Hydrobiological Research 1(1), 1-9, 2023
https://doi.org/10.5281/zenodo.8328941
https://hydrobiologicalresearch.com

Research Article

Hematological parameters in rainbow trout, *Oncorhynchus mykiss*, after exposure to ibuprofen

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Received: 05.06.2023 Accepted: 01.08.2023 Published Online: 11.09.2023

Abstract

Ibuprofen, a non-steroidal anti-inflammatory drug, has been found in the aquatic environment, but its effects on fish and other aquatic organisms remain largely unknown. The aim of this study was to investigate the effect of Ibuprofen on haematological parameters in rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to immersion two different doses of ibuprofen concentrations (50 and 500 µg/L) for 96 hours. Exposure to acute toxicity resulted in abnormal behavior of some fish. In this study, a significant decrease in red blood cells (RBC) and mean cellular hemoglobin concentration (MCHC) were observed throughout the study period. Hematocrit (Hct), Haemoglobin (Hb), white blood cells (WBC), mean cellular volume (MCV) and mean cellular hemoglobin (MCH) levels were increased. This study demonstrated that different doses of Ibuprofen were associated with different toxic effects on the hematological parameters in rainbow trout.

Keywords: Ibuprofen, Rainbow trout, Haematological parameters

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively utilized pharmaceutical drugs by individuals worldwide. They are readily available and commonly used, often accessible as over-the-counter medications, which contributes to their presence in various environmental settings (Ascar et al., 2013). Diclofenac, ibuprofen, indomethacin, naproxen, and phenazone are among the NSAID agents that have been detected in surface waters (Termes, 1998; Thomas & Hilton, 2004; Richards et al., 2011; Pluciennik-Koropczuk, 2014). Ibuprofen serves as an example of non-steroidal anti-inflammatory drugs (NSAIDs), which are extensively prescribed medications on a global scale (Green, 2001; Burke et al., 2006).

The use of hematological parameters has been widely employed in various studies to assess the toxic effects of chemical substances on the aquatic environment. Hematology has emerged as an important tool in understanding the impacts of contaminants in aquatic ecosystems. Researchers have investigated hematological abnormalities in fish species exposed to NSAIDs such as diclofenac (Saravanan et al., 2011; Ghelfi et al., 2016) in and, as well as ibuprofen (Saravanan et al., 2012) in. Similarly, changes in the hematological profile of exposed to dipyrone have been observed (Pamplona et al., 2011).

Ibuprofen has been detected in aquatic environments and has the potential to affect hematological parameters. This pharmaceutical compound can pose harm to fish species. Therefore, the objective of this study was to assess the hematological effects on rainbow trout following exposure to Ibuprofen.

MATERIALS AND METHODS

Experimental Fish and Chemical Supply

Rainbow trout for the experiment (approximate weight: 20 g) were obtained from İnönü University, College of Surgu, Aquaculture Station in Malatya (Türkiye). The fish were kept in a 100 L fiberglass tank. Prior to each experiment the fish were transferred tank containing aerated well water and acclimatised for a minimum for 14 days. Fish were fed *ad libitum* with a commercial (Ecobio, Türkiye) feed throughout the experiments.

The mean quality parameters of water used for preparation of test solutions were: dissolved oxygen 7.8 ± 0.5 mg/L, temperature 12±0.7°C, pH 7.4±0.8. No critical levels were detected for NO_2 and NO_3.

Ibuprofen and other all chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Experimental Plan and Hematological Assay

Ibuprofen was dissolved in dimethyl sulfoxide (DMSO). The experimental water was kept in the tank for 24 h before ibuprofen was added. Two different concentrations (50 and 500 µg/L) of ibuprofen were given to the fish for 96 h. DMSO was used as a solvent control for the ibuprofen treatment. Ten fish were used for each treatment. After each exposure period of diazinon, blood from the fish was collected from the caudal peduncle and immediately analyzed for the estimation of hematological parameters.

The erythrocyte level was determined in a hemocytometer using Natt & Herrick solution (1952), using the technique reported by Wintrobe (1934). The amount of hemoglobin (Hb) was determined spectrophotometrically at 540 nm using the cyanomethemoglobin method (Drabkin, 1946). Hematocrit (Hct) was determined as the volume occupied by erythrocytes in microhematocrit tubes. White blood cells (WBC) were detected according to Ispir & Dorucu (2005).

Statistical Analysis

All experiments were conducted mean values and standard deviations of the data of hematological parameters were calculated from the experimental data obtained. Mean significance of hematologic parameters was analyzed using analysis of variance for experimental groups. Differences between the mean values were considered significant when $p < 0.05$.

