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Research Article

Study on acute toxicity and haematological alterations induced by the exposure of ibuprofen to common carp (*Cyprinus carpio*)

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ABSTRACT

Indiscriminate discharge of pharmaceutical waste into the aquatic ecosystem may pose major health challenges to aquatic biota. The effect of acute exposure to ibuprofen was studied utilizing changes in haematological parameters under static bio-assay method in common carp (*Cyprinus carpio*). For this investigation, the fishes are split into four separate groups. Based on the sub-lethal toxicity research, fishes exposed to 1/10th of LC50 value of ibuprofen. The 96-h LC50 of Ibuprofen was 12.75 mg/L. in *C. carpio*. Therefore, for this experiment, fish were subjected to 0.25, 0.50 and 1 mg/L doses of ibuprofen. At the end of each exposure session (7th day, 14th day and 21st day), fish specimens were anesthetised with tricaine methane sulfonate to permit the collection of blood samples. The RBC count in group IV fishes of ibuprofen treated fishes, at 21 day of exposure, was lowered and it is statistically significant ($p<0.01$) to control fishes. WBC counts in fishes that had 7 and 14 days exposure to ibuprofen were not statistically altered, compared to control rats. Ibuprofen treated fish did not elicit any significant alterations ($p > 0.05$) with reference to monocytes, basophils and eosinophils. Based on the above results, it might be inferred that administration of ibuprofen, may elicit immunological perturbations, and their toxicity may rise depending on the dose.

1. Introduction

A propionic acid derivative, ibuprofen (IBU), is commonly used as a pain reliever, anti-inflammatory, and antipyretic. It's a non-discriminatory cyclooxygenase (i.e. cyclooxygenase 1, COX-1, and cyclooxygenase-2, COX-2) inhibitor that reduces the generation of prostaglandins (PGs), which are important in both animal and human sexual reproduction (Burdan et al. 2006). In mammals, PGs are linked to the regulation of Na+/K+-ATPase activity (Kreydiyyeh and Markossian 2006), and evidence of their significance in osmoregulation in fish gills is growing (Choe et al. 2006). The blocking of the PGs pathway, which affects both cortisol production and sodium pump activation, could be the cause of ion regulation problems in ibuprofen-treated fish (Gravel et al. 2009). The discovery that ibuprofen is a hsp70 inducer (hsp70 is a cellular stress protein that is essential for preventing stressor-mediated proteotoxicity in trout livers and gills) supports the theory that it disrupts ion control (Gravel et al, 2009).

Haematological indices are valuable markers for determining the health of living creatures, such as fish (Nwani et al. 2014). Because of its role as a source of essential nutrients, gases, ions, and endocrine factors, as well as its role as a reservoir for excretory metabolic products, changes in blood parameters are frequently indicative of the overall toxic effects of environmental contaminants, and thus its study can be a useful tool in the assessment of fish health. Haematological, histological, biochemical, and behavioral indicators are frequently employed to assess toxicant action mechanism, immune system integrity, and tissue damage, according to Kavitha et al. (2010).

Most native fish species in India have little scientific record of ibuprofen-induced harmful effects on behavior and haematological markers. There have been research on sublethal concentrations (Kasprzyk-Hordern et al. 2008), but there is little information on point sources (acute studies). The common carp was chosen as the study's model because it is extensively dispersed in tropical freshwater habitats and is well-liked by the local population. The fish is also resilient and adaptable to

laboratory conditions, and it generates sufficient blood for estimating haematological parameters. The goal of the study was to evaluate the ibuprofen 96-hour LC50 in C. carpio and look at the variance in haematological components that could affect the fish's health. The results of this study's endpoints could be used to track the acute and sublethal effects of ibuprofen in freshwater fish.

