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Original article

Effects of sublethal concentrations of tribenuron-methyl pesticide on some hematological, immunological and biochemical parameters in Siberian sturgeon (*Acipenser baerii*), and LC_{50} value

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Abstract

In this study, some toxicological effects of tribenuron-methyl pesticide on non-target aquatic animals were determined using Siberian sturgeon (*Acipenser baerii* Brandt, 1869) as the animal model. The effects of sublethal tribenuron-methyl concentrations on various hematological, biochemical and immunological parameters in the blood of *Acipenser baerii* were examined. Additionally, the LC_{50} (lethal concentration) value for this fish species was determined. The sturgeons were exposed to tribenuron-methyl for 15 days at concentrations of 0.0 (control), 50, 100 and 150 mg/L. Biochemical, hematological, and immunological alterations were observed in fish exposed to tribenuron-methyl. A statistically significant decrease was found in erythrocyte (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV) (at 100 and 150 mg/L) and protein levels (at 50, 100, and 150 mg/L) compared to the control group. Conversely, a significant increase was observed in leukocyte (WBC), glucose, cortisol, interleukin-1-beta (IL-1β), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) (at 50, 100, and 150 mg/L), monocyte, lymphocyte, neutrophil, interleukin-6 (IL-6) (at 100 and 150 mg/L), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (at 150 mg/L). No change was observed in eosinophil counts. The LC_{50} values for tribenuron-methyl were determined as 2827 mg/L for 24 hours, 1831 mg/L for 48 hours, 1474 mg/L for 72 hours and 1017 mg/L for 96 hours. In conclusion, long-term exposure to sublethal concentrations of tribenuron-methyl caused toxicity-induced hematological, biochemical and immunological changes in Siberian sturgeon.

Keywords: Acipenser baerii, hematology, non-specific immune parameters, tribenuron-methyl, toxicity

Introduction

Sturgeons evolved approximately 250 million years ago and have survived to the present day.

However, the Siberian sturgeon (*Acipenser baerii*) is currently among the species at risk of extinction due to various factors, including habitat destruction caused by pollutants such as pesticides, as well as poaching and illegal trade. The increasing use of toxic substances such as herbicides in agricultural activities has led to numerous adverse effects in aquatic ecosystems. Tribenuron-methyl is a widely used herbicide for controlling broadleaf weeds in agricultural settings (Baghfalaki et al. 2012). Herbicidal chemicals such as tribenuron-methyl, used to control undesirable plants, directly interact with fish and other aquatic organisms, causing significant harm to these species. Today, with advancements in agriculture, the application of pesticides has increased, leading to severe pollution in aquatic environments. Although pesticide applications are designed to target specific organisms, their toxicity to non-target organisms poses a significant potential hazard (Harabawy and Ibrahim 2014). Pesticide practices generally arise from intense agriculture and with a superficial current and under-surface percolation in the after application week many pesticides arrive in rivers, lakes, ground-waters and ponds and contaminate these waters (Nouri et al. 2000, Plhalova et al. 2012). Concern regarding the detrimental influences of pesticides and toxicant on fish and other aquatic organisms is growing and for this reason studies have been carried out on pesticides in the freshwater field (Iwafune et al. 2011, Kovarova et al. 2013). It is quite evident that pesticides with diverse cytotoxic features can distort multiple organ systems in fish and this condition can be seen in the immune system (Porter et al. 1999). Studies on the pathogenesis of diseases indicate that exposure to chemical pollutants leads to corruption of the immune system (Fatima et al. 2007). Fish blood is a pointer of whole body pathophysiological functions. Consequently, in fish subject to toxical material, blood parameters are pretty major in the identification of their functional and structural parameters (Zutshi et al. 2010). Van Vuren (1986), noted that when the aquatic standard breaks down due to toxic materials, any physiological modification in fish will affect the quantities of one or more of the hematological parameters. To understand the physiology of the organism, the immunological, biochemical and hematological construction of the blood is a substantial health pointer that ensures reliable data (Saravanan et al. 2011, Kazuń et al. 2020). Exposure to herbicides can cause changes in the immune system of fish, the number of white blood cells and the activity of some immune system enzymes (Gholami-Seyedkolaei et al. 2013).

