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# Assessment of ibuprofen toxicity on haematology of fish *Cyprinus carpio*

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**Abstract**
Water, crucial for human survival, faces constant degradation due to the release of various pollutants like pharmaceuticals, heavy metals, pesticides, and human activities. Present study observed behavioural and haematological changes in freshwater fish, *Cyprinus carpio*, after exposure to a sublethal concentration of Ibuprofen. To assess the impact of ibuprofen, the $LC_{50}$ was calculated at different time intervals *ie* 24 hrs, 48 hrs, 72 hrs, and 96 hr. Lethal concentration was observed 0.23 ml/L. Further investigation involved administering a sublethal concentration of ibuprofen $1/10^{th}$ of $LC_{50}$, *i.e.*, 0.023 ml/L to the fish. After exposure to over 7, 14, 21, and 28 days in treated four sets, significant behavioural alterations in *C. carpio* were observed. Haematological parameters as RBC count, haemoglobin levels, and haematocrit percentage, decreased significantly ($p<0.05$), while WBC count, MCH, and MCHC values increased significantly ($p<0.05$) compared to the control group. MCV values remained unchanged, possibly indicating RBC destruction due to Ibuprofen-induced stress. This research suggests that aquatic organisms, sharing physiological similarities with humans, can serve as models for understanding the impact of contaminants on human health. It highlights the potential consequences of pharmaceutical pollutant exposure to organisms in water bodies, with implications for human health.

**Keywords:** Water, Ibuprofen, toxicity, *Cyprinus carpio*, haematology

## Introduction
It is an old version that "there is more gold in water than on the land". Water is a very complicated and vitally significant substance. It is one of the Earth's most important resources that is essential part of all living beings but water is continually threatened by a variety of contaminants such as heavy metals, hydrocarbons, and pesticides and other anthropogenic activities. In recent years, pharmaceutical goods gained limelight and were termed as "contaminants of rising concern" as their concentration was very high in surface waters and is harmful for aquatic ecosystem. After administration, pharmaceuticals and their excreted metabolites are subsequently released to rivers and other water bodies.

One of the major types of pharmaceuticals is NSAID which are one of the greatest significant classes of medicines, as they are the most usually used in primary care and thus the utmost widely used worldwide (Saravanan *et al*. 2012; Zhang *et al*. 2020) [15, 19]. Ibuprofen is the most popularly used NSAID. It is the third most popular, highly prescribed and most saleable over the counter medicine in the world. It is one of the drugs listed in Essential Drugs list 2010 made by World Health Organization (WHO) which is available even without a prescription. Commonly used for pain relief, including headaches, dental pain, menstrual cramps, muscle aches, arthritis, flu, and inflammation, Ibuprofen is typically taken orally in forms like tablets, capsules, and liquids. It's available under various brand names such as Brufen and Advil for all kind of pain relief with some side effects. The impact of ibuprofen along with its metabolites, which are excreted through urine, further degrades the water making it unfit for consumption and aquatic creatures to live in.

Fish live in extremely close contact with their environment, and therefore they are very susceptible to any physicochemical changes. It is also a good bio indicator for revealing the water quality, highly adaptable and can thrive in various aquatic environments, including slow-moving or still waters like rivers, lakes, ponds, and even brackish waters. Pharmaceuticals are intended to be biologically active, and once in the water, they can harm fish.

These enter the fish body through skin, gills or by ingestion. Three native big carps and three exotic carps make up the majority of Indian aquaculture production. *Cyprinus carpio*, known as common carp, belongs to the cyprinid order and the cyprinidae family, which is the biggest freshwater fish family and wide geographic distribution. It is a versatile and adaptable fish species that plays a multifaceted role in ecosystems, aquaculture, cultivated and cultural traditions around the world and holds economic value in various ways, contributing to both local and global economies.

Carp fish is a nutritional powerhouse, offering essential fatty acids, protein, minerals, and fat-soluble vitamins like A, E, and D. With 127 calories and 17.8g of protein per 100g, it's a valuable dietary source. Carp provides moderate levels of omega-3 fatty acids crucial for nervous system development, particularly in infants and children, promoting heart health. This fish is also rich in B-complex vitamins, including niacin and pyridoxine (B-6), as well as vitamin-E, vitamin-B12, thiamine, and riboflavin. Furthermore, it's a natural source of essential minerals such as iodine, selenium, phosphorus, calcium, zinc, potassium, and magnesium. Iodine, vital for thyroid hormone production, is particularly noteworthy for human nutrition and hence its consumption is widespread in various parts of the world.