2. Material and Methods

2.1 Test organism

The specimens were around 16.111.02 cm long and weighed 505 gram. New fish supplies were received on a monthly basis, so fish material was rarely stored in the laboratory aquaria for more than a month. Healthy *Cyprinus carpio* was purchased and transported to the laboratory in plastic buckets with adequate air. The fish specimens were relocated from the plastic buckets to the glass aquaria for 20 days to acclimate, minimize transport-induced stress, and allow for capture-induced mortalities prior to compound treatment.

2.2 Fish Collection and Maintenance

Using common carp (*C. carpio*) as the test organism, a 21-day experiment was conducted in India from July to September 2019. Fish were captured and transported to the laboratory from fish hatchery ponds in Nanded, Maharashtra, India. Fish with an average body weight of 305 g and an average body length of 16.111.02 cm were stocked in aquaria (with a volume of 140 liters of water) two weeks before to the experiment, and aeration was provided with an air pump for 24 hours to allow for adaption. Following the adaption phase, fish of similar mean weight were separated and a survival test with three replications was carried out: At a density of 3.5g L-1, 20 fish were utilized in each repetition. Aeration was provided at all times, and a 12:12 (L:D) photo period was used. Fish were fed at a rate of 1% body weight per day, and 50% of the water was replaced daily.

2.3 Test Compound:

For the present study, ibuprofen was chosen as toxicant based partially on the probability of their having biochemical effects. Analytical grade ibuprofen was purchased from Fischer Scientific India Pvt. Ltd, India and 0.2 ml/l used to prepare the stock solution at different concentrations for acute toxicity study 5, 10, 15, 20, 25 and 30 mg/L due to their low water solubility.

2.4 Acute Toxicity Study:

Median lethal concentration:

Water reconstituted from the following salts: NaHCO3 (174 mg/L), MgSO4 (120 mg/L), KCl (8 mg/L), and $CaSO4.{}_2H_2O$ (120 mg/L) was kept at room temperature with constant aeration and a natural light/dark photoperiod in test systems consisting of 1208040-cm glass tanks filled with water reconstituted from the following salts:

Specimens were exposed to static systems with no food provided during the exposure duration. The median lethal concentration (LC50) of ibuprofen was estimated to set the target concentration to be employed in evaluating biochemical examination of tissue.

Six experimental systems containing different concentrations of ibuprofen (5, 10, 15, 20, 25, and 30 mg/L) in reconstituted water, as well as a seventh ibuprofen-free control system, were set up, and ten carp were randomly selected from the stock and placed in each system (using the random number method).

The LC50 was determined using 10 fish from each group. The exposure time was 96 hours, and the number of dead specimens in each system was counted at the end. The experiment was carried out in quintuplicate. Probit analysis was used to calculate the ibuprofen 96-h LC50 and its 95 percent confidence limits (P0.05).

Fish were exposed to 1/10th of the LC50 value of ibuprofen in sub-lethal toxicity experiments. In C. carpio, the 96-hour LC50 of ibuprofen was 12.75 mg/L. As a result, fish were given ibuprofen concentrations of 0.25, 0.50, and 1 mg/L in this experiment. In natural water, control fish were kept without any treatment.

2.5 Experimental groups and dosage:

Fish (n=42) were gathered and randomly placed into 7 glass aquariums after being fasted for 24 hours. Each treatment group had three tanks, each containing six fish and 25L of test fluid. The control fishes are in Group I. Ibuprofen 0.25 mg/L was given to Group II fish for 21 days. Ibuprofen 0.50 mg/L was given to Group III fish for 21 days. Group IV fish were given 1 mg/L ibuprofen for 21 days. Except for group I, the remaining three groups of fish were given sub-lethal doses of ibuprofen for 24, 48, 72, 96 hours, 10 days, and 20 days, respectively, from the above four groups. The control group, Group I, was kept. All of the groups were fed the same sort of food and had identical living conditions. Fish specimens were sedated with tricaine methane sulfonate at the end of each exposure period (7th, 14th, and 21st days) to allow blood sampling.

2.6 Haematological analysis:

Blood samples were collected from different surviving individuals exposed to the same concentration and transferred immediately (10–20 min) to the laboratory for haematological analysis.