This study aimed to investigate the effects of sublethal concentrations of tribenuron-methyl on various immunological (monocytes, lymphocytes, neutrophils, eosinophils, TNF-α, IFN-γ, IL-1β, IL-6, IL-8), biochemical (glucose, cortisol, protein), and hematological (WBC, RBC, Hb, Hct, MCV, MCH, MCHC) parameters in the blood of Siberian sturgeon (*Acipenser baerii* Brandt, 1869). Additionally, the biological response of Siberian sturgeon to this herbicide was evaluated, and the lethal concentration (LC_{50}) of tribenuron-methyl was determined for this species.

Materials and Methods

Experimental design and fish samples

The fish used in this study were obtained from a commercial sturgeon farm located in Karacalar Village, İmamoğlu District, Adana, Turkey, and all experiments were conducted at the same facility. Before being placed into concrete ponds, the fish underwent macroscopic (external visual examination) and microscopic (parasitological and bacteriological examination) health screenings. They were acclimatised to the concrete pond conditions for two weeks prior to the experiments. A photoperiod of 12 hours light and 12 hours dark (12h:12h L/D) was maintained throughout the trial. Sturgeon feed (size 4.5) was used, and the fish were fed four times a day at a rate of 1.5% of their live body weight (Chebanov and Galich, 2013). A total of 300 sturgeon (*Acipenser baerii*), weighing approximately 500-600 g (mean weight: 546.5±31.7 g; mean length: 51.4±6.2 cm), were used for LC_{50} and sublethal tribenuron-methyl concentration trials. The sturgeons were stocked at a density of 3.64±0.67 kg/m³ in 30 recirculating concrete ponds (dimensions: 2×1×1 m; water volume: 1500 L) supplied with oxygenated water. The sublethal dose experiment was designed with four groups, including one control group and three experimental groups, with each group tested in three repetitions; a total of 120 fish were used, which were randomly distributed into 12 concrete ponds, with 10 fish placed in each pond for the sublethal dose experiment. The same design procedure was followed for the LC_{50} experiment. 180 fish were used, with 10 fish stocked in each of 18 concrete ponds, which included one control group and five experimental groups in three repetitions. The experiments were conducted simultaneously under the same water parameters and stock conditions.

During the experiments, the pH was measured as 8.1±0.67, dissolved oxygen was 6.8±0.85 mg/L, water temperature was 19.2±0.6°C, NaCl concentration was

441.6±22.5 mg/L, conductivity was 509.5±36.2 µs and TDS was 452.9±27.1 mg/L. The nitrite (NO_2), nitrate (NO_3), and ammonia (NH_3) concentrations in the concrete ponds were determined as 0.02±0.01 mg/L, 17.44±12.3 mg/L, and 0.04±0.01 mg/L, respectively.

The study was approved by the Animal Experiments Local Ethics Committee (Ç.Ü-SABİDAM) dated 18.03.2021 (meeting number: 3, decision no: 1). The measurements for water parameters of the concrete ponds were measured daily with a EUTECH-PC 650 multiparameter instrument (pH, oxygen, temperature, NaCl, conductivity and TDS). Also, a Spectroquant NOVA 60 (Merck) was used for measuring ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3) (mg/L) values in the concrete ponds.

Toxicant

Tribenuron-methyl (Methyl 2- [4-methoxy-6-methyl - 1,3,5 - triazin -2- yl (methyl) carbamoyl-sulfamoyl] benzoate) was purchased from the Hektaş company. It has CAS number 101200-48-0 and is produced for herbicide purposes as an agricultural drug. Its commercial name is "Frame" (Active Ingredient: 75% Tribenuron-methyl, Water Dispersible Granules-WG). Tribenuron-methyl was dissolved in test water to prepare stock solutions. These stock solutions were subsequently diluted to achieve the desired experimental concentrations in the ponds.

