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Short Communication

Assessment of Haematological Alterations in *Labeo rohita* (Rohu) Exposed to Ibuprofen

Sridhar Sakthi[1], D Manimekalai[1]*, P Padmavathy[2], R Shalini[3], N Jayakumar[4] and M Ponmani[1]

[1]*Department of Aquatic Environment Management, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.*

[2]*Directorate of Extension Education, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.*

[3]*Department of Fish Quality Assurance and Management, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.*

[4]*Department of Fisheries Biology and Resource Management, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.*

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Ibuprofen, Haematological parameters, *Labeo rohita,* Erythrocyte indices, LC_{50}, NSAIDs

ABSTRACT

Pharmaceutical pollutants, particularly non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, have become significant contaminants in aquatic environments, posing risks to fish health. This study investigates the acute toxicity and haematological alterations in *Labeo rohita* (Hamilton, 1822) exposed to different concentrations of ibuprofen, and key haematological parameters was determined to be 5.658 mg/L, classifying ibuprofen as highly toxic to *L. rohita*. Significant reductions in RBC, Hb, and HCT were observed, indicating impaired oxygen transport and potential anaemia. Conversely, elevated WBC counts suggest an immune response to chemical stress. In particular, the impact of pharmaceutical industries operating within the study area warrants close examination, as their effluent discharges can significantly contribute to the contamination load in adjacent water bodies. It is crucial to assess the cumulative pharmaceutical residues in these freshwater systems, given their potential to disrupt aquatic ecosystems, impair fish health, and pose risks to human populations dependent on these resources.

Aquatic ecosystems are increasingly being exposed to pharmaceutical pollutants, with non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (IBP) being among the most commonly detected contaminants in freshwater bodies (Islas-Flores *et al*., 2014; Katzung, 2017). IBP enters aquatic environments primarily through wastewater discharge, pharmaceutical manufacturing effluents, and improper disposal of medicines. Due to its widespread presence and persistence, IBP poses significant risks to aquatic organisms, particularly fish, which are highly susceptible to its toxicological effects (Kasprzyk-Hordern *et al*., 2008).

Labeo rohita, a major freshwater carp species in aquaculture and a vital component of South Asian fisheries, has been widely studied for its sensitivity to environmental pollutants. Among various physiological parameters, haematological indices serve as reliable biomarkers for assessing fish health and stress responses under toxicant exposure. Changes in parameters such as red blood cell (RBC) count, haemoglobin concentration (Hb), haematocrit (HCT), white blood cell (WBC) count and differential leukocyte counts provide critical insights into the physiological disruptions caused by xenobiotics (Iheanacho *et al*., 2017).

Existing research has demonstrated that exposure to pharmaceuticals, including NSAIDs, can significantly alter the haematological profile of fish, leading to immunosuppression, oxidative stress and metabolic disturbances. However, data regarding the acute

* Corresponding author: manimekalai@tnfu.ac.in
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haematotoxic effects of IBP on *L. rohita* remain limited. Given the ecological importance of this species and the growing concern over pharmaceutical pollution, investigating the haematological responses of *L. rohita* to IBP exposure is imperative for understanding its potential impact on aquatic health.

This study aims to evaluate the haematological alterations in *L. rohita* subjected to acute concentrations of IBP. By examining key blood parameters, this research seeks to elucidate the physiological stress and toxic effects induced by IBP exposure, thereby contributing to the broader understanding of pharmaceutical-induced toxicity in freshwater fish species.

Materials and methods

Fingerlings of *L. rohita* (9±2 g, 8.5±1.5 cm) were obtained from Kallidaikurichi and Thanjavur Private Fish Farms, Tamil Nadu, India. They were acclimatized for 20 days in FRP tanks under laboratory conditions with continuous aeration and routine water exchange. Fish were fed a commercial diet (Coppens) with regular tank cleaning to remove waste. Water quality parameters were maintained per APHA (2017) standards: temperature 30.45±0.10°C, pH 6.42±0.08, dissolved oxygen 6.51±0.6 mg/L, total alkalinity 352±1.2 mg/L, and total hardness 131±1.5 mg/L as $CaCO_3$.