RESULTS

Changes in the hematological profiles of fish exposed to acute Ibuprofen exposure for 96h were given in Figure 1-7. Blood parameters namely erythrocyte, WBC, haemoglobin, hematocrit, MCV and MCH mean levels increased as dose-dependent in exposure to Ibuprofen of the fish, but the level of RBC and MCHC decreased. In this study, Ibuprofen demonstrated an enhancing effect on hematological parameters in fish. The exposure to 500 µg/L. Ibuprofen resulted in a significant increase in WBC, Hb, Hct, MCV, and MCH parameters. Exceptionally, the level of MCV after 96 h exposure of Ibuprofen was higher than in the control group. However, the level of RBC and MCHC after 96 h exposure time was found to be lower than the control (Figure 3 and Figure 7). There was also statistically significant differences in levels of blood parameters for different exposure doses ($p < 0.05$).

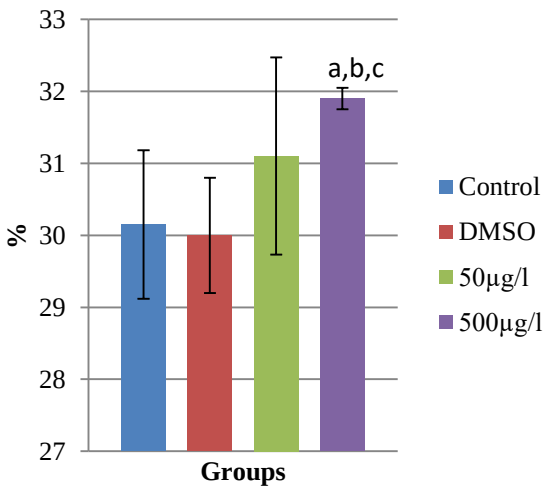

Figure 1: Changes in the Hct values in rainbow trout treated with concentration of IB (50 and 500 µg/L, 96 h). a: control; b:DMSO; c: 50 µg/L Ibrofen; d: 500 µg/L Ibrofen. Significant at $p < 0.05$.

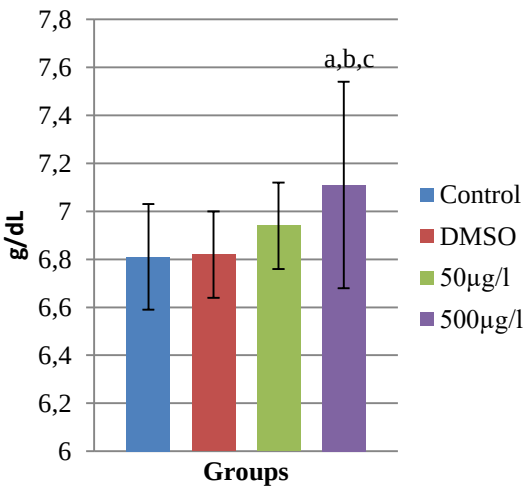

Figure 2: Changes in the Hb content in rainbow trout treated with concentration of IB (50 and 500 µg/L, 96 h). a: control; b:DMSO; c: 50 µg/L Ibrofen; d: 500 µg/L Ibrofen. Significant at $p < 0.05$.

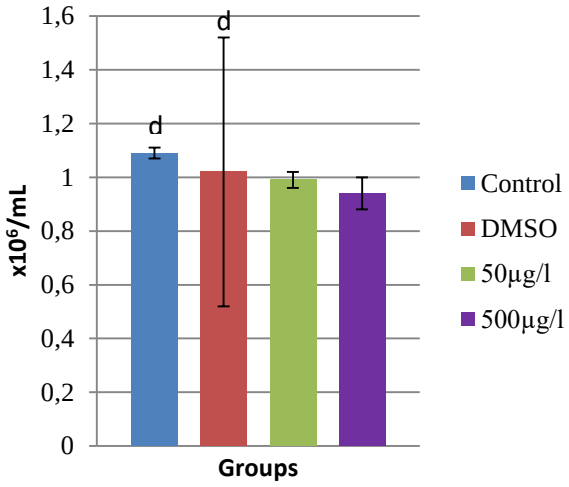

Figure 3: Changes in the RBC count in rainbow trout treated with concentration of IB (50 and 500 µg/L, 96 h). a: control; b:DMSO; c: 50 µg/L Ibrofen; d: 500 µg/L Ibrofen. Significant at $p < 0.05$.