They can tolerate a range of water conditions and temperatures and the impact of the toxicant can be well understood by analysing the fish's behaviour or blood or serum of the fish, because blood is a pathophysiological reflector of the whole body (Sharma and Singh, 200:2006). Fish exposed to chemical pollutants may experience fluctuations in their hematological levels, which can either increase or decrease. The toxicant induced changes mainly depended on the fish species, age, the cycle of the sexual maturity of spawners and diseases. It even causes modifications to cellular responses in the liver, kidney, and gills; disturbance of the endocrine system through changes in aromatase activity, affecting sex hormone balance.

The current study's primary goal was to examine the haematological changes in *Cyprinus carpio* fish after inducing sublethal doses of Ibuprofen for 7, 14, 21 and 28 days in treated sets.

## Materials and Methods

In the month of May, 2023, sixty freshwater fishes, *Cyprinus carpio*, were collected for experimental purpose from Johilla river located in Amarkantak near University campus, Lalpur, Anuppur district, Madhya Pradesh, India, located at 23°18 North 81°15 East.

Samples of adult fishes of both sexes, male and female, of approximately the same length (15-30 cm) and weight (150-240 g) procured using seine net of size 70 m x 80 m with mesh size of net 35-40 mm with the help of local fisherman.

After collecting, Fishes were brought to Aquatic Toxicology Laboratory, Department of Zoology, IGNTU, Amarkantak, by local transportation method in 15 Ltr oxygenated plastic containers disinfected (1% $KMNO_4$) Fishes were not subjected to any stress like temperature, dehydration and crowding.

*Cyprinus carpio* has its body covered with large dark yellow-gold cycloid scales. Inferior mouth and highly extendable to form a proboscis. It has a long snout and in the corners of the mouth are two pairs of short mandibular barbels these barbels help carp locate food by detecting odors and vibrations. Long dorsal fin and short anal fin. Identification of the fish has been done by the following fin formula.

### Acclimatisation and maintenance

The fishes were acclimated for 7 days before performing the experiment. In the Aquatic Toxicology Laboratory, eight aquaria marked as aquarium I, II, III, IV, V, VI, VII and VIII were disinfected with 1% $KMNO_4$ solution prior to bringing the fishes. After bringing the fishes in the laboratory they were disinfected with 1% $KMNO_4$ solution to avoid any dermal infection. Fishes were weighed with the help of a digital hook weighing machine (GLUN) and were carefully stocked in well aerated aquaria (76 x 37.5 x 37.5 cm) of capacity approximately 100 L containing seasoned tap water of temperature 28±2 ˚C measured by laboratory thermometer and pH 6.8-7.2 measured using a digital pH meter (Electronics India, Model – 111).

During the acclimation period, water was renewed daily. Aquarium was marked at 25 L water level and the evaporated water was refilled up till the mark. To maintain the temperature and avoid suffocation the fans of the lab were kept on as well as windows were kept open for the whole time. The fishes were fed twice daily at 10 AM and 5 PM with artificial food pellets, Tokyu, available from the market and about 3-4 food grains were given to each fish. Faecal matter was removed every day from the water in the experimental tanks with the help of a siphon pipe to avoid fouling.

### Test Compound

For the experiment purpose 20 film coated ibuprofen tablets, Brufen 400 mg, manufactured by Abbott pharmaceuticals, were purchased from a local pharmaceutical store in Rajendragram and was used to prepare different concentrations. Ibuprofen, an analgesic and non-steroidal anti-inflammatory drug (NSAID), is sold under many brand-names around the world. The most common are Brufen, Ibugesic, Advil, Motrin, and Nurofen. Chemical names 2-(p-isobutylphenyl) propionic acid and 2-(p-isobutylphenyl) propionic Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) with molecular formula $C_{13}H_{18}O_2$**,** IUPAC Name-(RS)-2-(4-(2-methylpropyl)phenyl) propanoic acid

The use of paracetamol and ibuprofen together for multimodal pain treatment produces better analgesia. The very common brand name in India is Combiflam (Ibuprofen 400mg + paracetamol 325 mg) and Aceclofenac.

Experimental design-The experiment was divided into two parts-Experiment I-For $LC_{50}$ determination-The data was analysed statistically by log dose/probit regression line method (Finney, 1971).

Experiment II-For haematological alteration determination, 30 healthy *C.carpio* fishes were selected for second experiment after acclimating. The selected fishes were kept in 5 different aquaria marked as control I, and treated II, II, IV and V with 6 fishes in each aquarium, to analyse the haematological alteration produced by exposure of sublethal (1/10) ibuprofen.