2.7 RBC count

The total red blood cell count was done by instrument-Neubauer Haemocytometer. 0.02 ml of blood was pipetted from the blood sample and added to 4 ml of the RBC diluting fluid (Toisson's solution). This was done to make a 1:200 dilution of the blood sample in a fresh test tube. The mixed blood sample was loaded onto a Neubauer counting chamber and all RBCs in the central area of the Neubauer improved cell counting chamber, were counted using a light microscope at 40 × objective. The red cells are counted in the five i.e. (25) groups of 16 small squares. The number of cells counted for each sample was multiplied by 10,000 to obtain the RBC count per ml of blood.

2.8 WBC count

The total white blood cell count is also made by using Neubauer haemocytometer slide and Turk's fluid is used as a WBC diluting fluid. The white cells are counted in the four large comer squares.

2.9 Differential leucocytes count (Leucogram)

For the determination of leucocytes, 0.02 ml of blood was pipetted into a small test tube containing 0.38 ml of WBC diluting fluid (Turk's) make a 1:20 dilution of the blood sample. The diluted blood sample was introduced into the Neubauer counting chamber and all cells located at the four corner squares were counted using a light microscope at 10 × objective. The total number of WBCs was calculated in mm3 × 104. While counting, the method of Hibiya (1982) was used for the identification and numbers of different classes of leukocytes (neutrophils, monocytes, lymphocytes, eosinophils and basophils) in the blood smears. The number of each type of leukocyte was calculated as a percentage.

2.10 Statistical analysis

Each blood parameter was analyzed three times, with the results subjected to statistical analysis using the students' t' test for significance. Haematological values were averaged and standard deviations were calculated. The normality of the data for haematological parameters was evaluated before a two-way analysis of variance (ANOVA) was used to discover if there were any significant differences between them. Statistical significance was considered as $p<0.05$ in all statistical analyses performed in Microsoft Excel.

3. Results and Dicussion

3.1 Determination of LC50.

Figure-1 and Table-1 demonstrate the median lethal concentrations (LC50) of Ibuprofen for C. carpio after 24, 48, 72, and 96 hours. Table-1 also includes the probit numerical values as well as their 95 percent confidence ranges. In C. carpio, the 96-hour LC50 of ibuprofen was 12.75 mg/L.

Table.1. Median lethal concentrations (24-96 h LC50) of ibuprofen for *C. carpio*

NSAID (mg/L)	Mean LC50 values			
	24 h	48 h	72 h	96 h
Ibuprofen	33.87	28.33	18.83	12.75

According to 96 h LC50=12.75 mg/L of Ibuprofen in *C. carpio*, Fish were exposed to nominal concentration of 0.25, 0.50, and 1 mg/L of Ibuprofen. Control fish were maintained without any treatment in natural water.

3.2 Effect of ibuprofen on RBC and WBC:

The hematocrit levels of ibuprofen-exposed and control fish did not differ significantly (Table-2). Red blood cell (RBC) counts in fish given 1 mg/L ibuprofen for 21 days were not substantially different from control fishes after 7 and 14 days of exposure, but there was a significant, P 0.01 decline in RBC count in fish given 1 mg/L ibuprofen for 21 days (Table 2).

When compared to control fishes, the RBC count in group IV fishes (1mg/L ibuprofen treated) reduced from 6.99+0.53 to 4.70±0.76 ($x10^6$ mm^{-3}) after 21 days of exposure, which is statistically significant (p0.01).

In addition, all concentrations of ibuprofen treatment fishes showed a rise in white blood cell (WBC) count. WBC counts in fish exposed to ibuprofen for 7 and 14 days were not substantially different from control fishes, but there was a significant, P 0.01,P 0.001 increase in WBC counts in fish exposed to ibuprofen for 21 days (Table 7).

