Evaluation of Hematological Parameters in two Different Species Trout Exposed to Chlorpyrifos

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Abstract: Environmental pollutants are a major problem for aquaculture. The aim of this study was to evaluate the effects of chlorpyrifos, a pesticide used by agricultural producers in plant production, on some hematological parameters (hemoglobin, hematocrit, erythrocyte, leukocyte, platelet, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo truttafario). Fish were exposed to CPF concentrations (1 µg/L and 2 µg/L) for periods of 21 days.

In rainbow trout; dosage effects were found very significantly for leukocyte, platelet, MCV and MCHC among hematologic parameters (P<0.01), whereas dosage main effects were found significantly for other parameters (P<0.05). In brown trout; hemoglobin, hematocrit, erythrocytes, leukocytes, platelets, MCH and MCHC were significantly affected by the treatments (P<0.01). In chlorpyrifos applications, the values regarding hematologic parameters showed some differences such as increasing-decreasing and decreasing-increasing.

In this study, hematological parameters will be valuable to facilitate as a potential biomarker for assessing the health status of fish.

Keywords: Rainbow trout, Hematological parameters, Brown trout, Chlorpyrifos.

1. INTRODUCTION

Chlorpyrifos (CPF) is intensively used pesticide based on organophosphate insecticide. CPF includes a phosphor thiol functional moiety, has been broadly used in agricultural applies to increase crop yields [1-2]. It was shown that CPF was the pollutants in surface and ground water of many countries. Furthermore, CPF has also been detected in many aquatic ecosystems, including natural and urban streams, rivers and lakes [3]. CPF is very toxic for fish even at low concentrations [4]. Therefore, fish and aquatic organisms and water ecosystems are negatively affecting the balance. There are many studies on the adverse effects of CPF toxicity in fish [5-6-7]. Fish have proved to be good models to evaluate the toxicity and effects of contaminants in previous studies. Rainbow trout and brown trout are an economically important fish species, commonly found in rivers and Dam Lake in Turkey and considered as a potential bio indicator species [8]. Exposure of fish to various chemicals can cause changes in various hematological parameters [9]. Examination of the hematological parameters of organism in studies carried out in order to determine toxic effects is important for achieving correct knowledge [8-9]. Hematology is a science that not only gives information about fish diseases but also determines the effects of nutrition and environmental factors. Fish affects all kinds of stresses, diseases, nutritional deficiencies, toxic substances, changes in water quality and other environmental factors [10]. Hematological parameters are very important diagnosis of diseases. Erythrocyte and leukocyte counts, hemoglobin, hematocrit and platelet counts can easily respond to determining stress caused by environmental pollutants. The measurement of hematological changes in the blood has an important role in predicting the toxic effects of toxic substances [11]. The study of hematology parameters in fishes has been widely used for the detection of physio pathological alterations in different conditions of stress. Consequently, in the present investigation an effort has been made to study the effect of CPF on alterations in hematological parameters of rainbow trout and brown trout to the same stressor were compared.

2. MATERIALS AND METHODS

2.1. Fish Maintenance and Experimental Design

Rainbow trout (O. mykiss) and brown trout (S. t. fario) (average weight 160±10 g and mean standard length of 19±2 cm) were supply from Ataturk University, Faculty of Fisheries. The water was
distributed to the 450-liter indoor fiberglass tanks (A=15 rainbow trout, B=15 rainbow trout, C=15 rainbow trout, D=15 brown trout, E=15 brown trout, F=15 brown trout) as having a minimum of 0.5 l/min for each kg of fish. Water parameters has been measured during the study (water temperature 9 ±1.5 °C, dissolved oxygen 9.2 mg/l and pH 7.2). 15 fish have been distributed per tank for a total of 6 tanks. 2 of the tanks control (one of their O. mykiss and the other S.t.fario) group and the other 4 tanks (O. mykiss and S.t.fario) have been defined as the treatment groups. As per the trial procedure for defined volume retrials, the concentrations have been reapplied every 12 hours (continuous flow system) in order the dose. We determined the constants that were lower than the negative effect level observed for the trout. Fishes were exposed to CPF concentrations of 1 µg/L and 2 µg/L for 21 days. After 21 days (chronic exposure), the five fish from each tank were randomly selected and sampled.