### Sample collection

Blood sample was collected to study haematological alterations after 7th, 14th, 21st and 28th days of exposure by using the method of Bello *et al* (2014) from each fish of aquaria. Fish was taken out from the aquarium using a fish net and placed in a fish tray. Fish sacrificed by cervical dislocation by a little struck on its head with the help of hand. Blood was collected from the gills using sterilised Dispo van

needles and heparinized 5 ml (BD Emerald$^{TM)}$ syringes. Blood was collected using gentle aspiration until approximately 3 ml was recovered. The needle was carefully withdrawn and blood stored in EDTA coated Eppendorf tubes which is an anticoagulant and transferred immediately (10–20 min) to the central laboratory for haematological analysis of the parameters Total Erythrocyte Count (RBC)-Rusia and Sood method (1992), Total Leukocyte Count (WBC)-Rusia and Sood method (1992) [14], Haemoglobin-Wintrobe method (1974), Hematocrit-Nelson and Morris (1989) [12] and Erythrocyte indices-Dacie and Lewis (2011) [7] was observed, For statistical analysis t Test applied.

## Results

$LC_{50}$ was observed after 24, 48, 72, and 96 hours after exposing the fish to ibuprofen at different concentrations 0.1, 0.2, 0.4 and 0.8mg in aquaria I, II, III and IV respectively. $LC_{50}$ was observed after 96 hours $LC_{50}$ = 0.234 (Table I) and sub lethal dose 1/10 (0.023 ml/L) was taken for study.

The exposure of sub lethal concentration of Ibuprofen to *C. carpio* induced a change in the behaviour of the fish after 28 days. Abnormal behaviour was characterised by hyperactive and jerky movements, loss of equilibrium, irregular fin movements, increased mucus secretions and erosion of fins and settling at bottom in groups. These behavioural responses were dose dependent i.e., the higher the concentration, the stronger the response.

The decrease in the values of RBC, Hb, Hematocrit with the increase in days of exposure of Ibuprofen is very highly significant after 7, 14, 21 and 28 days (Table 1 and Fig I) The increase in the values of WBC, MCH, MCHC with the increase in days of exposure of Ibuprofen is significant after 7 days and very highly significant after 14, 21 and 28 days

**Table 1:** Toxicity evaluation of Ibuprofen to *Cyprinus carpio* specifying fiducial

| Experimental Animal | Compound | Regression Equation | $LC_{50}$ | Variance | Fiducial limit |
|---|---|---|---|---|---|
| *Cyprinus carpio* | Ibuprofen | Y = 5.08 + 1.81(Xm-2.43) | 0.0234 ml/L | 0.027 | $M_1$ = (+) 2.43<br>$M_2$ = (+) 2.33 |

**Table 2:** Hematological parameters in blood of *Cyprinus carpio* after Ibuprofen toxicity

| Parameters | Control | Exposure time (treated group) | | | | Result |
|---|---|---|---|---|---|---|
| | | 7 days (Group I) | 14 days (Group II) | 21 days (Group III) | 28 days Group IV) | |
| RBC | 6.85±0.08 | 5.85± 0.08*** | 4.73± 0.11*** | 3.80±0.08*** | 2.98±0.08*** | ↓ |
| WBC | 4.77± 0.04 | 5.30±0.11* | 6.34± 0.13*** | 7.26±0.20*** | 7.92±0.11*** | ↑ |
| Hemoglobin | 11.74±0.17 | 10.74±0.15*** | 9.74±0.17*** | 8.74±0.16*** | 7.73±0.17*** | ↓ |
| Hematocrit | 52.68±0.76 | 44.99±0.68*** | 36.40±0.83*** | 29.1±0.94*** | 22.94±0.62*** | ↓ |
| MCH | 17.16±0.02 | 18.38±0.12*** | 20.65±0.55** | 23.10±0.51*** | 25.96±0.37*** | ↑ |
| MCHC | 22.30±0.14 | 23.89±0.45* | 26.85±0.59*** | 30.03±0.69*** | 33.76±0.54*** | ↑ |

Values are Mean± SE and t value, * Significant ($p$<0.05), **-Highly Significant ($p$<0.01), *** – Very highly significant ($p$<0.001)

## Discussions

In this study, fish *C. carpio* were exposed to varying concentrations of Ibuprofen for different durations i.e., 24, 48, 72, and 96 hours, to assess its toxicity and calculate the lethal concentration ($LC_{50}$), which was observed 0.234 ml/L and sublethal concentration of Ibuprofen (1/10) of $LC_{50}$ was 0.023ml/L. The toxicity of Ibuprofen can vary significantly among different species and even within strains of the same species. For instance, previous studies have reported $LC_{50}$ values of 132.6 mg/l in *Daphnia magna* by Han *et al*. (2006) [9], 22.3 mg/l in *Hydra attenuate* Quinn *et al*., (2008) [13], 142 mg/l in the fish *Cirrhinus mrigala* (Saravanan *et al*., 2011) [16], and 175.6 mg/L in *Cyprinus carpio* by Islas-Flores *et al*. (2014) [10]. $LC_{50}$ in freshwater fish *Clarius gariepinus* was 0.38mg/L. Additionally, $LC_{50}$ values can be influenced by factors such as fish age, drug formulation, fish weight, and water quality, potentially explaining the lower $LC_{50}$ value observed in current investigation (0.234 ml/L).