LC_{50} experiments

LC_{50} stands for Lethal Concentration 50, representing the concentration of a substance that causes mortality in 50% of a given population (OECD 2019). An attempt was made to determine the LC_{50} value for periods of 24, 48, 72 and 96 hours. A total of 180 sturgeons were used in the acute toxicity tests. The acute toxicity tests were performed in accordance with the Organization for Economic Cooperation and Development (OECD) Guideline No. 203 (OECD, 2019), following static-renewal test conditions (Finney, 1971). The ponds were labelled as follows: A-1, A-2, and A-3 (control group); A-4, A-5, and A-6 (exposed to 400 mg/L); A-7, A-8, and A-9 (exposed to 1200 mg/L); A-10, A-11, and A-12 (exposed to 2000 mg/L); A-13, A-14, and A-15 (exposed to 2800 mg/L); A-16, A-17, and A-18 (exposed to 3600 mg/L). The sturgeons were not fed during the experiment. Each pond was monitored twice daily to record any dead fish at 24, 48, 72, and 96 hours after the start of the study.

Sublethal toxicity experiments

To perform sublethal toxicity tests, 120 sturgeons were distributed in 12 concrete ponds (n = 10) and they were exposed to tribenuron-methyl at concentrations of 0.0 (control), 50, 100 and 150 mg/L for 15 days. The amounts of toxic substance were calculated based on the total volume of water in the ponds. The concrete ponds were labelled S-1, S-2, and S-3 (control group); S-4, S-5, and S-6 (exposed to 50 mg/L); S-7, S-8, and S-9 (exposed to 100 mg/L); S-10, S-11, and S-12 (exposed to 150 mg/L). Sublethal concentration doses were determined based on previously conducted acute toxicity tests. During the sublethal tribenuron-methyl exposure trials, two-thirds of the water in the ponds was slowly drained each day and immediately replaced with fresh water to remove metabolites. Tribenuron-methyl was then added to the experimental ponds (except for the control group) in proportion to the volume of water added to maintain a consistent herbicide concentration. Feeding was stopped 24 hours before blood sample collection began. No fish mortality was recorded in sublethal toxic dose applications. Before the immunological, biochemical, and hematological analyses, sturgeons from each experimental concentration were anesthetized with 2-phenoxyethanol at a concentration of 0.1 mL/L after 15 days of exposure (Priborsky and Velisek 2018).

Hematological analyses

Blood samples were collected from the caudal vein using a syringe (Gomułka et al. 2015) and transferred into EDTA-containing tubes for hematological analysis. Natt-Herrick solution was used to determine RBC and WBC counts. Using this solution, erythrocytes and leukocytes were counted simultaneously under a light microscope (Olympus BX51) at 400× magnification. The results were expressed as $\times 10^6/mm^3$ for erythrocytes and $\times 10^3/mm^3$ for leukocytes (Blaxhall and Daisley 1973, Kocabatmaz and Ekingen 1984). Cyanmethemoglobin and microhematocrit methods were used to determine hemoglobin (Hb) and hematocrit (Hct) values (Kocabatmaz and Ekingen 1984).

The RBC indices determined were as follows; MCV (Mean Corpuscular Volume); MCH (Mean Corpuscular Hemoglobin); MCHC (Mean Corpuscular Hemoglobin Concentration). The formulas for these indices are as follows:

$MCV\ (\mu^3) = (Hct)\ (\%) / RBC\ (10^6/mm^3) \times 10$

$MCH\ (pg) = Hb\ (g/100\ mL) / RBC\ (10^6/mm^3) \times 10$

$MCHC\ (g/dL) = Hb\ (g/100\ mL) \div (Hct)\ (\%) \times 100$

(Ighwela et al. 2012, Duman and Şahan 2023).