2.2. Chemical
Chlorpyrifos (480g/L chlorpyrifos, O, O-diethyl-O-(3,5,6-trichlor-2-pyridyl) phosphorothioate) was attained from a commercial manufacturer (Akdeniz Chemical Companies, Erzurum, Turkey). Chlorpyrifos were solubilized in test water. This stock was diluted in tanks to obtain experimental concentrations. The LC50 value for trout (9 µg/l) of chlorpyrifos was determined according to EPA [13]. For each fish species one tank was used as control (no application of pesticide), and the others were added 1 µg/l and 2 µg/l chlorpyrifos, respectively. We have chosen these concentrations because it is lower than lethal concentrations for rainbow trout. Fish deaths were not observed during chemical exposure.

2.3. Hematological Analysis
Nearly 2 cc blood was collected from caudal vena to determine of hematological parameters. Fish from each treatment were for collection of blood by puncture of the caudal vessel using a syringe containing 10% EDTA solution. The collected blood was separated into aliquots for different analyzes: the red blood cell (RBC), white blood cell count (WBC) and platelets (Plt) were determined according to Vanietal. [14] and Parlak[15], for analysis of hematocrit percentage (Hct), Goldenfarbet al.[16]method was used. Hemoglobin concentration (Hb) was measured by the Collier [17] method. Hematological parameter such as the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe[18] method.

2.4. Statistical Analyses
The SPSS 20.0 software was used to perform all analyses and was assessed by Duncan. The data are expressed as the mean ± SD. Significant difference was acknowledged when P<0.05.

3. RESULTS
The changes in hematological parameters of the fish rainbow trout and brown trout exposed to chronic toxicity of chlorpyrifos are presented in Table 1. Chlorpyrifos exposure in rainbow trout led to a significant increased (P<0.05) in WBC, RBC, Hb, MCH and MCHC, but MCV decreased. Chlorpyrifos exposure in brown trout led to a significant increased WBC, RBC, Hb, and MCHC but MCV and MCH decreased (P<0.05) (Table 1).

Table1. Hematological parameters of rainbow trout and brown trout.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rainbowtrout</th>
<th>Brown trout</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1 µg/L</td>
</tr>
<tr>
<td>WBC</td>
<td>4.167</td>
<td>4.225</td>
</tr>
<tr>
<td>RBC</td>
<td>2.120</td>
<td>2.468</td>
</tr>
<tr>
<td>Hb</td>
<td>2.428</td>
<td>4.002</td>
</tr>
<tr>
<td>MCV</td>
<td>243.396</td>
<td>221.102</td>
</tr>
<tr>
<td>MCH</td>
<td>11.412</td>
<td>16.214</td>
</tr>
<tr>
<td>MCHC</td>
<td>4.689</td>
<td>7.334</td>
</tr>
<tr>
<td>Plt</td>
<td>9.127</td>
<td>10.123</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1 µg/L</td>
</tr>
<tr>
<td>WBC</td>
<td>2.992</td>
<td>3.780</td>
</tr>
<tr>
<td>RBC</td>
<td>2.006</td>
<td>2.362</td>
</tr>
<tr>
<td>Hb</td>
<td>6.102</td>
<td>6.326</td>
</tr>
<tr>
<td>MCV</td>
<td>284.262</td>
<td>217.929</td>
</tr>
<tr>
<td>MCH</td>
<td>30.418</td>
<td>26.333</td>
</tr>
<tr>
<td>MCHC</td>
<td>10.700</td>
<td>12.095</td>
</tr>
<tr>
<td>Plt</td>
<td>6.994</td>
<td>7.482</td>
</tr>
</tbody>
</table>