Behavioural changes after exposure of C. carpio to sublethal concentration may be due to the stress produced by Ibuprofen on fish's body. Observed similar behavioural changes in Clarias gariepinus. Gautam & Rani in the year (2008) assessed same finding in Labeo rohita observed drastic changes in behaviour of *Channa punctatus* after nuvan toxicity.

## Hematological Alterations

Blood serves as a comprehensive physiological indicator of the entire body, making blood measurements valuable in assessing the structural and functional status of organs affected by exposure to toxic substances. As a result, the current investigation focused on change in alteration in the haematology of common carp, *Cyprinus carpio*, subjected to Ibuprofen for 7, 14, 21 and 28 days in 4 different treated aquaria with the control set, RBC, WBC, Hb, Hematocrit (PCV) and erythrocyte indices (MCV, MCH and MCHC) in *Cyprinus carpio* changed significantly after sublethal exposure of Ibuprofen. Present findings observed were highly significant at p values ($p$<0.001) and ($p$<0.01).

The results of present research on blood parameters showed a reduction in the count of red blood cells in fishes exposed to pharmaceuticals for 7, 14, 21 and 28 days respectively (Table I, Fig I). This might be due to stress produced by the toxicant in the body of the fish and hence there might be a destruction of RBC. Besides transport of oxygen, red cells have other functional tasks in the body; therefore, an insufficient quantity and quality of erythrocytes would consequently have several additional effects on metabolism beyond the simple oxygen supply task for tissue metabolism. In general, reduction in red cell number is directly proportional to the amount of haemoglobin and is therefore deleterious for oxygen transport. Any blood dyscrasia and degeneration of the red blood cells could be ascribed as pathological in fishes exposed to toxicants. Changes in red blood cell counts have been reported by Clark *et al*. (1979) to be strong indicators of stress due to presence of toxicants or pollutants in the aquatic environment. Similarly, Saravanan *et al*. (2011) [16] investigated the Eco toxicological impacts of clofibric acid and diclofenac in common carp *C. carpio* and demonstrated a reduction in RBC count caused either by the inhibition of

erythropoiesis or by the destruction of red blood cells by the drugs CA and DCF. In another research by Ambili *et al.* (2013) [2] on the effects of the antibiotic oxytetracycline on Indian major carp *Labeo rohita* it was reported that due to prolonged exposure of the antibiotic, the gill region was affected, resulting in impaired osmoregulation and anaemia and leading to a decrease in RBC count. In the year 2004, Adhikari *et al*. revealed remarkable decline in the level of RBC, Hb content and Ht along with significant elevation in MCV and MCH in Cypermethrin exposed fresh water Carp *Labeo rohita.* Also, Velisek *et al*. (2009) reported 96-hour acute response of Alimethrin 10EM on rainbow trout (*O. mykiss*) observing significant decrease in developmental forms of myeloid sequence and segmented neutrophil granulocyte in the experimental group.

The WBC count in treated fishes exposed to the drug were increased after 7 days, 14 days, 21 days and 28 days than control set (Table I, Fig II). This might be due to the stimulatory effect of the toxicant on the immune system and release of lymphocytes from lymph myeloid tissue which act as a defence mechanism which may increase WBC count in fishes.

Primarily, WBCs are involved in the regulation of immunological function in many organisms and the changes in WBC number to pollutants reflect the decrease in the non-specific immunity of the fish. In the present study also the increase in WBC count in both CA and DCF treatments indicate a generalised immune response to drug toxicity or the immune system may be stimulated by the drugs to protect against toxicity.