Immunological analyses

For non-specific immunological analysis, leukocyte differential counts (lymphocytes, monocytes, neutrophils, and eosinophils) and cytokine levels (TNF-α, IFN-γ, IL-1β, IL-6, and IL-8) were measured from blood samples collected from the fish. To determine the percentage of leukocyte cells, a drop of blood without an anticoagulant was collected from the fish's caudal vein and spread onto a slide using a second slide. The slides were stained using the May-Grünwald-Giemsa technique, and the entire field was examined. In the selected area, 100 leukocytes were counted, and the percentages of monocytes, lymphocytes, neutrophils, and eosinophils were determined (Blaxhall 1972, Fujimaki and Isoda 1990). For serum TNF-α, IFN-γ, IL-1β, IL-6 and IL-8 cytokine levels, enzyme-linked immunosorbent assay (ELISA) kits were used, and the results were assessed using the double-antibody sandwich method (Voller et al. 1978). This method does not require a native antigen but relies on a reporter-labeled detection antibody (Kohl and Ascoli 2017). Commercial ELISA kits were used for the analyses: Fish Interleukin 1β (IL-1β) ELISA Kit (Catalog no. MBS700230), Fish Interferon-γ (IFN-γ) ELISA Kit (Catalog no. MBS702530), Fish Tumor Necrosis Factor-α (TNF-α) ELISA Kit (Catalog no. MBS024441), Fish Interleukin 6 (IL-6) ELISA Kit (Catalog no. MBS702353), and Fish Interleukin-8 (IL-8) ELISA Kit (Catalog no. MBS700055). The catalog numbers for these kits were obtained from MyBioSource, Inc.

Biochemical analyses

The cortisol level was measured using the competitive inhibition enzyme immunoassay technique (Fish Cortisol ELISA Kit, Catalog No. CSB-E08487f) (Baßmann et al. 2017). Total protein and plasma glucose levels were measured using enzymatic methods with commercial assay kits (Sigma-Aldrich Quanti-Pro™ BCA Assay Kit, QPBCA; Sigma-Aldrich Glucose and Sucrose Assay Kit, MAK013) (Akins et al. 1992, Bartoňková et al. 2017).

Statistical analyses

For the evaluation of the results, One-Way ANOVA and Duncan's multiple range tests were performed using SPSS 10.0 for the statistical analysis of each parameter in the control and sublethal dose groups (Hayran and Ozdemir 1996). Probit analysis was performed as suggested by Finney to find the LC_{50} value (Finney 1971).

Results

Lethal concentration toxicity tests

The median lethal concentration (LC_{50}) was examined in static-renewal tests over 24, 48, 72, and 96 hours. LC_{50} values significantly decreased with increasing exposure time, from 2827±157 mg/L at 24 hours to 1017±112 mg/L at 96 hours. The LC_{50} values of tribenuron-methyl herbicide for *Acipenser baerii* at 24, 48, 72 and 96 hours are presented in Table 1. Fish mortality increased proportionally with the rise in pesticide concentration (Fig. 1).

Immunological, hematological and biochemical responses to sublethal toxicity concentrations

Cytokine levels (TNF-α, IFN-γ, IL-1β, IL-6 and IL-8) and leukocyte cell percentages (lymphocytes, monocytes, neutrophils and eosinophils), analyzed as non-specific immune system parameters, are presented in Table 2. According to the table, when comparing these values between the control and experimental groups, a significant increase was observed in IL-1β, IL-8, TNF-α and IFN-γ levels in the groups exposed to 50, 100 and 150 mg/L tribenuron-methyl doses ($p<0.05$). On the other hand, monocyte, lymphocyte and neutrophil values showed similar increases in the groups exposed to 100 and 150 mg/L doses. No change was observed in eosinophil counts ($p<0.05$).

In terms of hematological parameters, a statistically significant decrease was found in RBC, Hb, Hct and MCV levels in the groups exposed to 100 and 150 mg/L tribenuron-methyl doses compared to the control group ($p<0.05$). Conversely, a significant increase was observed in WBC levels in the groups exposed to 50, 100 and 150 mg/L doses. Moreover, MCH and MCHC levels increased significantly in the group exposed to the 150 mg/L dose compared to the control group ($p<0.05$). The hematological parameters are presented in Table 3.

Variations in biochemical parameters, such as glucose, cortisol and protein levels, are presented in Table 4. Compared to the control group, a statistically significant increase in glucose and cortisol levels was observed in the groups exposed to 50, 100 and 150 mg/L tribenuron-methyl doses, while a significant decrease in protein level was detected.

Discussion

Siberian sturgeon, due to the high commercial value of their eggs (caviar) and meat, whose cultivation is rapidly increasing worldwide, is among the most widely cultured sturgeon species (Duman 2020).