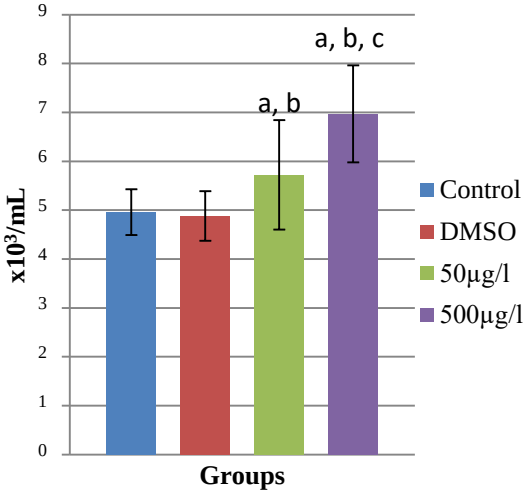

Figure 4: Changes in the WBC count in rainbow trout treated with concentration of IB (50 and 500 µg/L, 96 h). a: control; b:DMSO; c: 50 µg/L Ibrofen; d: 500 µg/L Ibrofen. Significant at $p < 0.05$.

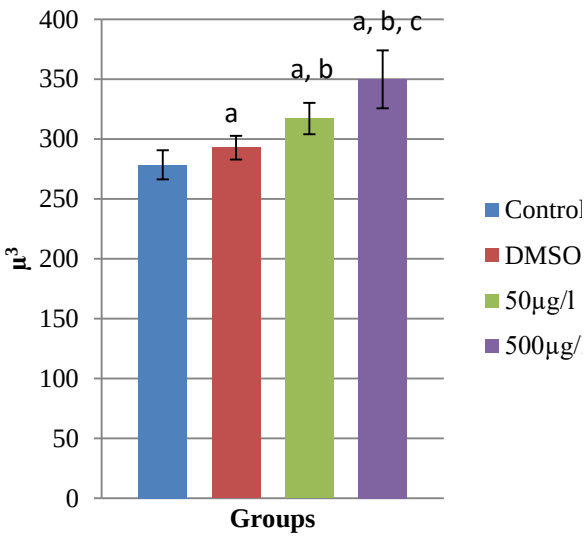

Figure 5: Changes in the MCV value in rainbow trout treated with concentration of IB (50 and 500 µg/L, 96 h). a: control; b:DMSO; c: 50 µg/L Ibrofen; d: 500 µg/L Ibrofen. Significant at $p < 0.05$.

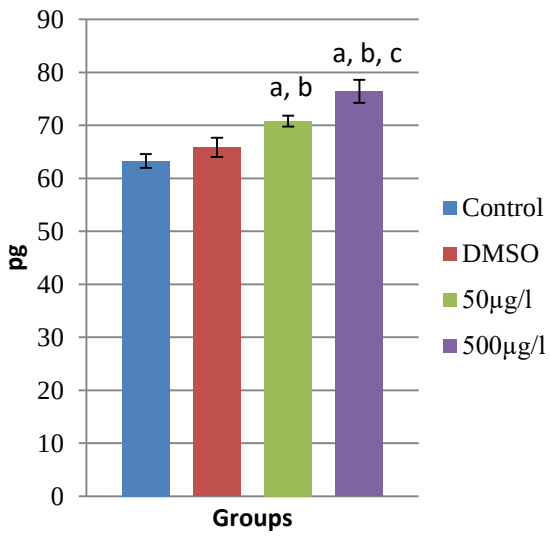

Figure 6: Changes in the MCH values in rainbow trout treated with concentration of IB (50 and 500 µg/L, 96 h). a: control; b:DMSO; c: 50 µg/L Ibrofen; d: 500 µg/L Ibrofen. Significant at $p < 0.05$.

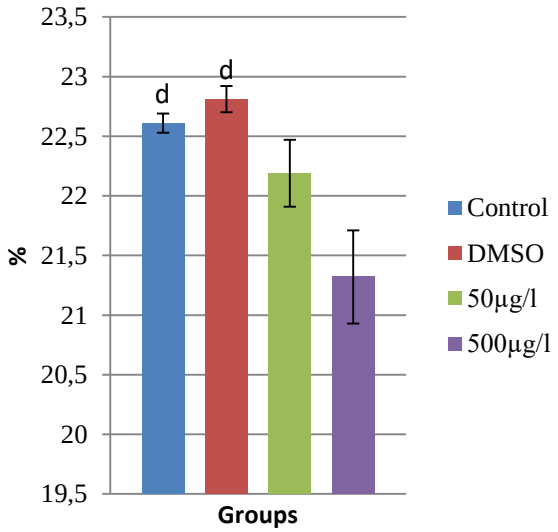

Figure 7: Changes in the MCHC values in rainbow trout treated with concentration of IB (50 and 500 µg/L, 96 h). a: control; b:DMSO; c: 50 µg/L Ibrofen; d: 500 µg/L Ibrofen. Significant at $p < 0.05$.

