Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Effect of Cypermethrin on Blood Hematology and Biochemical Parameters in Fresh Water Fish *Ctenopharyngodon idella* (Grass Carp)

Mujeeb Ullah[1], Ali Muhammad Yousafzai[1], Ijaz Muhammad[2], Sobia Atta Ullah[1], Muhammad Zahid[1], Muhammad Ismail Khan[1], Khalid Khan[1], Khayyam[1], Gul E Nayab[2], Michael Aschner[3], Khalaf F Alsharif [4], Khalid J Alzahrani [4], Haroon Khan[5*]

[1] Department of Zoology Islamia College University 25120, Peshawar, Pakistan
[2] Department of Zoology, Abdul Wali Khan University 23200, Mardan, Pakistan
[3] Department of Molecular Pharmacology, Albert Einstein College of Medicine Forchheimer 209 1300 Morris Park Avenue, Bronx, NY 10461, USA
[4] Department of Clinical Laboratory (Sciences), College of Applied Medical Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[5] Department of Pharmacy, Abdul Wali Khan University Mardan, 23200, Mardan, Pakistan

ARTICLE INFO

Original paper

Article history:
Received: April 22, 2022
Accepted: September 15, 2022
Published: September 30, 2022

Keywords:

Ctenopharyngodon idella, Cypermethrin, hematology, biochemistry, exposure time

ABSTRACT

The insecticide cypermethrin adversely affects biochemical parameters in blood and behavior in grass carp (*Ctenopharyngodon idella*). Fish were obtained from the hatchery and reared in the laboratory. Different concentrations of cypermethrin were applied. Blood was collected and hematological and biochemical parameters were measured. Biochemical parameters such as protein levels, cholesterol, phosphorous and calcium in both acute and chronically cypermethrin-treated groups decreased, with increasing exposure time from 24h to 15 days with more pronounced effects in the acute groups. Increased glucose, urea, serum glutamic pyruvic transaminase (SGPT), creatinine, and lactate dehydrogenase (LDH) levels were found in both acute and chronic groups with increasing exposure time. Hematological parameters, such as red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MHCH), and red cell distribution width (RDW) were significantly reduced in both groups as the exposure time increases. However, the number of white blood cells (WBC) and platelets increased. This study established both the acute and chronic toxicity of cypermethrin in grass carp, which likely occurs secondary to altered biochemical and blood parameters.

Doi: http://dx.doi.org/10.14715/cmb/2022.68.10.3

Introduction

The *Ctenopharyngodon idella* belongs to the Cyprinidae family, an herbivorous fish. It's native to Eastern Asia and also has a native range from Northern Vietnam to the Amur River (1). The rapid increase in the population has decreased the available land for cultivation. To solve this problem, different chemicals are used to increase the production of vegetables, fruits and crops. These chemicals are present in the form of fertilizers, insecticides and herbicides. Fast industrialization has led to pollution affecting aquatic ecosystems. In Pakistan, effluents from industries, wastes from domestic activities and runoffs are commonly released into streams, ponds and other water bodies. These chemicals are the main source of pollution, and they alter the physical as well as the chemical composition of the water, and are toxic to aquatic life, especially to fish diversity (2-7).

Cypermethrin is an artificial pyrethroid used for scheming diverse kinds of creepy-crawly pests of fiber, fruits, crops and vegetables (8-10), copepod bloodsucker invasion (11), marine and earthly ectoparasites (12). It enters waterways as a result of runoffs and affects aquatic organisms (13). Cypermethrin is extremely poisonous to fish (in laboratory tests, 96 h LC_{50} were generally within the range of 0.4–2.8 µg/l) and marine invertebrates with LC_{50} in the range of 0.01–5 µg/l (14, 15). Cypermethrin alters fish metabolism and hematological parameters (16). Long-term exposure decreases the fish life span (17).

Pyrethroids are highly toxic to a number of life forms, including fish, lobster, shrimp, mayfly nymphs and numerous species of zooplankton (18), affecting hematological indices in fish (19), such as hematocrit, hemoglobin, number of erythrocytes and white blood cells (20).

Hematological parameters have been studied to explain the physical condition of fish (21), and assess environmental stresses (22, 23). Hematological indices are known to act in response to changes in ecological conditions and have been considered in *C. idella* (24) and *Heteropneustes fossilis* (25). Our study aimed to determine the toxic effects of cypermethrin exposure on the common carp.

