Determination of diazinon effects on some haematological parameters in rainbow trout, oncorhynchus mykiss

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ABSTRACT

The aim of this study was to determine the effects of diazinon on some haematology indices in rainbow trout. During the four day, 10 rainbow trout were exposed to two different concentration of diazinon, 40% (0.66mg/L) and 80% (1.32mg/L), respectively (LC50 =1.65mg/L). The values of erythrocytes (RBC), packed cell volume (PCV) and hemoglobin (Hb) were significantly (P<0.05) decreased. Whereas, leukocytes (WBC) value increased significantly (P<0.05) and then decreased. The values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly (P<0.05) increased with diazinon administration. However, the increase in the mean corpuscular hemoglobin content (MCHC) was statistically insignificant (P>0.05). Lymphocyte decreased significantly (P<0.05). Neutrophils increased and then decreased significantly (P<0.05). In conclusion, the changes blood parameteres of the fish exposed to diazinon may be related to decreased immune system.

Key words: Diazinon, Effect, Haematological parameters, Organophosphorous pesticide, Rainbow trout.

INTRODUCTION

Pesticides are the main cause of environmental problems due to the lower dilution capacity of the water systems which leads to the risk of high concentrations of toxic substances. Recent days, pesticides are main causes of toxicity in fish. Pollute can pass water sources by two ways: through direct application of pesticides in water systems and indirect uses like erosion in cultivated areas and filtration of agricultural sewage and eventually passed into ground waters and ecosystem. Moreover, pesticides have harmful effects for many animals including fish and bird, and the accumulation of undesirable toxic substance in fish tissue can affect health of people who eat fish (Adedeji et al., 2000; Shamoushaki et al., 2012). Diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimiinyl) phosphorothioate] is mostly used in agriculture to control the insects (Adele and et al., 2009). Diazinon has functions as an acetylcholinesterase (AChE) inhibitor in the organism. When diazinon enters the body, it leads to a permanent stimulation within the organism by inhibition acetylcholine esterase and results in the paralysis of the nervous system of insects (Miron et al., 2005). At the same time, it penetrates in to the interstitial fluid of fish, affects fish reproduction and reduces the population (Far et al., 2012). The important point is that increasing time of exposure of fish to reduce pesticide resistance has more damaging effects on fish community (Oh et al., 1991). The presence of diazinon in water and its negative effects on aquatic organisms has been reported (De-Vlaming et al., 2000). Blood and tissues of living organisms are very sensitive to changes and are widely used in Ichthyology research (Adedeji et al., 2000). Many studies conducted in recent years have showed the harmful effects on fishes and the aquatic ecosystem of pesticides. Haematological parameters in fish living in the different environmental conditions are very important for toxicological studies (Schlenk, 2005; Banaee et al., 2008; Banaee et al., 2011). This study was to determinate the effects of diazinon on some haematological indices in rainbow trout.

MATERIALS AND METHODS

Collection and maintenance of fish: Fish and Chemical treatments were performed in the Ataturk University, Faculty of Aquaculture Inland Water Fish Research and Application Center. The live Rainbow trout (n = 30) used in this study were an average weight of 150–190 g. The average temperature of the water was 12 ± 2°C during the experiments. Fish were fed with 2% of body weight fish feed once a day. Before attempting, the fish divided into groups were kept in 1 x 1.2m (wide-deep) fiber-glass tanks one week for adaptation. Aeration was allowed during the trial. The quality parameters of water were measured as O2 = 8.5 ppm, pH = 7.4, SO42- = 0.30 mg/L, PO43- = trace, NO3- = 3.15 mg/L, NO2- = trace and conductivity = 230 ls/cm (Karatas et al., 2015).

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**Acute Toxicity:** Regarding acute effects of agricultural chemicals, Diazinon was identified as LC50 (96h). For this, 3 treatment groups including control group were created to test toxicity; each treatment was 3 replicated with 10 fish per tank with 60 L water capacity. Fishes were exposed to Diazinon at a nominal concentration of 0.0 mg/L (control group), 40% (0.66mg/L) and 80% (1.32mg/L), respectively, which were equivalent to approximately 40% and 80% of 96 h LC50 value (1.65mg/L) for 4 days toxicity testing. Fish deaths were saved (24, 48, 72 and 96 h) after the beginning and died fishes were taken instantly from the tank (Banane et al., 2011).

**Haematology assay:** After the test period, fish were taken out of the water rapidly and held securely in a loom with a soft cloth covering the head for taking blood samples. Blood samples collected with the help of a syringe from the tail vein were transferred to haematology (with anticoagulant) tubes and shaken a few times in order to prevent freezing. Retention time of fish was less than 1 min in order to avoid the effects of stress (Karatas et al., 2014). At the time of blood sampling, suitable smears created were air-dried, kept in 96% ethanol during 30 min and painted with Giemsa dyeing for 30 min. Then, the leucocyte count was performed under microscope. The haematological parameters were examined for erythrocyte count (RBC, ×10⁶/mm³), hematocrit (Hct, %), haemoglobin (Hb, g/dL), mean corpuscular haemoglobin (MCH= Hb in g/ RBC in millions × 10pg), mean corpuscular volume (MCV= packed cell volume as percentage/RBC in millions × 10³3, fl), mean corpuscular haemoglobin concentration (MCHC= Hb in g/ packed cell volume × 100 g per 100 mL, %), and leucocyte count (WBC×10⁶/mm³) (Shamoshaki et al., 2012; Klont et al., 1994).