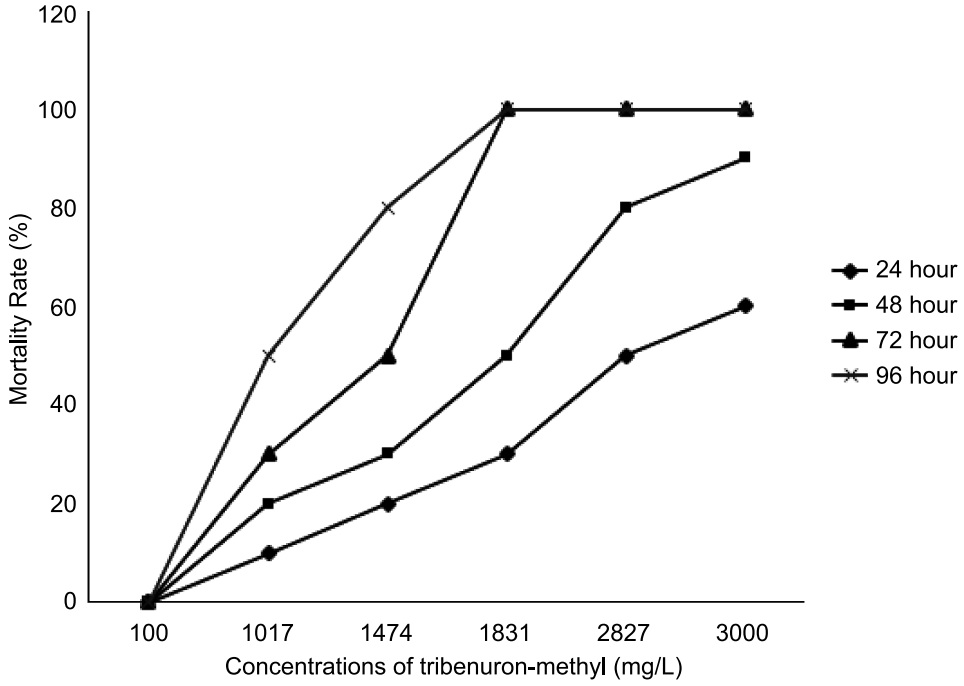

Fig. 1. Mortality rate of *Acipenser baerii* exposed to tribenuron-methyl doses.

Table 1. Median lethal concentrations of tribenuron-methyl to Siberian sturgeon (*Acipenser baerii*).

LC_{50} (mg/L)	Duration of exposure (hour)
2827±157 (2544–3166)	24
1831±129 (1593–2106)	48
1474±120 (1253–1710)	72
1017±112 (803–1423)	96

Acute tribenuron-methyl toxicity was determined in *Acipenser baerii* after 24, 48, 72 and 96 hours of toxicity. LC_{50} was calculated using the Probit Analysis test. Results are shown as mean ± S.D. with the maximum and minimum values.

Table 2. Non-specific immune parameters of *Acipenser baerii* exposed to sublethal concentrations of tribenuron-methyl for 15 days.

Parameters	Control Group	Tribenuron-Methyl Exposure Groups		
	0.0 mg/L	50 mg/L	100 mg/L	150 mg/L
Lymphocyte (%)	48.84±2.25^a	51.73±1.48^a	59.24±4.11^b	63.43±2.35^b
Monocyte (%)	10.13±1.37^a	12.15±2.11^a	15.87±1.76^b	16.18±1.81^b
Neutrophil (%)	31.17±1.41^a	34.85±1.72^a	42.94±1.76^b	49.15±3.64^b
Eosinophil (%)	1.7±0.14^a	1.4±0.12^a	1.9±0.17^a	2.1±0.18^a
TNF-α(pg/ml)	31.12±9.23^a	40.21±15.51^b	48.29±12.71^b	59.97±18.3^c
IFN-γ (pg/ml)	65.87±10.25 ^{a}	79.17±15.11^b	83.39±14.47^b	89.18±18.27^b
IL-1β (pg/ml)	1.06±0.171^a	1.89±0.257^b	1.93±0.715^b	1.97±0.513^b
IL-6 (pg/ml)	11.3±2.1^a	13.9±2.5^a	27.3±5.8^b	30.2±8.2^b
IL-8 (pg/ml)	41.12±7.1^a	68.24±9.8^b	74.9±15.6^b	79.7±16.3^b

Data are represented as mean ± SD. The values in the same line with different letters are significantly different ($p<0.05$).