Materials and Methods

Fish were obtained from the Carp hatchery Mardan. During collection, the same size and healthy fishes were selected and brought to the laboratory in plastic bags containing a sufficient amount of oxygen. Fish were housed in aquariums 5 feet in length, 2.5 feet in width, and 3 feet in height containing 700-liter water (26).

* Corresponding author. Email: haroonkhan@awkum.edu.pk

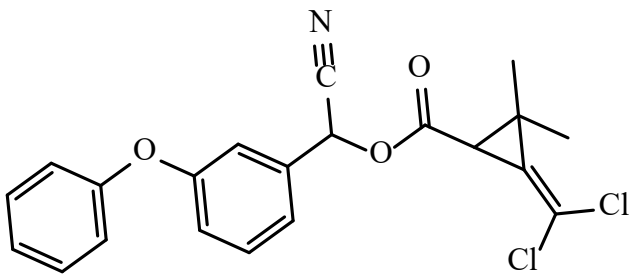

Figure 1. Chemical Structure of Cypermethrin.

Pre experimental management

Cypermethrin, $C_{22}H_{19}C_{l2}NO_3$ (a- Cyano- (3-phenoxy-phenyl)- methyl 3- (2,2- dichloro-vinyl)- 2,2- dimethylcyclo-pro-pane- carboxylate) (CAS Number 52315-07-8, – 98%, Molecular Weight 416.30) was purchased from Sigma-Aldrich (Germany). The molecular structure of cypermethrin is shown in Figure 1. Aquariums were washed, cleaned and disinfected and filled with water. Aerators were used to keep the water fully aerated. Water was changed every 20 hrs. The fish were placed in the aquarium for a period of 20 days to acclimatize to the laboratory conditions and were fed once a day. The provision of food was stopped 48 h prior to the start of toxicity experiments (26).

Experimental Design

Fish were divided into three groups. Group I was not exposed to any chemicals and was considered a control group; group II was treated with cypermethrin considered. Fish of the control group were kept in a separate aquarium provided with oxygen by aerators, food and exchange of water each 24 h. The treated fish were housed under similar conditions along with various concentrations of cypermethrin for various periods. After the exposure to cypermethrin, the blood was collected from fish in ethylenediaminetetraacetic acid (EDTA) for further hematological and biochemical analysis (27). The number of fish, the treatments and the time of exposure are shown in Table 1.

Collection and preservation of Fish blood

Blood was collected from the caudal vein midline just posterior to the anal fin. Collected blood was transferred to Gel and EDTA tubes containing anticoagulants (28) and subjected to hematological and biochemical analyses.

Hematological parameters

Blood samples (5ml) were collected by direct prick near the caudal vein. The length and weight of the fish were recorded before the collection of the blood. The following parameters were analyzed: RBC, WBC, HGB, HCT, MCV, MCH, MHCH and RDW were analyzed by the d6580 auto hematology analyzer.

Biochemical parameters

Analyzed biochemical parameters, including total protein, cholesterol, glucose, phosphorous, SGPT, urea, creatinine, calcium and LDH, were determined by following standard methods(29-31).

Statistical Analysis

Statistical analysis was carried out by using SPSS, version 16. Continuous data are expressed as mean ± S.E. One factor experiment carried out in completely randomized design data were compared by using one-way ANOVA. Significant differences were defined at $P<0.05$.