DISCUSSION

Biological monitoring techniques, such as assessing hematological and biochemical variables, have become valuable tools for monitoring environmental quality, water pollution, and the health status of aquatic organisms (Kohler et al., 2007). Hematological parameters such as hematocrit (Hct), hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), and others are utilized to assess the oxygen transport capacity in the bloodstream and have been utilized as pollution indicators in aquatic environments (Nussey et al., 1995) In our study, the findings indicate that Ibuprofen exhibits moderate toxicity to rainbow trout. The toxic effects of Ibuprofen on rainbow trout were observed to increase with higher concentrations. Limited information is available regarding the hematological impacts of Ibuprofen on fish.

Ibuprofen showed a significant effect on Red blood cells. Significant ($p < 0.05$) decrease in RBC count was observed at 96 h. In the present study, the decrease in RBC counts might have resulted from inhibition of RBC production by the Ibuprofen. Similarly, Pamplona et al. (2011) reported a depletion of total content of RBC in the blood of Rhamdia quelen when exposed to dipyrone, a NSAIDs. Saravanan et al. (2011) and Saravanan et al. (2012) recorded a decrease in the RBC levels in common carp (*Cyprinus carpio*) and Indian major carp (*Cirrhinus mrigala*) when exposed to clofibric acid, diclofenac and Ibuprofen. Generally, accumulation of toxicants in the gill region may damage the structure of gill resulting hemolysis and toxicant induced impaired osmoregulation may leads to a reduction in RBC counts (Saravanan et al., 2011).

In our study significant increase in WBC was also observed with high dose of Ibuprofen. The significant ($p < 0.05$) increase in WBC was observed at 96 hours post Ibuprofen administration. The increase of WBC count could be related to the presence of tissue damage such as necrosis in fish (Oliveira Ribeiro et al., 2002). WBC increases could be due to an induced proliferation, and may be a consequence of depletion of circulating differentiated cells (Al-Rudainy, 2015). The total white blood cells (WBCs) count plays a major role in the defense system of fish. In the present investigation the significant increase in leucocyte count cell during Ibuprofen treatment indicated the presence of tissue damage such as necrosis in fish. Saravanan et al. (2011) reported a significant increase in WBC content in common carp exposed to clofibric acid, a lipid regulator, and diclofenac, a NSAID. Similar to our findings, Saravanan et al. (2012) observed that Indian major carp exposed to Ibuprofen showed a significantly increased of WBC values in blood. Our results for this parameter showed similarity to the results in previous studies.

In the present study, hemoglobin increased in in experimental groups, due to Ibuprofen exposure. Increase of hemoglobin may be due to reduction of the circulating red blood cells and consequence of red blood shrinkage, hemolysis and/or the reduction of the red blood cells. Further, the gradual increase in the immature erythrocytes indicated the activation of compensatory erythropoietic process of the kidney to increase the oxygen carrying haemoglobin as an adaptation to altered respiratory homeostasis (Nussey et al., 2002). A significant increase in Hb levels has been reported in rainbow trout exposed to carbamazepine, a human pharmaceutical (Li et al., 2011) and in common carp exposed to Ibuprofen (Saravanan et al., 2012).

In the this study, Hct levels of fish exposure to 500 µg/L Ibuprofen were significantly higher than those of fish the control or 50 µg/L Ibuprofen groups. Increased Hct level rainbow trout after exposure to carbamazepine were also reported by Li et al. (2010) and Li et al. (2011). Saravanan et al. (2011) reported a increase of Hct volume in common carp after exposure to Ibuprofen. Ribas et al. (2015) observed that *Hoplias malabaricus* exposed to the higher dose of Dexamethasone showed a increased of Hct levels. With rainbow trout, (Steinbach et al., 2016) found an increase in Hct level with administration of diltiazem. The results in this study are in agreement with the results in previous investigations.

The observed increase in mean corpuscular volume (MCV) in rainbow trout individuals exposed to Ibuprofen can potentially be attributed to the presence of a higher quantity of older or larger red blood cells, as described by Hardig & Hoglund (1983). In this study, the notable increase in MCV observed during Ibuprofen treatment indicates the potential swelling of red blood cells due to hypoxia or impaired water balance, leading to reduced red blood cell lysis in circulation. The swelling of red blood cells due to hypoxic conditions in organisms exposed to the toxicant can lead to a noteworthy increase in MCV values. Therefore, the significant increase in both MCV and mean corpuscular hemoglobin (MCH) observed during Ibuprofen treatment in our study may be attributed to the aforementioned factors.

Acknowledgement

This study was presented as a oral presentation at the 1st International Black Sea Congress on Environmental Sciences, 31 Agust-3 September 2016. Giresun, Turkey.

Authors' Contributions

UI: Manuscript design, writing, statistical analyses

EF: Laboratory experiments, reading, draft checking

Conflict of interest

The authors declare no conflict of interest for this study.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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