Ten fish were exposed to different concentrations of the pharmacological agent IBP (CAS number 15687-27-1, > 98% purity) purchased from Sigma-Aldrich in a 35-liter trough holding 10 L of water for each test concentration. Throughout the testing phase, the experimental organisms were not fed. Each concentrations were done in triplicate manner. Wide range finding test for IBP was calculated to analyse the LC_{50} value for acute toxicity. The range for the concentrations (100, 10, 1, 0.1 and 0.01 ppm) were estimated by the OECD guidelines.

Blood samples were collected on days 1-4 of exposure by pelvic fin puncture using a heparinized syringe (21-gauge needle) and stored in EDTA vials to prevent clotting. The blood samples were analyzed according to Blaxhall and Daisley (1973). Packed cell volume (PCV) was measured using the micro-hematocrit method, while haemoglobin (Hb) levels were assessed with Sigma Diagnostics kits using the cyanohemoglobin technique. RBC and WBC counts were determined with a Neubauer haemocytometer (Dacie and Lewis, 2006), and lymphocyte differentials were analyzed on May-Grunwald-Giemsa-stained blood smears (Mirale, 1982). Erythrocyte indices (MCHC, MCH, MCV) were calculated using RBC count, Hb, and PCV following Dacie and Lewis (2006).

The probit method (Finney, 1971) was used to analyze acute toxicity data concerning quantal responses (mortality). A two-way ANOVA was performed on the haematological parameters with Tukey post hoc test using SPSS version 20 to compare means and identify significant differences at the 5% probability level.

Results and discussion

LC_{50} of IBP was found to be 1.54 ppm by using the method of Log Probit Analysis software. The Commission of the European Communities (1996) established EU guidelines (93-67-EEC) that divide chemicals into various categories based on their LC_{50} (for aquatic animals, less than 1 mg/l is extremely poisonous, 1–10 mg/l is toxic, and 10-100 mg/l is hazardous). This result indicates that IBP is severely toxic for *L. rohita*. The toxicity however varies across species, with LC_{50} values reported as 0.38 ppm for *Clarias gariepinus* (Malarvizhi *et al*., 2012), 173 ppm for *Lepomis macrochirus*, and 132.6 ppm for *Daphnia magna* (Islas-Flores *et al*., 2017), reflecting species-specific and exposure-related differences.

The blood parameters such as WBC, RBC, HCT, MCV, MCH and MCHC were analysed for the drug IBP. There were significant differences ($p<0.05$) in all parameters of the treated fish compared to the control group in terms of both concentration and duration. In case of IBP, WBC shows the higher value in 96 h at concentration of 3.092 mgl^{-1} and lower in 48h at 0.386 mgl^{-1}. The higher value for RBC was attained in 1.546 mgl^{-1} concentration and the lower was in 96h at 0.386 mgl^{-1} concentration. Whereas in case of MCH and MCHC higher level occurs in the 48 h at 6.184 mgl^{-1} and lower level occurs in 24 h at 1.546 mgl^{-1} and 0.386 mgl^{-1}. In HCT, the higher concentration (6.184 mgl^{-1}) was at 24 h showed higher value and lower concentration (0.386 mgl^{-1}) showed lower value. And in MCV, it showed higher value at (1.546 mgl^{-1}) at 96 hour and lower value occurred in concentration of 6.184 mgl^{-1} at 48 h which was mentioned in the Table I. The Table I indicates the four days parameters (WBC, RBC, HCT, MCV, MCH and MCHC) with the accordance to five different concentrations (6.184, 3.092, 1.546, 0.773 and 0.386 mgl^{-1}).

Blood parameters, linked to energy, respiration, and defense, serve as physiological indicators of environmental changes (Nwani *et al*., 2014; Iheanacho *et al*., 2017). The various haematological parameters sensitive to pollutants and help assess an organism's physiological status (Adhikari *et al*., 2014).