Results

Hematology

Cypermethrin increased WBCs count as the exposure time increased from 24 h (140.32 x10ˆ3/µL) to 15 days (178.50x10ˆ3/µL). A trend towards decreased Hb was noted as the exposure time increased from 24 h (6.82 g/dl) to 15 days (4.55g/dl). RBC mean value in the 24-h exposure group was 2.20 x10ˆ6/µL and 0.25x10ˆ6/µL in the 15-day exposure group and decreased with the increasing exposure time. In the 24-h exposure group, HCT was 4.42% and increased to 13.15% in the 15-day exposure group. The amount of MCV was reduced by cypermethrin exposure, with a mean value of 151.75fL in the 24-h and a mean value of 69.50fL in the 15-day exposure group. The highest value of MCH was observed in the 24-h at 168.02 pg and 82.85pg in the 15-day exposure group. MHCH mean value in the 24-h exposure group was 113.75 g/dL and decreased to 77.75g/dL in the 15-day exposure group. RDW-CV percentage decreased with the exposure time. In the 24-h and the 15 days exposure group, RDW-CV percentages were 29.00% and 20.60%, respectively. PLT value increased with the time of exposure. In the 24-h exposure group, 66.00 x10ˆ3/µL, while in 15 days exposure group, it was 136.00x10ˆ3/µL. Observed values of hematological parameters in the different exposure groups at 0.8 µl/L, 0.7 µl/L, 0.6µl/L, and 0.5 µl/L concentrations are shown in Table 2.

Blood Biochemistry

Serum proteins decreased with increased exposure time from 7.4500±.017078 after 24 h to 3.6000±.07071 after 15 days at 0.08 and .05 µl/L concentration, respecti-

Table 1. Number of fish used in control and each treated group, amount of cypermethrin used and time of exposure.

S. No	Number of Fishes	Amount of cypermethrin	Time of exposure
Group 1	10	Control group	
Group 2	10	0.8 ul/L	24 h
Group 3	10	0.7 ul/L	48 h
Group 4	10	0.6 ul/L	72 h
Group 5	10	0.5 ul/L	96 h
Group 6	10	0.7 ul/L	5 days
Group 7	10	0.6 ul/L	10 days
Group 8	10	0.5 ul/L	15 days

Table 2. Showing results of the hematological parameters such as WBC, HGB, RBC, HCT, MCV, MCH, MCHC, RDW-CV, and PLT of control, acute and chronic group. The (P<0.05) was considered significant.

Hematological Parameters	Control	Treated groups						
		24 h 0.8µl/L	48 h 0.7µl/L	72 h 0.6µl/L	96 h 0.5µl/L	5 days 0.7µl/L	10 days 0.6µl/L	15 days 0.5µl/L
WBC(x10^3/µL)	118.75±1.75000	140.32±1.149	136.600±1.009	142.750±1.3768	145.250±.8539	155.500±.6455	163.00±.8165	178.50±.9574
HGB (g/dl)	8.85±.06455	6.825±.0853	6.250±.0645	5.525±.853	4.9750±.0853	4.625±.0478	4.750±.0645	4.750±.0645
RBC (x10^6/µL)	2.79±.01109	2.200±.0736	1.795±.0210	1.500±.0219	1.1975±.0377	.7350±.0253	0.3200±.0129	0.2500±.0255
HCT (%)	8.22±.07071	4.425±.0853	10.225±.02495	8.475±.0853	8.700±.00000	12.100±.02857	10.150±.02986	13.150±.03840
MCV (fL)	178.2800±2.67	151.75±1.108	130.25±1.4930	121.50±1.707	107.00±1.8257	102.00±1.290	86.500±1.707	69.500±1.707
MCH (pg)	197.200±.7348	168.02±.1.037	150.12±.8750	129.75±.1.1086	129.75±2.160	103.57±.6786	71.175±.04230	82.850±0.7135
MCHC (g/dL)	121.50±.9574	113.75±.1.738	106.00±1.290	96.50±1.099	82.000±1.290	75.750±1.1086	64.750±1.652	77.750±1.108
RDW-CV (%)	35.50±.02041	29.000±.6455	22.87±.04787	16.300±.03366	14.857±.02393	12.925±.02561	15.5000±.03559	20.600±.05369
PLT (x10^3/µL)	24.50±.6455	49.50±.1.1902	66.00±.1.290	80.000±1.290	92.500±1.7078	110.25±1.3768	124.00±1.290	136.00±1.290

Table 3. Showing results of blood biochemical parameters such as protein, cholesterol, phosphorus, Urea, creatinine, Glucose, Calcium, LDH and SGPT of control, acute and chronic group. The (P<0.05) was considered significant.

