal of haematology*, *178*(6), 888–895. https://doi.org/10.1111/bjh.14830

Mukherjee, S.; Islam, S.; Sarkar, O. and Chattopadhyay, A. (2025). Oxidative Stress in Kidney of Zebrafish due to Individual and Combined Exposure to Amoxicillin, Arsenic, and Fluoride: Involving Nrf2-Keap1-ARE Pathway. Journal of Applied Toxicology.

Namiq, K.; Albassam, N. H. and Wahab, N. K. (2025). Assessment of Pesticide Residues in Fish Tissues and Water at Various Times of the Year. *Diyala Agricultural Sciences Journal*, *17*(1), 178-193.

Omar, I. and Al Sulivany, B. (2025). Sustainable Aquaculture Nutrition: Impact of Chlorella vulgaris and Iron Nanoparticles and their Combination on Growth, Hematology, and Immune Stability in the Common Carp (*Cyprinus carpio*). *Egyptian Journal of Aquatic Biology and Fisheries*, 29(2), 916-938. doi: 10.21608/ejabf.2025.418035.

Owais et al., M. (2024). The Pangas Catfish *Pangasius pangasius*; Growth Efficiency and Nutritional Composition Under Variety of Saltwater Challenges. *Egyptian Journal of Aquatic Biology and Fisheries*, 28(6), 1-13. doi: 10.21608/ejabf.2024.389994

Owais, M.; Ali, B. N.; Azam, H. A.; Al Sulivany, B. S. A.; Naeem, M.; Zahra, E.; Ishtiaq, N. & Hassan, A. (2025). Impact of Phytogenic Feed Additives on Growth Performance, Antioxidant Defense, and Serum Biochemical Indices in *Cirrhinus*

mrigala. *Journal of Education and Science*, *34*(4), 86–96.

Owais, M.; Mohammed, D. A.; Mhammad, H. A.; Al Sulivany, B. S. A.; Dernekbaşı, S. and Fazal, R. M. (2025). The Role of Silica Nanoparticles in Modulating Growth Performance, Enzyme Activity, and Heavy Metal Accumulation in Muscle Tissue of Common Carp (*Cyprinus carpio*. L). *Science Journal of University of Zakho*, *13*(2), 197–205. https://doi.org/10.25271/sjuoz.2025.13.2.1495

Perera, T.; Ranasinghe, S.; Alles, N. and Waduge, R. (2018). Effect of fluoride on major organs with different times of exposure in rats. *Environmental health and preventive medicine*, *23*(1), 17. https://doi.org/10.1186/s12199-018-0707-2

Potiris, A.; Moustakli, E.; Trismpioti, E.; Drakaki, E.; Mavrogianni, D.; Matsas, A. and Stavros, S. (2025). From Inflammation to Infertility: How Oxidative Stress and Infections Disrupt Male Reproductive Health. Metabolites, 15(4), 267.

Radwan, M.; El-Sharkawy, M. A.; Alabssawy, A. N.; Ghanem, S. F.; Mohammadein, A.; Al Malki, J. S. and Darweesh, K. F. (2023). The synergy between serious parasitic pathogens and bacterial infestation in the cultured Nile tilapia (Oreochromis niloticus): a severe threat to fish immunity, causing mass mortality and significant economic losses. Aquaculture International, 31(5), 2421-2449.

Rai, S. N.; Tiwari, N.; Singh, P.; Singh, A. K.; Mishra, D.; Imran, M. and Singh, M. P. (2022). Exploring the Paradox of COVID-19 in Neurological Complications with Emphasis on Parkinson's and Alzheimer's Disease. *Oxidative medicine and cellular longevity*, *2022*(1), 3012778.

Refsnes, M.; Skuland, T.; Schwarze, P. E.; Øvrevik, J. and Låg, M. (2008). Fluoride-induced IL-8 release in human epithelial lung cells: relationship to EGF-receptor-, SRC- and MAP-kinase activation. *Toxicology and applied pharmacology*, *227*(1), 56–67. https://doi.org/10.1016/j.taap.2007.09.022

Ribeiro, O.; Pinto, M. Q.; Tavares, D.; Ferreira-Cardoso, J. V.; Correia, A. T. and Carrola, J. S. (2024). Copper and Temperature Interaction Induced Gill and Liver Lesions and Behaviour Alterations in Mozambique Tilapia (*Oreochromis mossambicus*). Water, 16(17), 2499.

Rind, K. H.; Majeed, S.; Ujan, J. A.; Habib, S. S.; Batool, A. I.; Naz, S. and Mohany, M. (2025). Effect of Fluoride Concentrations on Proximate Composition and Serum Biochemistry of Labeo rohita Across Varying Exposure Durations. Fluoride, 58(11), 1-9.