In our study, haematological responses showed an increase in WBC and MCHC under lethal IBP concentrations throughout the study. The elevation in WBC count, linked to immune regulation across organisms, indicates a general immune response and protective reaction to IBP (Saravanan *et al*., 2011). The toxicant's immune-stimulatory effect likely triggered lymphocyte release as a defense mechanism, contributing to the elevated WBC count in fish. Additionally, the study noted an increase in MCHC

Table I. Effect of different concentrations of IBP on haematological parameters of *L. rohita* exposed for 1- 4 days.

Tissue	Day of exposure	Control	6.184 ppm	3.092 ppm	1.546 ppm	0.773 ppm	0.386 ppm
WBC	24 h	15.27± 0.47^{a}	1.73±0.08^{a}	1.67±0.04^{a}	0.91±0.04^{a}	0.61±0.05^{a}	0.913±0.08^{a}
	48 h	20.24±0.76^{a}	2.59±0.31^{a}	1.67±0.20^{a}	1.56±0.23^{a}	0.73±0.28^{a}	0.49±0.29^{a}
	72 h	22.77±0.41^{a}	23.27±0.60^{a}	11.3±0.5^{a}	4.28±0.16^{a}	3.22±0.12^{a}	2.85±0.10^{a}
	96 h	24.24±0.30^{a}	130.47±0.30^{a}	269.07± 0.65^{a}	256.74±2.14^{a}	50.37± 0.4^{a}	7.43±0.49^{a}
RBC	24 h	7.033±0.70^{b}	3.42±0.40^{a}	4.50±0.19^{a}	5.50±0.26^{a}	3.35±0.31^{a}	4.7±0.53^{a}
	48 h	5.13±0.25^{b}	1.30±0.44^{a}	3.96±0.13^{a}	1.71±0.06^{a}	1.71±0.04^{a}	1.67±0.04^{a}
	72 h	6.27±0.56^{b}	1.63±0.09^{a}	0.87±0.09^{a}	0.61±0.05^{a}	0.7±0.04^{a}	0.64±0.04^{a}
	96 h	4.46±0.49^{b}	0.7±0.07^{a}	2.11±0.12^{a}	0.91±0.04^{a}	0.38±0.026^{a}	0.14±0.06^{a}
HCT	24 h	52.13±0.70^{d}	23.03±1.09^{c}	18.1±0.55^{bc}	17.36±0.75^{abc}	15.67±0.45^{ab}	14±0.56^{a}
	48 h	55.56±1.20^{d}	13.27±0.47^{c}	12.37±0.61^{bc}	10.97±0.20^{abc}	10.73±0.77^{ab}	10.27±0.15^{a}
	72 h	50.27±0.40^{d}	11.77±0.41^{c}	9.97±0.80^{bc}	7.52±0.26^{abc}	5.47±0.40^{ab}	5.5±0.2^{a}
	96 h	47.73±0.90^{d}	6.1±0.2^{c}	5.4±0.55^{bc}	5.37±0.25^{abc}	1.53±0.35^{ab}	0.75±0.13^{a}
MCV	24 h	128.13±0.73^{b}	105.7±0.79^{a}	108.13±0.80^{a}	100.77±1.17^{a}	101±0.65^{a}	103.67±0.95^{a}
	48 h	121.83±1.40^{b}	99.13±0.37^{a}	108.27±0.60^{a}	110.53±0.80^{a}	107.13±0.64^{a}	104.47±0.71^{a}
	72 h	131.1±0.87^{b}	113.57±0.98^{a}	106.47±1.15^{a}	111.9±0.91^{a}	105.77±2.09^{a}	104.53±0.55^{a}
	96 h	124.83±1.25^{b}	109.4±0.5^{a}	100.97±0.81^{a}	115.47±2.10^{a}	106.2±0.98^{a}	105.63±0.61^{a}
MCH	24 h	47.2±0.65^{c}	42.13±0.92^{bc}	36.73±0.35^{ab}	31.97±0.40^{ab}	34.83±0.94^{a}	35.5±0.82^{a}
	48 h	46.37±0.61^{c}	44.83±0.35^{bc}	30.93±0.75^{ab}	35.63±0.68^{ab}	34.23±0.47^{a}	33.47±1.001^{a}
	72 h	45.23±0.81^{c}	37.67±0.45^{bc}	36.73±1.45^{ab}	43.17±0.55^{ab}	34.5±1.276^{a}	33.7±1.11^{a}
	96 h	39.17±0.61^{c}	38.17±0.60^{bc}	37.93±1.01^{ab}	35.9±0.43^{ab}	33.4±0.62^{a}	32.87±0.96^{a}
MCHC	24 h	36±0.79^{a}	41.57±0.61^{a}	34.77±0.73^{a}	34.17±0.75^{a}	33.3±0.36^{a}	34.53±0.38^{a}
	48 h	35.17±0.25^{a}	47.53±1.20^{a}	33.9±0.69^{a}	27.87±0.7^{a}	27.9±0.87^{a}	23.7±0.79^{a}
	72 h	33±0.65^{a}	44.17±0.45^{a}	36.3±0.60^{a}	34.7±0.45^{a}	34.5±0.52^{a}	35.8±0.264^{a}
	96 h	36±0.75^{a}	38.43±0.47^{a}	38.13±0.80^{a}	33.3±0.40^{a}	35.03±0.49^{a}	33.33±0.40^{a}