The increasing use of toxic herbicides in agricultural areas adjacent to aquatic environments has become a major concern for aquaculture producers and fisheries workers due to their potential impact on aquatic ecosystems. Various toxicity tests have been conducted on many herbicides to assess their effects on non-target organisms, such as fish (Velisek et al. 2010, Ndimele et al. 2015, Kondera et al. 2018). Aquatic toxicity tests are often used to assess the potential toxicological effects of harmful substances on aquatic organisms and to monitor water quality (Brungs et al. 1977, Choudri 2017). The effects of tribenuron-methyl on the immune system of fish and other physiological alterations remain largely unexplored. A study evaluated the acute

Table 3. Hematological parameters of *Acipenser baerii* exposed to sublethal concentrations of tribenuron-methyl for 15 days.

Parameters	Control Group	Tribenuron-Methyl Exposure Groups		
	0.0 mg/L	50 mg/L	100 mg/L	150 mg/L
WBC ($\times 10^3$/mm^3)	10.41±0.22^a	15.17±0.35^b	16.97±0.34^b	17.61±0.13^b
RBC ($\times 10^6$/mm^3)	1.15±0.14^a	1.03±0.29^a	0.89±0.16^b	0.75±0.32^b
Hb (g/dL)	9.5±0.30^a	9.1±0.23^a	7.4±0.12^b	7.1±0.32^b
Hct (%)	29.2±0.61^a	27.5±0.67^a	22.4±0.75^b	17.9±0.31^b
RBC Indices				
MCV (μ^3)	263.1±11.7^a	266.9±13.2^a	247.6±9.2^b	238.7±8.9^b
MCH (pg)	82.6±4.1^a	88.3±4.9^a	83.1±4.3^a	94.6±5.8^b
MCHC (%)	32.5±2.8^a	33.1±2.9^a	33±3.1^a	39.6±4.2^b

Data are represented as mean ± SD. The values in the same line with different letters are significantly different ($p<0.05$).

Table 4. Biochemical parameters of *Acipenser baerii* exposed to sublethal concentrations of tribenuron-methyl for 15 days.

Parameters	Control Group	Tribenuron-Methyl Exposure Groups		
	0.0 mg/L	50 mg/L	100 mg/L	150 mg/L
Cortisol (ng/mL)	9.37±0.5^a	28.7±5.1^b	45.2±6.6^b	75.4±8.1^c
Glucose (mg/dL)	51.9±2.3^a	90.8±7.5^b	139.1±12.7^c	173.8±14.3^c
Protein (g/dL)	2.9±0.2^a	1.91±0.2^b	1.85±0.3^b	1.71±0.6^b

Data are represented as mean ± SD. The values in the same line with different letters are significantly different ($p<0.05$).

toxicity of tribenuron-methyl (95% purity) on *Hypophthalmichthys molitrix*, *Cyprinus carpio* and *Rutilus rutilus caspicus*. The 96-hour LC_{50} values for these species were determined as 152.74 mg/L, 289.08 mg/L, and 139.45 mg/L, respectively (Baghfalaki et al. 2012). Ertuğ et al. (2021), determined the 120-hour LC_{50} value of tribenuron-methyl (WG, %75) in zebrafish as 1.850 mg/L. Different studies on tribenuron-methyl toxicity have reported that the 96-hour LC_{50} values are 738 mg/L for *Oncorhynchus mykiss*, >1000 mg/L for *Lepomis macrochirus*, and >560 mg/L for *Salmo gairdneri* (FAO 2011). In the present study, the 96-hour LC_{50} value for *Acipenser baerii* exposed to tribenuron-methyl was determined to be 1017 mg/L. Studies have shown that tribenuron-methyl exhibits high toxicity to fish. The toxicity to fish may vary depending on several factors, including the age and developmental stage of the fish, species, physiological condition, nutritional status, water temperature, pH level, dissolved oxygen, water hardness, ion content, concentration and exposure duration.