Ruliffson, W. S.; Burns, L. V. and Hughes, J. S. (1963). The Effect of Fluoride Ion on Fe^{59} Iron Levels in Blood of Rats. *Transactions of the Kansas Academy of Science (1903-)*, *66*(1), 52–58.

Sadiqa, A.; Parveen, S.; Riaz, H. F.; Ayoub, U.; Shaheen, A.; Rasheed, S. and Khan, M. S. (2024). Toxic Impact of Detergent (Bright) On Growth, External Morphology, Behaviour, Liver Toxicology, Haematological Parameters and Histopathology of Nile Tilapia (*Oreochromis niloticus*). Journal of Survey in Fisheries Sciences, 11(4),

45-54.

Saha, N. C.; Banerjee, P.; Chatterjee, A.; Bhattacharya, R.; Saha, S. and Pastorino, P. (2024). Haematological, biochemical, enzymological changes and mitochondrial dysfunction of liver in freshwater climbing perch *Anabas testudineus* during their acute and chronic exposure to sodium fluoride. Environmental Toxicology and Pharmacology, 106, 104360.

Samah R.K.; Chuntian, Z.; Shimaa, M. A. Z.; Mayada, R. F.; Hesham, S.E.; Mouhamed, S.S. and Basma, A.E. (2023). Modulatory effect of thymol on the immune response and susceptibility to *Aeromonas hydrophila* infection in Nile tilapia fish exposed to zinc oxide nanoparticles. Aquatic Toxicology, 259, 1-12.

Sankar, J.; Thangam, Y.; Umamaheswari, S.; Kowsalya, S.; Syed Ali Fathima, K. M.; Sathyavathi, R.; Suvetha, L.; Gayathiri, E.; Prakash, P. and Nagarajan, S. (2025). Impact of fluoride on thyroid function and histopathology in *Cyprinus carpio*: Implications for aquatic ecosystems. *Toxicology reports*, *14*, 101964.

Singh, R.; Hussain, M. A.; Kumar, J.; Kumar, M.; Kumari, U. and Mazumder, S. (2017). Chronic fluoride exposure exacerbates headkidney pathology and causes immune commotion in *Clarias gariepinus*. *Aquatic toxicology (Amsterdam, Netherlands)*, *192*, 30–39. https://doi.org/10.1016/j.aquatox.2017.09.006

Terova, G.; Ceccotti, C.; Al-Sulivany, B. S. A. and Micaela, A. (2018). The Dietary Taurine Addition In Fish Feed; Its Protective Role To The Reactive Oxygen Species (Ros) Production In The European Seabass (*Dicentrarchus labrax*) During A Forced. Conference Aqua 2018.

Tietz, N.W. (1986): Textbook of clinical chemistry, (Saunders, W.B., And Ed.), Philadelphia, pp: 1271-1281.

Tietz, N.W. (1995): Clinical guide to laboratory tests, 3 Ed., (Saunders, W.B., [2] Ed.), Philadelphia, USA, 130-273.

Umbuzeiro, A. G. and Collier, T. K. (2019).Environmental science and pollution research. *Environmental science and pollution research international*, *26*(27), 27555–27557.

Wedemeyer, G. A. (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. *Federal Government Series*, *18*

Wood, C. M.; Kelly, S. P.; Zhou, B.; Fletcher, M.; O'Donnell, M.; Eletti, B. and Pärt, P. (2002). Cultured gill epithelia as models for the freshwater fish gill. *Biochimica et biophysica acta*, *1566*(1-2), 72–83.

Yadav, S. S.; Kumar, R. and Tripathi, M. (2014). Effects of fluoride exposure on some enzymatic and histopathological changes in the liver of *Heteropneustes fossilis* (Bloch). International Journal of Fauna and Biological Studies, 1(5), 80-84.

Zhang, R.; Yan, Z.; Zhong, H.; Luo, R.; Liu, W.; Xiong, S. and Liu, M. (2024). Gut microbial metabolites in MASLD: Implications of mitochondrial dysfunction in the pathogenesis and treatment. Hepatology communications, 8(7), e0484.

Zhou, B, H.; Zhao, J.; Liu, J.; Zhang, L, J.; Li, J. and Wang, H. (2015). Fluoride-induced oxidative stress is involved in the morphological damage and dysfunction of liver in female mice. Chemosphere. 139; 504-511.

Zimmerman, H. and Henry, J. (1979): Clinical Enzymology. In: Clinical Diagnosis and Management by Laboratory Methods 16 (J. B. Henry, Ed.), Saunders, Philadelphia, pp: 365-368.

Zuhra, N.; Akhtar, T.; Yasin, M. R.; Mushtaq, Z.; Sajjad, H.; Javed, S.; and Baran, M. F. (2024). Impact of Fluorides and Fluorocarbons Contamination on the Environment and Human Health. In Fluoride and Fluorocarbon Toxicity: Sources, Issues, and Remediation (pp. 121-161). Singapore: Springer Nature Singapore.