Saravanan *et al*. (2012) [15] reported an increase in the WBC count in fishes treated with Ibuprofen for 35 days showing that the stimulatory effect of the toxicant on the immune system and release of lymphocyte from lymph myeloid tissue act as a defence mechanism which may increase WBC count in fishes. Similarly, reported a significant increase in WBCs in *Clarias gariepinus* exposed to pharmaceutical chloramphenicol and Reddy (2013) [25] found the same for the freshwater fish *Catla catla* exposed to cadmium. The increase in WBC count can also be due to increased antibody production which helps in survival and recovery of fish exposed to sublethal concentration of toxicant. Haemoglobin results showed that the values of haemoglobin reduced consecutively when compared to the control set for 7 days, 14 days, 21 days and 24 days respectively (Table I, Fig III) which indicates poor oxygen transport by blood caused by damage of red cells or due to increased accumulation of $CO_2$ in blood. Decrease in red blood cell count of goldfish *Carassius auratus* was noticed when subjected to hypoxia (anoxia). This was evidently due to red cells aggregating in the gills. Decrease in Hb indicates poor oxygen transport by blood caused by damage of red cells or due to increased accumulation of $CO_2$ in blood.. *Clarias gariepinus* treated to malathion reduced red blood cell count, haemoglobin concentration, and packed cell volume values.

Haematocrit exposure of Ibuprofen resulted in decreased level of Hematocrit or Packed Cell Volume as for 7, 14, 21 and 28 days (Table I, Fig IV). These results signify haemodilution anaemia which may result from excessive fluid in the blood. It also depicts anaemia in certain cases. Fall of haematocrit value may also result from hydraemic or haemodilution or excessive fluid in the blood. On the contrary increase in PCV values indicates an increase in red cell population which may occur as a result of decrease of oxygen supply. 'Stress' in fish produces haemoconcentration initially with elevated blood lactate, increased glucose concentration with higher Ht and Hb concentration (haemoconcentration). An alteration in the fish metabolism would have also led to decrease values of Ht value (Srivastava and Mishra, 1979) [17]. Erythrocyte indices- In the present investigation the increase of MCH and MCHC were seen in Ibuprofen treated fish which was simultaneous with time increase but no significant difference was seen in the value of MCV of treatments in all the concentrations (Table I, and Fig V, VI). Most common reason for high levels of MCH and MCHC values is macrocytic anaemia, which is a blood disorder in which the body fails to produce enough red blood cells.

Erythrocyte indices (MCV, MCH and MCHC) are widely used for determining the size, content and density of Hb in red blood cells. (Ambili *et al*., 2013) [2].

The increase in MCV may be due to the increase of immature RBC. In addition, anaemia due to disorders in gas exchange across the gills may lead to the increase of MCH and MCV An increase in MCV and MCH levels also indicates the swelling of RBCs which is caused by the toxicity of the drug.