When compared to control, the higher WBC count in group IV (1 mg/L ibuprofen) treated fish increased from 4.35+0.26 to 8.20+0.61 after 21 days of exposure, which is statistically significant (p0.001). When ibuprofen-treated fishes are compared to non-ibuprofen-treated fishes, the elevation is significantly higher (88 percent) (86 percent).

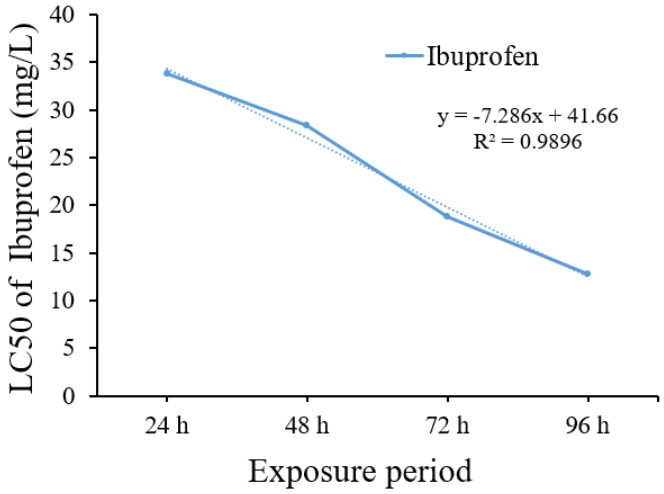

Figure.1 Median lethal concentration (24-96 h LC_{50}) of Ibuprofen for *C. carpio*

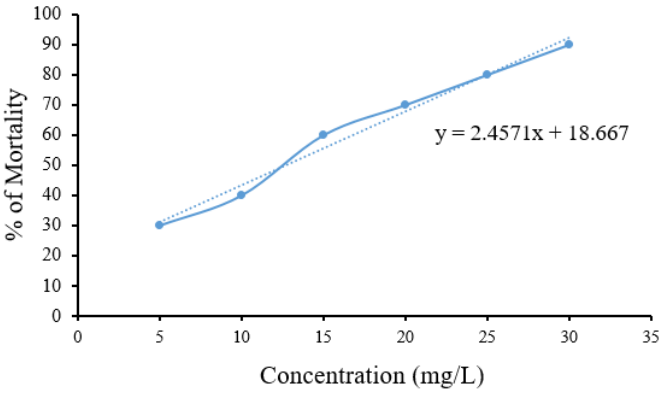

Figure-2. Linear graph of Percentage of mortality at different concentration of ibuprofen

3.3 Effect of ibuprofen on Differential Count of WBC:

Table-2 shows changes in the white cell differentials of C. carpio. The neutrophil count was reduced by I buprofen in a dose and duration-dependent manner. At 21 days after exposure, the maximum neutrophil count in group iv fishes reduced from 34.44±2.75 to 23.07±1.06 in group iv fishes (1 mg/L ibuprofen treated). At the 21st day of exposure, the levels of neutrophils in group IV fish were extremely statistically significant (p<0.01). When comparing group II and group III fishes to group I (control) fishes, there is no statistical significance in the neutrophil count after 7 and 14 days of exposure.

Table 3 also revealed that ibuprofen (1 mg/L) treatment resulted in a rise in lymphocyte count from 55.92+4.32 to 71.44±2.05 relative percentage after 21 days of exposure, which is highly statistically significant at p0.01.

Table-2. Effect of ibuprofen in fish *Cyprinus carpio* with reference to RBC and WBC (x10⁶ mm⁻³) (Values are mean ± SE)

Tissue	Day of Exposure	Experimental Groups			
		Group I (Control)	Group II (0.25 mg/L)	Group III (0.5 mg/L)	Group IV (1 mg/L)
RBC (x10⁶ mm⁻³)	7	6.99±0.53	6.81±0.21	6.55±0.13	5.95+0.54*
	14	6.99±0.53	6.74±1.05	6.21±0.53	5.53+0.79*
	21	6.99±0.53	6.47±0.63	5.38±0.15	4.70+0.76*
WBC (x10⁶ mm⁻³)	7	4.35±0.26	4.55±0.60	4.93±0.64	5.07+0.12*
	14	4.35±0.26	5.80±0.43	5.83±0.88	5.60+0.99*
	21	4.35±0.26	5.99±0.73*	6.90±0.50**	8.20+0.61**

Data expressed as mean ± SEM. * Significant at P < 0.01; ** Significant at P < 0.001.