**Statistical analysis:** The variations through the medium of ±SEM between groups were evaluated using Independent Samples-t test. 95% confidence limits were considered important. SPSS 15.0 software was used for data analysis (Karatas, 2015).

**RESULTS AND DISCUSSION**

Concentrations of diazinon were equivalent to approximately 40% and 80% of 96 h LC50 value for 4 day toxicity testing. In some cases, affected fish from diazinon exhibited vertical and downward swimming and swimming near to surface. Haematological parameters studied in diazinon administered trout are shown in Table 1. RBC, PCV and Hb levels decreased significantly (P<0.05) after exposure to Diazinon. Nevertheless, WBC value increased significantly (P<0.05) and then decreased. The MCV and MCH values increased significantly (P<0.05) and MCHC values were insignificant. During experimental period, level of lymphocyte decreased significantly (P<0.05). Whereas, level of neutrophil increased and then decreased significantly (P<0.05). Haematological parameters show different susceptibility against various environmental factors and chemicals. Toxic substances may cause a change in haematological parameters (Zhiteneva et al., 1989; Golovina, 1996; Luskova et al., 1997). The purpose of hematological studies is to determine the sensitivity to environmental conditions of fish (Fernandes et al., 2003). High concentration and short-term exposure to sub lethal concentration generally reduces erythrocyte indices. During experiment of diazinon toxicity, mortality was not observed. Levels of RBC, PCV and Hb decreased both at 40% and 80% in comparison with the control group. But, level of WBC increased in 40% and decreased at 80%. Significant (P<0.05) differences in WBC, RBC PCV and Hb, diazinon toxicity were observed between 40% and 80%. Similar results were observed in O. mykiss exposed to cypermethrin (Atamanalp et al., 2002), in Brook trout and Fathead minnows exposed to Diazinon (Allison et al., 1987) and in Cyprinid watsoni exposed to malathion (Khattak et al., 1996), in S. glanis exposed to Diazinon (Koprucu et al., 2006), in C. carpio exposed to Diazinon (Luskova et al., 2002), in C. gariepinus exposed to Diazinon (Adeje et al., 2000). In the present study, the values obtained for the hematological indices showed significant change in MCV and MCH (P<0.05). Neutrophils are important in terms of protect the body and evaluation of phagocytic activity, against bacterial infection. Neutrophilia formed as nonspecific response against stress in animals. The percentage of neutrophils generally decreases during exposure to pollutants (Nussey et al., 1995; Svoboda et al., 1994). Toxic substances can cause a weakness in immune system of fish. The results of this study showed that the duration of Diazinon exposure may cause a destruction in hematopoietic tissue or weaker immune system with increasing concentrations (Svoboda et al., 2001). Toxic substances accumulated in fish tissues and adversely effect of the health condition of the fish. Therefore, blood parameters are important in terms of determination of the effect of toxins (Cyriac et al., 1989). Stressors like pesticide Diazinon may be potentially the cause of decreased number of leukocytes, lymphocytes and neutrophils. In the present study, lymphocyte decreased significantly (P<0.05). Neutrophils increased and then decreased significantly (P<0.05). In conclusion, haematological parameters could be varied within the effects of many

<table>
<thead>
<tr>
<th>Indices</th>
<th>Control</th>
<th>40%</th>
<th>80%</th>
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<tbody>
<tr>
<td>WBC(10⁶/mm³)</td>
<td>357.0±19.4²</td>
<td>248.1±99.2²</td>
<td>275.2±113.4²</td>
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<tr>
<td>RBC(10⁶/mm³)</td>
<td>1.35±0.10²</td>
<td>0.86±0.36²</td>
<td>0.85±0.32²</td>
</tr>
<tr>
<td>PVC (%)</td>
<td>27.2±2.12²</td>
<td>18.7±6.82²</td>
<td>15.5±6.80²</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>8.5±0.54²</td>
<td>6.65±1.97²</td>
<td>5.05±2.10²</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>69.6±26.6²</td>
<td>79.0±21.1²</td>
<td>87.5±2.1²²</td>
</tr>
<tr>
<td>MCV (µm³)</td>
<td>22.8±8.20²</td>
<td>286.7±65.4²</td>
<td>342.2±97.4²</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>24.0±5.43²</td>
<td>27.2±1.70²</td>
<td>30.7±7.47²</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>38.9±1.05²</td>
<td>43.12±15.5²</td>
<td>26.8±13.0²</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>84.7±4.46²</td>
<td>68.7±21.3²</td>
<td>75.8±19.8²</td>
</tr>
</tbody>
</table>

*Results are given as mean±standart deviation. There is significant difference between parameters given as different superscripts (P<0.05).*
environmental factors such as temperature, season, water quality and sex, the type of culture and chemicals. Stressors like pesticide diazinon may be potentially the cause of decreased number of leukocytes, lymphocytes and neutrophils. It is revealed that the evaluation of blood parameters is significant in terms of having information about the health status of fish.

REFERENCES


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