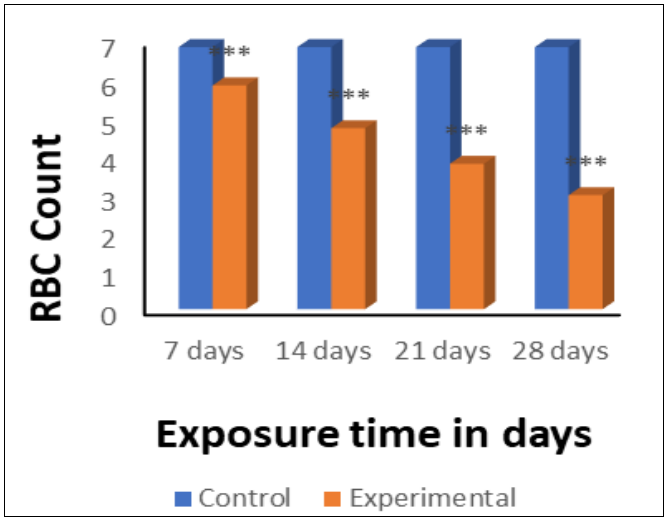


**Fig 1:** Effect of Ibuprofen toxicity on RBC of *Cyprinus carpio*

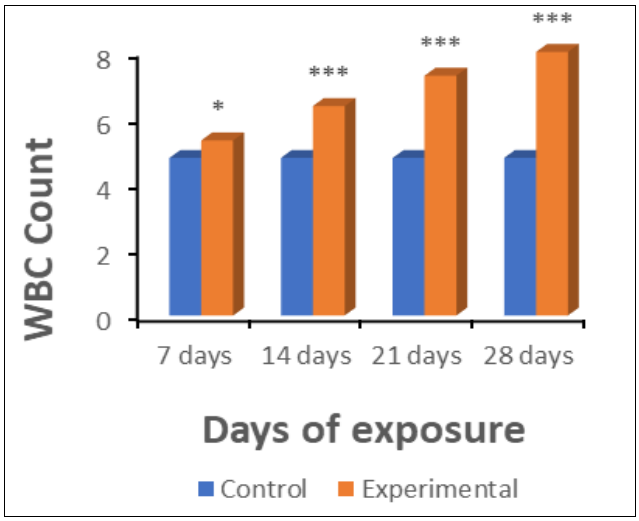


**Fig 2:** Effect of Ibuprofen toxicity on WBC of *Cyprinus carpio*

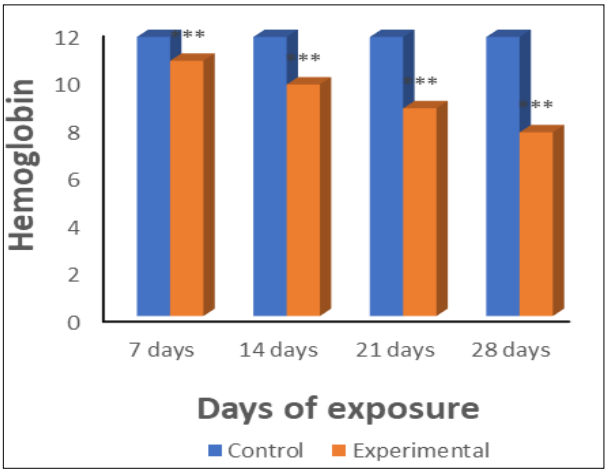


**Fig 3:** Effect of Ibuprofen toxicity on Hemoglobin of *Cyprinus carpio*

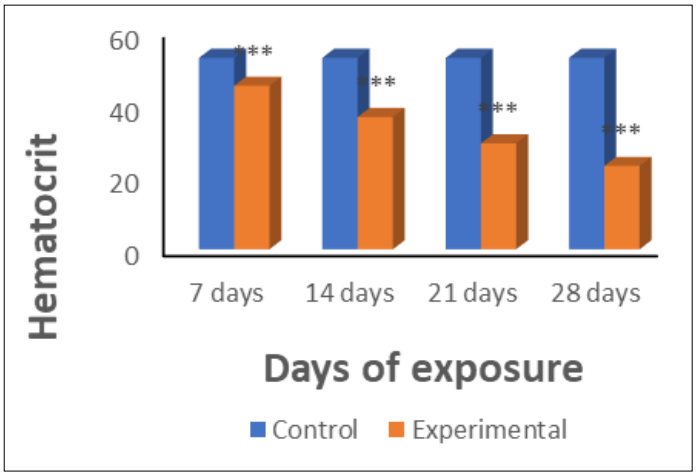


**Fig 4:** Effect of Ibuprofen toxicity on Hematocrit of *Cyprinus carpio*

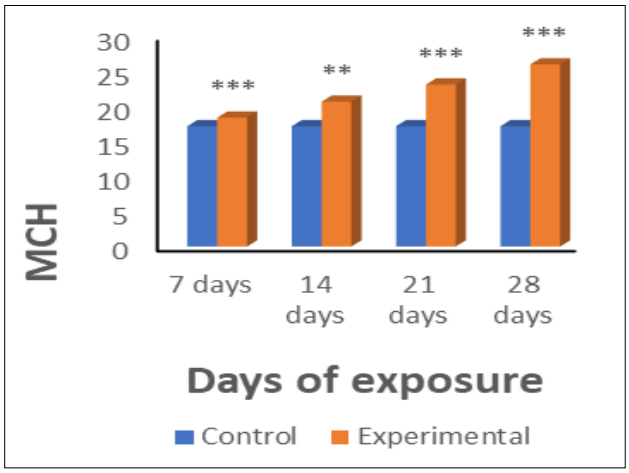


**Fig 5:** Effect of Ibuprofen toxicity on MCH *Cyprinus carpio*

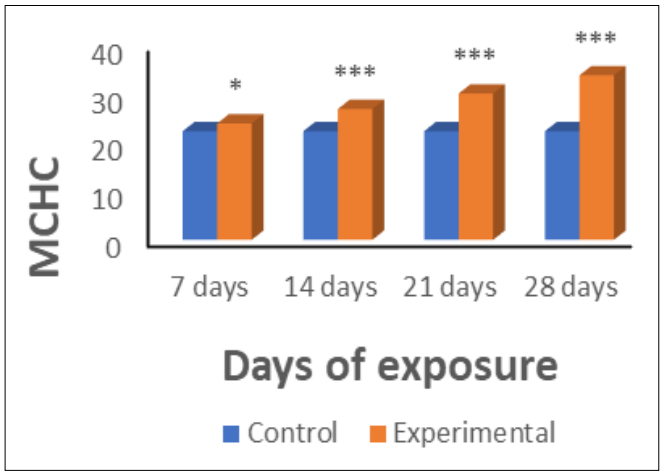


**Fig 6:** Effect of Ibuprofen toxicity on MCHC of *Cyprinus carpio*

Values are Mean± SE and t value, * Significant ($p$<0.05), **- Highly Significant ($p$<0.01), *** – Very highly significant ($p$<0.001)

**Conflict of interest**

It is certified that all authors have contributed significantly and all authors agree with the content of the manuscript and have no conflicts of interest.