Table.-3 Effect of ibuprofen in fish *Cyprinus carpio* with reference to WBC differential count (Values are mean ± SE and in relative %)

Tissue	Day of Exposure	Experimental Groups			
		Group I (Control)	Group II (0.25 mg/L)	Group III (0.5 mg/L)	Group IV (1 mg/L)
Lymphocyte count	7	55.92±4.32	55.43±2.70	56.90±2.10	61.44±2.05*
	14	55.92±4.32	56.07±2.20	60.47±2.50	65.66±3.60**
	21	55.92±4.32	65.44±3.80*	66.14±3.19	71.44±2.05**
Neutrophil count	7	34,44±2.75	34.10±2.10	32.04±3.50	26.05±1.16
	14	34,44±2.75	32.61±1.20	29.90±2.30	25.50±2.40
	21	34,44±2.75	27.06±3.67	27.14±3.20	23.07±1.06
Monocyte count	7	1.5±0.03	1.5±0.08	1.4±0.07	1.5±0.04
	14	1.5±0.03	1.4±0.02	1.6±0.03	1.4±0.09
	21	1.5±0.03	1.4±0.03	1.5±0.07	1.6±0.02
Basophil count	7	1.3±0.06	1.4±0.02	1.3±0.08	1.4±0.01
	14	1.3±0.06	1.5±0.04	1.3±0.07	1.5±0.04
	21	1.3±0.06	1.2±0.05	1.2±0.09	1.3±0.03
Eosinophil count	7	1.4±0.09	1.4±0.03	1.5±0.06	1.5±0.08
	14	1.4±0.09	1.6±0.02	1.5±0.09	1.3±0.03
	21	1.4±0.09	1.4±0.03	1.5±0.02	1.4±0.02

Data expressed as mean ± SEM.
Statistically significant comparison of control group and other treated groups (p < 0.05), ***highly significant (p < 0.01)

Ibuprofen-treated fish did not show any significant changes in monocytes, basophils, or eosinophils (p 0.05). Neutrophils are important in the body's defense against harmful invading pathogens, and tissue damage caused by toxicant stress can cause an increase in their number in the blood. Ibuprofen caused neutropenia and lymphocytosis in this study, which was dose and duration dependent. The duration-dependent declines and increases in lymphocyte and neutrophil percentage subpopulations could be linked to drug-induced stress and stress defense. As a result, lymphocytosis is a fish's response to stress caused by the medicine. Neutrophils are phagocytotic, hence a decrease indicates that the fish blood's phagocytotic function has been damaged. In experiments involving fish exposed to fenthion, the neutrophil count was likewise suppressed (Nwani et al. 2016). Diminished neutrophil counts, according to Lohner et al. (2001), may indicate reduced or disturbed phagocytotic capacity and disease resistance. Nwani et al. (2016) discovered a substantial rise in WBCs in *Clarias gariepinus* exposed to pharmaceutical chloramphenicol, while Reddy (2013) found the same in *Catla catla*, a freshwater fish exposed to cadmium.

4. Conclusion

Pharmaceuticals discharged from sewage treatment plants are one of the most common sources of medications in the aquatic environment. The consequences on human, aquatic, and animal health must be thoroughly explored through bio-safety and toxin research. To lessen the problem, conscious efforts are required, as well as proper legislation to assess and control it. The 96-h LC50 of Ibuprofen in *C. carpio* was 12.75 mg/L in this study, indicating that the fish is very susceptible to the medication even at low acute doses. Though this is modest in comparison to literature values, numerous variables could have contributed to the difference, including the drug's heterogeneous metabolism by different fish species. The toxicity of ibuprofen was tested in *Cyprinus carpio* at the behavioral and haematological levels, and fish health was negatively affected at each of these levels. Based on the existing findings, it is possible to conclude that ibuprofen administration may cause immunological disturbances, with toxicity increasing with dose. More research is needed to determine their toxicity in the biological system. Additional

research on the toxicokinetics and toxicodynamics of ibuprofen and ibuprofen in freshwater fishes is needed, however, to have a complete knowledge of the drug's mechanism.