Values are the Mean ±SD, ($p<0.05$), two-way ANOVA with Tukey post hoc test. Significantly different from control group. Where MCV is Mean Corpuscular Volume, MCH is Mean Corpuscular haemoglobin and MCHC is Mean Corpuscular Haemoglobin Concentration. The a, b and c of the values indicates the subset of the values in which each value comes under a different category (Tukey test).

which may suggest the onset of spherocytosis (Umamaheshwari and Senthilnathan, 2014). Similar observations of elevated MCHC levels were seen in *M. cephalus* (Grey Mullet) exposed to pesticides (Francesco *et al*., 2012). Furthermore, a slight decrease in the HCT parameter, moving from higher to lower IBP concentrations, indicates anemia (Thakur and Sahai, 1987). Comparable reductions in RBC count, HCT, and Hb levels have been reported in carps exposed to various toxicants (Lavanya *et al*., 2011). The accumulation of toxicants in the gill area may lead to haemolysis due to gill structure damage and compromised osmoregulation, potentially causing RBC reduction (Saravanan *et al*., 2011). In line with these findings, the present study observed a reduction in RBC count under lethal IBP exposure, likely due to IBP's suppression of RBC production.

MCV assesses the size or condition of RBCs and indicates whether cell division during erythropoiesis is normal or abnormal. Conversely, MCH indicates the average haemoglobin content in each red blood cell within a blood sample. In our drug IBP toxicity test, the MCV and MCH showed the decrease in mean count throughout the study period which indicates the hypochromic microcytic anaemia. MCH values typically align with MCV values (Kumar and Banerjee, 2016). The substantial increase in mean RBC, along with a decrease in MCV and MCH observed in this study, may indicate erythrocyte damage and gill tissue damage, leading to impaired oxygen transport and hindered gaseous exchange (Burgos-Aceves *et al*., 2019).

Conclusion

The present study highlights the adverse effects of ibuprofen exposure on the haematological profile of *L. rohita*. The significant reduction in RBC count, haemoglobin levels, and haematocrit indicates impaired oxygen transport and potential anaemia, while the increase in WBC count and altered leukocyte distribution suggest an immune response to chemical stress. These findings provide strong evidence that pharmaceutical contaminants like IBP can severely impact the physiological health of fish, leading to potential ecological consequences. Given the widespread presence of IBP in aquatic environments, this study emphasizes the urgent need for monitoring and

regulating pharmaceutical pollutants in water bodies.

DECLARATIONS

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Ethical approval

This study was conducted based on the approval of the ethical committee of TNJFU, Nagapattinam, Tamil Nadu, India.

Statement of conflict of interest

The authors have declared no conflict of interest.

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