*MCV = [PCV (%) / RBC (10⁶)] x 10 µm³; MCH = [Hb (g) / RBC (10⁶)] x 10 pg; MCHC = [Hb (g) / PCV (%)] x 100 g per 100 ml, RBC: Red blood corpuscle count; Hb: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; WBC: White blood cell count. Means sharing the same letter in the same row are not significantly different (p > 0.05)
Table 1 shows blood parameters changes such as erythrocyte count, leukocyte count, platelet count, hemoglobin, hematocrit, MCV, MCH and MCHC in rainbow and brown trout. The data revealed decreasing or increasing differences in all hematological parameters, only the differences in RBC, Hb and MCV were statistically significant (P<0.05) in rainbow and brown trout. After the exposure period, compared with those obtained in the control samples, the MCV in rainbow trout samples were relatively decreased, while other parameters were relatively increased. After the exposure period, compared with those obtained in the control samples, the MCV and MCH in brown trout samples were relatively decreased, while other parameters were relatively increased.

4. DISCUSSION

Hematology is the science that investigate the organs that make up blood cells and blood cells. Hematological analysis is very important for the diagnosis of fish diseases. Factors such as hemoglobin breakdown, decrease in erythrocytes and amount of hematocrit can be an indicator of anemia [19]. Hematologic parameters are indicators used to identify physiological and biochemical changes caused by stress factors in fish [8-20].

Reductions in hemoglobin value are related to the destruction of cells due to the harmful effect of pesticide. The decrease in hemoglobin value of fish exposed to xenobiotics is thought to be due to the deterioration of iron synthesis mechanism due to the inhibition of aerobic glycolysis [21]. The increase in hemoglobin level is considered as a kind of adaptation. Disruption of the Osmoregulation balance, hypoxia and decreased blood fluidity cause an increase in hemoglobin level [22]. Since hematocrit level is proportional to the number of erythrocytes, blood is an important parameter that informs the oxygen transport capacity and the functions of erythropoietic tissues [23]. Hematocrit is generally reduced as blood fluid increases in the anemia. In fish studies, it was determined that the number of erythrocytes and hematocrit changes depending on species, medium concentration and duration of exposure [24].

As a consequence of chronic administration, it is considered that the hematocrit level is decreased by decreasing the number of red blood cells or by hemodilution. Xenobiotics cause changes in the nucleus and cell structure by changing the membrane properties of erythrocytes [25-26]. The increase in the number of RBCs in fish treated with chlorpyrifos after chronic treatment showed that osmoregulation and gill tissue were not affected by pesticide. In the study, the results obtained for RBC are similar to those obtained in other studies [27-28-29]. The increase in the number of WBCs can result from protective response against immune response and pesticide stress. Leukocytes are the most important cells of the immune system because of their defense system function. Pesticide exposure increases lymphocyte release from related tissues under toxic stress, which can lead to an increase in WBC count [30]. The increase in leukocyte count can be explained by the toxic damage caused by the defense system response to the fish or by stressed fish trying to establish homoeostasis balance [31]. The increase in platelet count can be demonstrated by the amount of xenobiotic substance in the body, the adverse effects on the blood-dependent immune system, the decrease in the amount of oxygen in the blood cells, and the response to the spleen [32]. In the case of hypoxia, fishes increase the amount of MCV and MCH of erythrocytes to get through this condition. The increase in these values is due to the destruction or non-production of erythrocytes. They reported that the stress factor caused an increase in MCV and a decrease in MCHC [33]. The increase in MCV was defined as endosmosis, and hemodilution would occur if this value increased more. The fish respond by increasing the MCV and MCH of erythrocytes to combat the hypoxia that the stress factor produces. In addition, toxic stress can lead to a decrease in Hg values at the same time as the breakdown of Erythrocytes [34]. The decrease in Hg content may be due to the hemoglobinization or shrinkage of erythrocytes due to the toxic effect of chlorpyrifos in the erythropoietic tissue [35-36]. Consequently, the resulting data indicate that chlorpyrifos exposure caused hematological changes in both fish species. Hematological parameters give information about the physiological status of the fish. Determining the level of pollution in the aquatic environment will be a step in the prevention of fish diseases and deaths. The data we obtained in our study indicate that hematology parameters are effective in determining pesticide toxicity. These data will contribute to toxicology studies and can be used in pollution monitoring programs and ecotoxicological risk assessment studies.
REFERENCES


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