Conflicting Interests

The authors have declared that no conflicting interests exist.

References

[1] Burdan F, Szumilo J, Dudka J, Korobowicz A, Klepacz R. 2006. Congenital ventricular septal defects and prenatal exposure tocyclo-oxygenase inhibitors.Brazilian. *Journal of Medical and Biological Research* 39: 925–934

[2] Choe KP, Havird J, Rose R, Hyndman K, Piermarini P, Evans DH. 2006. COX2 in a euryhaline teleost, *Fundulus heteroclitus*: Primary sequence, distribution, localisation and potential function in gills during salinity acclimation *Journal of Experimental Biology* 209: 1696–1708.

[3] Gravel A, Vijayan MM. 2007. Non-steroidal anti-inflammatory drugs disrupt the heat shock response in rainbow trout. *Aquatic Toxicology* 8: 197–206

[4] Hibiya T. 1982. *An atlas of fish histology: normal and pathological features*. Tokyo: Kodansha

[5] Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Research Journal* 42: 3498–3518

[6] Kavitha C, Malarvizhi A, Senthil Kumaran S, Ramesh M. 2010. Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. *Food and Chemical Toxicology* 48: 2848–2854.

[7] Kreydiyyeh SI, Markossian S. 2006. Tumor necrosis factor alpha down-regulates the Na+-K+-ATPase and the Na+-K+2Clcotransporter in the kidney cortex and medulla. *Cytokine* 33:138–144

[8] Lohner TW, Reash RJ, Willet VE, Rose LA. 2001. Assessment of tolerant sunfish populations (*Lepomis* sp.) inhabiting seleniumladen coal ash effluents. 1. Hematological and population level assessment. *Ecotoxicology and Environmental Safety* 50: 203–216

[9] Modra H, Svobodova Z, Kolafova J. 1998. Comparison of differential leukocyte counts in fish of economic and indicator importance. *Acta Veterinaria Brno* 67: 215–226.

[10] Nwani CD, Mkpadobi BN, Onyishi G, Echi PC, Chukwuka CO, Olua SN, Ivoke N. 2014. Changes in behavior and hematological parameters of freshwater African catfish *Clarias gariepinus* (Burchell 1822) following sublethal exposure to chloramphenicol. *Drug and Chemical Toxicology* 37: 107–130

[11] Nwani, CD, Somdare PO, Ukonze JA, Ejere VC, Nwadinigwe AO, Nwani JC Odo GE, Ugbor ON. 2016. Subchronic exposure to fenthion induces hematological changes in liver tissue of African catfish *Clarias gariepinus*. *Journal of Aquatic Animal Health* 28: 229–234.

[12] Ranzani-Paiva, M. J. T. 1995. Células do sangue periférico e contagem diferencial de leucócitos de tainha *Mugil platanus*, Gunther, 1880 (Osteichthyes, Mugilidae) da região estuarinalagunar de Cananéia SP (Lat. 25° 00′ S–Long. 47° 55′ W). *Boletin Instituto de Pesca* 22: 23–40

[13] Reddy SJ, Reddy DC. 2013. Impact of cadmium toxicity on behavioural and haematological biomarkers of freshwater fish, *Catla catla*. *International Journal of Bioassays* 2: 1199–1204

[14] Romao S, Donatti L, Freitas OM, Teixeeira J, Kusma J. 2006. Blood parameter analysis and morphological alterations as biomarkers on the health of *Hoplias*