Toxic substances can alter the blood parameters of fish and hematological indices serve as important data for evaluating the physiological status of fish (Saravanan et al. 2011, Docan et al. 2018). As a result of exposing Nile tilapia to sublethal concentrations of glyphosate-based herbicides for 14 days, decreases were observed in RBC, Hb, Hct and protein levels, while glucose level increased (Acar et al. 2021). Saravanan et al. (2011) reported a decrease in RBC, Hct and Hb levels, while WBC level increased on the 10th day of exposure to sublethal toxicity of lindane pesticide in *Cyprinus carpio*. Similarly, Oluah et al. (2020) found that toxicant-induced stress (exposed to sublethal concentrations of Ronstar herbicide for 14 days) led to a decrease in RBC, Hb and Hct levels, while the WBC level increased in *Clarias gairepinus*. Similarly, to the increase in leukocytes we determined in our study, leukocyte increases were reported in *Clarias gariepinus* exposed to a mixture of atrazine and metolachlor for 14 days (George et al. 2017), to paraquat herbicide for 20 days (Nwani et al. 2015) and in *Cyprinus carpio* exposed to linuron herbicide for 7 days (Lutnicka et al. 2019). In this study, the increase in the number of WBCs, a fundamental component of the immune system, can be considered a protective immune-adaptive response to tribenuron-methyl herbicide exposure. This response may serve to enhance antibody production, allowing fish to adapt to ecological conditions and improve their chances of survival (Mokhtar et al. 2023). In an experiment with *Clarias gariepinus*, decreases in MCV levels and increases in MCH and MCHC levels were reported after a two-week exposure to Primextra herbicide (Ebari et al. 2023). Ural (2013) stated that 14 days of exposure to a sublethal dose of Chlorpyrifos pesticide in *Cyprinus carpio* caused a decrease in MCV. Moreover, Bojarski et al. (2022), reported that exposure of *Cyprinus carpio* to sublethal doses of Roundup herbicide for 10 days resulted in increased MCH and MCHC levels. In our research, similar results to those of the studies of Acar et al. (2021), Saravanan et al. (2011), Oluah et al. (2020),

Ebari et al. (2023), Ural (2013) and Bojarski et al. (2022) for RBC, Hb, Hct, MCV, MCH and MCHC values were obtained. Red blood parameters are sensitive to toxical factors. Exposure to toxic substances may cause damage to gill function and morphology. A decrease in red blood cell count was observed indicating the presence of an anemic response to the use of tribenuron-methyl pesticide as a toxic agent. Also, it is believed that the decrease in RBC, Hb and Hct levels in fish exposed to toxic substances results from disruptions in hematopoietic processes and the accelerated breakdown of red blood cell membranes due to toxic exposure (Kori-Siakpere and Ubogu 2008). Monocytes play a crucial role in the immune response to toxins that harm the body. In cases of poisoning caused by various toxins, these cells become activated and migrate to damaged tissues, where they differentiate into macrophages. Macrophages phagocytize cellular debris, contribute to tissue repair, clear toxins, and help prevent further damage. Harabawy and Ibrahim (2014) reported an increase in monocyte and glucose levels, along with a decrease in protein levels, in *Clarias gariepinus* exposed to sublethal toxicity of the pesticide carbofuran. Administration of sublethal doses of of the herbicide pendimethalin to *Cyprinus carpio* for 14 days resulted in an increase in neutrophil and monocyte counts, as well as cortisol levels (Lutnicka et al. 2018). Kondera et al. (2018) examined the effects of sublethal exposure to Roundup herbicide over a 7-day period in *Cyprinus carpio*, and reported an increase in monocyte and neutrophil levels. Rowley et al. (1988) declared that monocytes and neutrophils play a crucial role in the nonspecific defense system of fish, forming the first line of cellular defense against stress. Our results in terms of monocyte and neutrophil values were consistent with those reported by Harabawy and Ibrahim (2014), Lutnicka et al. (2018) and Kondera et al. (2018). Studies have reported that on *Cyprinus carpio* exposed to sublethal concentrations of Roundups for 16 days (Gholami-Seyedkolaei et al. 2013), and on *Huso huso* to 14-21 days of sublethal toxic stress with diazinon (Khoshbavar-Rostami et al. 2006), there was no change in eosinophil levels. In other studies, it was determined that lymphocyte counts increased in *Oreochromis niloticus* exposed to sublethal doses of the herbicide Propanil for two weeks (Yajı et al. 2018) and in *Clarias gariepinus* subjected to subchronic exposure to the pesticide Fenthion for seven days (Nwani et al. 2016). Similar to the results of our study, the increase in lymphocyte levels may represent an adaptive immune response mechanism that enhances the survival ability of fish in sublethal toxic environments.