Biochemical Parameters	Control Group	Treated groups						
	No treatment	24 h 0.8µl/L	48 h 0.7µl/L	72 h 0.6µl/L	96 h 0.5µl/L	5 days 0.7µl/L	10 days 0.6µl/L	15 days 0.5µl/L
Serum Protein	8.7750±.011087	7.4500±.01707	6.4750±.012500	5.4250±.014930	4.4675±.020006	5.5500±.02887	4.5500±.07071	3.6000±.07071
Blood Cholesterol	1.8225±1.2500	1.5300±3.1622	1.2500±2.58199	1.5000±2.7537	1.2125±2.17466	1.2938±.89849	1.0988±1.28087	1.3750±1.280
Serum alkaline Phosphatase	9.0000±.10801	3.1250±.06292	5.2500±.10408	6.0500±.06455	7.0750±.08539	3.4750±.016520	4.3500±.04787	6.4250±.04787
Blood Urea	4.0475±.03038	13.7500±.0322	11.0000±.64550	8.9750±.020565	7.8750±.023936	15.1250±.02393	11.5000±.20412	9.5000±.020412
Serum Creatinine	0.8775±.01109	1.7500±.01040	1.4750±.06292	1.2075±.07398	0.6250±.011087	1.3500±.02887	0.5500±.02394	0.3625±.02394
Blood Glucose	43.5000±.6455	85.8750±.0239	80.7500±.03227	75.6250±.04269	65.3750±.055434	60.5000±.02041	57.7500±.20412	49.0000±.02041
Calcium	9.0725±.04922	7.5000±.02041	6.0000±.020412	6.0000±.020412	6.5000±.020412	7.5250±.06292	6.6250±.06292	5.5250±.06292
LDH	3.5000±2.0816	9.3125±6.2500	8.3475±3.42479	7.7950±5.42371	6.8750±6.61438	7.3050±2.5000	7.0100±1.70783	6.5650±1.7078
SGPT	46.2500±.3227	82.7500±.0322	76.2500±.03227	71.7500±.03227	67.3750±.023936	62.3750±.746	55.5000±.32275	51.2500±.0322

vely. An increase in blood cholesterol level was observed with increased exposure time after 24 h at 1.5300±3.1622 to 1.3750±1.280 after 15-days. Serum alkaline PO_4 value in the 24-h group was 3.1250±.06292 and 6.4250±.04787 in the 15-day treated group. Blood urea decreased with exposure time and was 13.7500±.3227 after 24-h and 9.5000±.020412 after 15-days of exposure. Serum creatinine was 1.7500±.010408 after 24-h exposure and significantly decreased to 0.3625±.02394 after 15-days exposure. A reduction in blood glucose level was found as the exposure time increased. After 24-h treatment, it was 85.8750±.02393 and reduced to 49.0000±.02041 after 15-days. Calcium level follows the same trend as blood glucose. After 24-h exposure, calcium level was 7.5000±.020412 and 5.5250±.06292 after 15-days. In the 24-h treated group LDH was 9.3125±6.2500 and reduced to 6.5650±1.7078 in the 15-days treated group. The observed value of SGPT in the 24-h cypermethrin-treated group was 82.7500±.0322 and decreased to 51.2500±.0322 in the 15-day cypermethrin treated group. Results are shown in Table 3.

Discussion

The effect and toxicity of cypermethrin on *C. idella* have not been previously reported. For the first time, we report on the toxic effect of cypermethrin on hematology and blood biochemistry in *C. idella.* We found that cypermethrin affects the hematological and biochemical parameters of *C. idella* blood. This study provides a more effective understanding of the toxic effect of cypermethrin on *C. idella* hematology and biochemistry. Insecticides are one of the main causes of aquatic pollution, leading to adverse effects on all aquatic organisms, including fish (32).

Fish blood reflects its physiology and health (33) (34, 35). The application of cypermethrin in agriculture for the control of insects and pests has to its runoff into aquatic bodies (36). Here, we addressed the effects of cypermethrin on the grass carp, determining its effects on hematological and biochemical parameters in blood.

Here we show that cypermethrin significantly increased WBC, MCV, and MCHC compared to the control group. Neelima *et al*, (37) conducted a static-renewal bioassay to assess the acute and sublethal toxicity of cypermethrin on some hematological parameters of white carp (*Cirrhinus mrigala*), observing a significant increase in WBCs count. To overcome the hypoxic conditions in high toxic medium, fish increase their MCV and MCH levels (38). The decrease in PCV and MCV shows that cypermethrin may interfere with the normal physiology of RBC. A significant decrease in the hematocrit values after exposure to cypermethrin is indicative of anemia and hemodilution possibly due to gill damage or/and impaired osmoregulation (37).