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**Author Contribution:** Equally

**References**

1. Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S. Effects of cypermethrin and carbofuran on haematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton, 1849). Ecotoxicol Environ Saf. 2004;58:220–226.
2. Ambili T, Saravanan N, Ramesh M, Abhijith D, Poopal R. Toxicological effects of the antibiotic Oxytetracycline to an Indian Major Carp *Labeo rohita*. Arch Environ Contam Toxicol. 2013;64:494–503. DOI:10.1007/s00244-012-9836-6.
3. Bartoskova M, Dobsikova R, Stancova V, Zivna D, Blahova J, Marselek P, *et al*. Evaluation of Ibuprofen toxicity for zebrafish (*Danio rerio*) targeting selected biomarkers of oxidative stress. Neuroendocrinology Letters. 2013;34(Suppl. 2):102–108.
4. Bello OS, Olaifa FE, Emikpe BO. Haematological and blood biochemical changes in African catfish, *Clarias gariepinus* fed Walnut (*Tetracarpidium conophorum* Mull Arg.) Leaf and Onion (*Allium cepa* Linn) Bulb supplemented diets. Am J Exp Agric. 2014;4:1593–1603.
5. Bendz D, Paxéus NA, Ginn TR, Loge FJ. Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden. J Hazard Mater. 2005;122:195–204.
6. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. J Fish Biol. 1973;5:771-78.
7. Buckley JA, Whitmore CM, Matsuda RI. Changes in blood chemistry and blood cell morphology in coho salmon, *Oncorhynchus kisutch* following exposure to sublethal levels of total residual chlorine in municipal wastewater. J Fish Res Board Can. 1976;33:776-782.
8. Clark S, Whitmore, DH, Jr, McMahon RF. Considerations of blood parameters of largemouth bass, *Micropterus salmoides*. J Fish Biol. 1979;14:147-158.
9. Dacie JV, Lewis SM. Practical haematology. 11th ed. Churchill Livingstone; c2011.
10. Finney DJ. Probit analysis. Cambridge University Press; c1971. p. 123–125.
11. Guiloski PC, Coelho Ribas JL, Pereira LDS, Neves APP, Silva HC. Effects of trophic exposure to dexamethasone and diclofenac in freshwater fish. Ecotoxicol Environ Saf. 2015;114:204-21.
12. Han GH, Hur HG, Kim SD. Ecotoxicological risk of