Several studies have determined an increase in TNF-α, IL-1β, and IL-8 levels in *Oreochromis niloticus* exposed to the pesticide chlorpyrifos for four weeks (Zahran et al. 2020), an increase in IFN-γ, TNF-α, IL-1β, IL-6 and IL-8 levels in *Cyprinus carpio* exposed to the herbicide paraquat for seven days (Ma et al. 2018), and an increase in IL-1β, IL-8, TNF-α and IFN-γ levels in *Cyprinus carpio* exposed to a low concentration of the pesticide indoxacarb for 21 days (Ghelichpour et al. 2019). Cytokines are immunoregulatory molecules that modulate the balance between proinflammatory and anti-inflammatory responses. Proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α, are primarily produced by activated macrophages and play a key role in the positive regulation of inflammatory reactions (Cestonaro et al. 2022). In our study, it was observed that IL-1β, IL-6, IL-8, IFN-γ and TNF-α levels increased in the tribenuron-methyl exposed fish groups due to the stimulation of the non -specific cellular defense system in response to the sublethal toxicant.

In a study on *Catla catla*, exposure to sublethal concentrations of the pesticide methyl parathion for 14 days resulted in an increase in glucose levels and a decrease in protein levels (Abhijith et al. 2012). Glucose is released in response to corticosteroids and is considered a primary reaction to stress factors. In fish, exposure to environmental stressors generally leads to an increase in glucose levels (Donaldson 1981, Barton 2002). Ghosh and Chatterjee (1989) stated that the decrease in protein level under toxic stress may result from the formation of lipoproteins used for the repair of damaged cells and tissue organelles, increased proteolysis and direct utilization by cells to meet energy demands. In the present study, the decrease in protein levels, along with changes in the biochemical mechanisms of the fish, may have increased their sensitivity to the toxic substance. The results obtained by Abhijith et al. (2012) and Harabawy and Ibrahim (2014) for glucose and protein levels in their study were consistent with the findings of our research. In another study, an increase in plasma cortisol levels was observed in the *Labeo rohita* exposed to sublethal concentrations of the pesticide deltamethrin for a period of 14 days (Suvetha et al. 2015). Waring and Moore (2004) reported that the elevation of serum cortisol concentrations in *Salmo salar* following a 7-day sublethal exposure to atrazine herbicide indicates the fish's failure to adapt to toxic stress. Cortisol plays a crucial role in maintaining hydromineral balance (Wendelaar Bonga 1997). In the present study, the significant increase in plasma cortisol levels throughout sublethal tribenuron-methyl exposure over 15 days might have evolved from osmoregulatory dysfunction, as indicated by restored hydromineral balance (Cericato et al. 2008).

The results indicate that hematological, biochemical and immunological parameters were affected by sublethal concentrations of tribenuron-methyl. It has been shown that acute and sublethal exposure to tribenuron-methyl causes significant changes in the hematological, biochemical and immunological parameters of *Acipenser baerii*. Furthermore, the findings of this study make possible the inclusion of the LC_{50} value for tribenuron-methyl in this fish species in the scientific literature. These results provide a better understanding of the toxicological endpoints of this pesticide, aiding in the establishment of its safe levels in aquatic environments and the protection of aquatic organisms. Concerns about the adverse effects of pesticides on fish and other aquatic organisms are increasing; therefore, further research on the impact of pesticides in aquatic ecosystems is strongly recommended.

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