The toxic effects of cypermethrin (25 percent EC) on tilapia liver and gills were studied by Devi and Leon (39). Cypermethrin affects gills epithelial cells, along with pronounced changes in the liver (increased vacuolization, necrosis, cytoplasmic vacuolization, decreased red blood corpuscles, excessive mucus secretion). An analogous effect on RBCs was observed in the current study. The low Hb concentration noted herein may be attributed to cypermethrin's propensity to adversely affect the oxygen-carrying capacity of RBCs.

In a study, grass carp were randomly exposed to different concentrations of cypermethrin, observing its effect on serum and spleen alkaline phosphatase (ALP) activity. ALP activity in serum and spleen was significantly increased, showing that cypermethrin was toxic to grass carp serum and spleen. In the current study, a similar effect of cypermethrin was observed. finding a reduction in serum ALP activity (40). Cypermethrin has also been studied by Carpio L. (41). Cypermethrin caused oxidative stress, apoptosis and immunodeficiency in the spleen of C. idella and led to oxidative damage, decreasing serum protein (42) analogous to the decrease in serum protein noted in the present study. Serum proteins may be reduced due to liver hypofunction, as the majority of the serum proteins are synthesized in the liver.

The levels of LDH were increased upon cypermethrin treatment in both acute and chronic groups, but the increased level was highly reported in acute groups as compared to chronic groups, according to Das and Mukherjee (43) in *L. rohita*. LDH levels may be increased because of liver hypofunction. Cypermethrin damages hepatocytes were, resulting in the rise of LDH. The increase in LDH activity may indicate activation of the glycolytic process and anaerobic metabolism.

Significant increases in creatinine, urea, glucose, cholesterol, and alkaline phosphatase levels, and reduction in total proteins and triglycerides in serum were reported in Jundiá, a South American teleostean fish. The observed results corroborate the findings of the present study (44). The effect may reflect liver necrosis and continuous leakage of alkaline phosphatase into blood vessels. In fish, a rise in serum glucose level is considered a general response to stress and it is known as an environmental stress indicator. Cypermethrin-exposed fish showed low hemoglobin content and hyperglycemia, especially after long-term exposure to high concentrations. Analogous results showing decreased Hb and hyperglycemia followed by hypoglycemia were observed herein. A significant decrease in Hb levels may lead to impaired oxygen supply to fish tissues, thus resulting in a slow metabolic rate and low energy production. Cypermethrin caused increased levels of serum alkaline phosphatase, as shown herein. Moreover, reduced levels of serum total protein, cholesterol, and a higher level of glucose are attributed to increased demand for energy by fish under stress to cope with detrimental conditions imposed by chronic exposure to the toxicant (45).

Conclusion

This study sought to determine the acute and chronic toxicity of cypermethrin on grass carp biochemical and blood parameters. In addition, to test cypermethrin toxicity at various concentrations and exposure times, both acute and chronic groups' protein, cholesterol, phosphorus, and calcium levels decreased, but acute groups decreased more than chronic groups. Glucose, urea, SGPT, creatinine, and LDH levels increased in both acute and chronic groups. The MCV, MCH, MHCH and RDW-CV values were decreased. WBC and platelets were increased. It was concluded that cypermethrin significantly alters fish hematology and biochemistry. This study will provide a more effective understanding of the toxic effect of cypermethrin on fish. The parameters studied are sensitive to toxicants and can be used as an indicator of the toxicological impacts.

Conflict of Interest

The authors did not declare any conflict of interest.

Acknowledgment

This work is supported by Taif University Researchers Supporting Program (Project Number: TURSP-2020/153) Taif University Saudi Arabia.

List of abbreviations

Serum glutamic pyruvic transaminase= SGPT, Lactate dehydrogenase = LDH, Red Blood Cells = RBC, Hemoglobin = HGB, Hematocrit = HCT, Mean Corpuscular Volume = MCV, Mean Corpuscular Hemoglobin = MCH, Mean Corpuscular Hemoglobin Concentration = MHCH, Red Cell Distribution Width = RDW, White Blood cells = WBC.

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