pharmaceuticals from wastewater treatment plants in Korea: occurrence and toxicity to *Daphnia magna*. Environ Toxicol Chem. 2006;25:265–271.
13. Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, García Medina S, Neri-Cruz N, Dublán-García O. Effect of Ibuprofen exposure on blood, gill, liver, and brain on common carp (*Cyprinus carpio*) using oxidative stress biomarkers. Environ Sci Pollut Res; c2014. DOI: 10.1007/s11356–013–2477–0.
14. Jhosi P, Bose M. Effect of Lindane and Malathion Exposures to Certain Blood Parameters in a Fresh Water Fish *Clarias batrachus*. Pollut Res. 2003;21:55-57.
15. Li ZH, Zlabek V, Li P, Grabic R, Velisek J, Machova J, Randak T. Biochemical and physiological responses in liver and muscle of rainbow trout after long-term exposure to propiconazole. Ecotoxicol Environ Saf. 2010;73(6):1391-1396.
16. Li ZH, Velisek J, Grabic R, Li P, Kolarova J, Randak T. Use of hematological and plasma biochemical parameters to assess the chronic effects of a fungicide propiconazole on a freshwater teleost. Chemosphere. 2011;83(4):572-578.
17. Luskova V. Annual cycles and normal values of hematological parameters in fishes. Acta Sciantiarum Naturalium. 1997;31:70.
18. Nwani CD, Mkpadobi BN, Onyishi G, Echi PC, Christian CO, Oluah SN. Changes in behavior and hematological parameters of freshwater African catfish *Clarias gariepinus* (Burchell 1822) following sublethal exposure to chloramphenicol. Drug Chem Toxicol. 2014;37(1):107-113.
19. Nelson DA, Morris MW. Basic methodology, haematology and coagulation. In: Nelson DA, Henry JB, eds. Clinical diagnosis and management by laboratory methods. 17th ed. WB Saunders; c1989. p. 578–625.
20. Ogueji EO. Toxicity of Chlorpyrifos-ethyl and Lambda-cyhalothrin (Pesticides) to juveniles of African catfish *Clarias gariepinus* (Burchell, 1822). PhD thesis, Ahmadu Bello University, Zaria; c2008.
21. Ogueji EO, Nwani CD, Iheanacho SC, Mbah CE, Okeke CO, Yaji A. Acute Toxicity Effects of Ibuprofen on Behaviour and Haematological Parameters of African Catfish *Clarias gariepinus* (Burchell, 1822). African J Aquat Sci. 2018;43(3):293–303. doi:10.2989/16085914.2018.146539.
22. Quinn B, Gagné F, Blaise C. An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, *Hydra attenuate*. Sci Total Environ. 2008;389:306–314.
23. Rani R, Gautam RK. Effect of Nuvan on Kidney Lipids of Freshwater Fish *Labeo rohita*. J Natcon. 2007;19(2):259–263.
24. Rusia V, Sood SK. Routine hematological tests. In: Mukerjee KL, ed. Medical laboratory technology. Tata McGraw Hill; c1992. p. 252–258.
25. Saravanan M, Devi KU, Malarvizhi A, Ramesh M. Effects of Ibuprofen on hematological, biochemical and enzymological parameters of blood in an Indian major carp, *Cirrhinus mrigala*. Environ Toxicol Pharmacol. 2012;34:14–22.
26. Saravanan M, Karthika S, Malarvizhi A, Ramesh M. Ecotoxicological impacts of clofibric acid and diclofenac on common carp (*Cyprinus carpio*) fingerlings: hematological, biochemical, ionoregulatory and enzymological responses. J Hazard Mater. 2011;195:188.
27. Srivastava AK, Mishra S. Blood dyscrasia in a teleost fish, *Colisa fasciatus*, associated with cadmium poisoning. J Comp Pathol; c1979. p. 609-613.
28. Wintrobe MM, Landsberg JW. A standardized technique for the blood sedimentation test. Am J Med Sci. 1935;189:102,1.
29. Zhang S, Wang J, Zhu DQ. Evaluation of Drug-Related Problems Among Elderly Chronic Disease Patients in Communities and Influence Factors. Cent South Pharm. 2020;18(6):148–153.
30. David A, Pancharatna K. Effects of acetaminophen (Paracetamol) in the embryonic development of zebrafish, *Danio rerio*. J Appl Toxicol. 2009;29:597–602.
31. Ebele AJ, Abdallah MA, Harrad S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerging Contaminants. 2017;3:1–16.
32. Zhang JS, Zhang X, Giesy JP. Endocrine disruption and consequences of chronic exposure to Ibuprofen in Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna* and *Moina macrocopa*. Aquat Toxicol. 2010;98:256–264.
33. Kavitha C, Malarvizhi A, Senthil Kumaran S, Ramesh M. Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. Food Chem Toxicol. 2010;48:2848–2854.
34. Praskova E, Voslarova E, Siroka Z, Macova S, Plhalova L, Bedanova I, *et al*. Comparison of acute toxicity of ketoprofen to juvenile and embryonic stages of *Danio rerio*. Neuroendocrinol Lett. 2011;32:117–120.
35. Reddy SJ, Reddy DC. Impact of cadmium toxicity on behavioural and haematological biomarkers of freshwater fish, *Catla catla*. Int J Bioassays. 2013;2:1199–1204.
36. Somdare PO, Nwani CD, Nwadinigwe AO, Nwani JC, Odo GE, Ugbor ON, *et al*. Fenthion induced toxicity and histopathological changes in gill tissue of freshwater African catfish, *Clarias gariepinus* (Burchell, 1822). Afr J Biotechnol. 2015;14:2103–2113.
37. Stancova V, Zivna D, Blahova J, Marselek P, Zelnickova L, *et al*. Evaluation of Ibuprofen toxicity for zebrafish (*Danio rerio*) targeting on selected biomarkers of oxidative stress. Neuroendocrinol Lett. 2013;34(Suppl. 2):102–108.
38. Velisek J, Svobodova Z, Piackova V, Sudova E. Effects of acute exposure to metribuzin on some hematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). Bull Contam Toxicol. 2009;82:492-495.
39. Wintrobe MM, Lee GR, Boggs DR, Bithei T, Attens JW, Foerster J. Clinical hematology. Lea & Febiger, Philadelphia; c1974. p. 1-3.
40. Zaki MS, Mostafa SO, Nasr S, Noor El-Deen AI, Ata NS, Awad IM. Biochemical, clinicopathological and microbial changes in *Clarias gariepinus* exposed to pesticide malathion and climate changes. Reports Opinion; c2009. p. 6-11.