Dimethoate Toxicity on Ions and Hematological and Enzymatic Parameters of Nile Fish Oreochromis niloticus (L. 1758)

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Abstract

The objective of this study was to evaluate the acute toxicity of dimethoate (0.8 mg/L, 8.0 mg/L and 16.0 mg/L) in Nile fish Oreochromis niloticus (L. 1758) for 24, 48 and 96 hours. The dimethoate toxicity was investigated in fish blood ions (Na, K, Ca and Cl), hematologic (Htc, Hb, RBC and WBC) and enzymatic parameters (ALT and AST). Autoanalyser spectrophotometric methods were used in this experiment. The result of ion levels such as Na, Cl and Ca were decreased, but K was increased in dimethoate exposure. The most decrease of these ions was Ca, and it was followed by Na and Cl. The blood Hb, RBCc and WBCc levels, except of Hct levels, decreased dimethoate exposed fish at time periods. The blood ALT and AST levels were found to be significantly elevated for dimethoate exposed condition. The hematological parameters were significantly altered by dimethoate. These blood parameters indicated that O. niloticus were sensitive of dimethoate on aquatic systems.

Keywords: Dimethoate; Oreochromis niloticus; Blood; Hematological Parameters; Ions; Enzymes

Introduction

Generally, pesticides are widely used in agriculture and animal husbandry. However, if misused, they may be harmful to humans, animals and the environment [1]. Organophosphorus (OP) insecticides are generally the most preferred pesticides for short-lived and tend not to accumulate in plant or animal tissues to any great extent. Organophosphorus (OP) insecticides are considered as anticholinesterase insecticides and the mechanism by which they elicit their toxicity is identified and is associated with the inhibition of nervous tissue [1-3] and other neurophysiological abnormalities [4]. Dimethoate, an organophosphate insecticide, was first described in 1951 and marketed [5]. The insecticide dimethoate (IUPAC name: O,0-dimethyl-S-methyl carbamoyl methyl phosphoro-dithioate) was selected for this study because it is a widely used to kill mites, mites and leaf pests. Dimethoate is water soluble and can leach into water sources and affect aquatic organisms such as fish [6-8]. And also, dimethoate brings about lesions in fish gill, liver and kidney [9]. In fish, ions such as sodium, potassium and calcium play an important role in osmoregulator systems [10]. David., et al. (2003) [11] shown that the ions, sodium, potassium, calcium in fish tissues, were decreased by pesticide. However, magnesium levels were also observed to increase by Schreck (1981) [12].

Hematological indices such as hematocrit (Hct), hemoglobin (Hb), red blood cells (RBCs), and white blood cells (WBCs) have been used as an indicator of pollution in the aquatic environment [13]. Ions, causing serious problem for healty in any change of their levels, are very
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important indices for health of fish. Pesticides may change of this parameters [14]. Therefore, it was emphasized that serum ion parameters can be useful as a diagnostic and general health status of fish. The activities of plasma enzymes such as ALT and AST are important biomarkers in assessing toxicity in aquatic ecosystems [14,15]. Nile tilapia, *Oreochromis niloticus*, has a worldwide economic prescription and is widely used in toxicological studies [16]. And also, fish blood determines the health of the organism. There are various reports on toxicity of different organophosphorus pesticides of fish [17]. The present study aimed to investigate the ions, hematological and enzymatic parameters effects of dimethoate toxicity of *O. niloticus*.

Materials and Methods

*Oreochromis niloticus* fish were obtained from Çukurova University Fisheries Faculty pools and their adaptation was ensured in five stock aquariums of 40 x 100 x 40 cm and acclimatized in the laboratory for two month at 25 ± 1°C. The adaptation of fish and experimental periods were used photoperiod in 12 light/12 dark lights. After this period the mean length and weight of the animals were 14.9 ± 1.25 cm and 50.5 ± 1.16 gr., respectively. The study was approved by the Animal Experimentation Ethics Committee of Çukurova University (Protocol 4-9/2015).

Water quality characteristics in tanks; pH: 8.4 ± 0.7, temperature: 25 ± 1°C, dissolved oxygen: 7.56 ± 0.6 mg/L, total hardness: 182.5 ± 4.21 CaCO₃ mg/L, total alkalinity: 268.2 ± 6.7 CaCO₃ mg/L.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control C</td>
<td></td>
</tr>
<tr>
<td>0.8 mg/L</td>
<td>T1</td>
</tr>
<tr>
<td>8.0 mg/L</td>
<td>T2</td>
</tr>
<tr>
<td>16.0 mg/L</td>
<td>T3</td>
</tr>
</tbody>
</table>

Table

Fish were divided into four groups and each group containing 18 fish (N = 6). Group I was held in tap water as control (C), and other groups were exposed to 0.8 mg/l dimethoate (T1), 8.0 mg/l dimethoate (T2) and 16.0 mg/l dimethoate (T3) for 24, 48 and 96 hours. The concentration of each toxicant was selected as sublethal concentration and based on available literature data (LC₅₀ for *O. niloticus* is 40 mg/L [18]). Fish were maintained in static conditions, where water and pesticide were not replaced during to 96 hours, freshly prepared toxicants solutions. Throughout the experiments, control and experimental fish were fed daily with a commercial fish food (Pinar Yem, İzmir, Turkey), at approximately 3% of their body weight.

The end of the each exposure time (24, 48 and 96 h), fish were anesthetized with MS-222 blood was collected from each fish by cutting the caudal peduncle. Fish blood was collected and divided, for hematological parameters and other parameters. The blood was centrifuged at 4000 rpm over 10 minutes at 15°C for the serum. Hematological parameters (Htc, Hb, RBCc and WBCc) immediately were determined by otoanalizatory (Beckman Coulter LH 750 hematology analyzer) at Cukurova Universty, Balcalı Hospital, centre laboratory. The serum samples were frozen and stored at -20°C until required for assays. The analyses of ALT and AST activities were assayed using UV test technique by Bergmeyer, Hoder, and Rej (1985) [19]. Ions such as Na, K, Ca, Mg and Cl were determined by ROCHE Hitachi E-170 and DPP at Cukurova Universty, Balcalı Hospital, centre laboratory.

Data are presented as mean ± standard error. For the statistical analysis, a one-way Analysis of Variance (ANOVA) was used, followed by Student Newman–Keul’s test, using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL). Differences were considered significant if p < 0.05.

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Results and Discussion

No mortality was observed at during the experiments. The statistical analysis was done with "SNK" at p < 0.05 and showed table 1-3 and figure 1-6.

Figure 1-4 show distribution of hematological (Hb, Hct, RBCc and WBCc) parameters dimethoate exposed to *O. niloticus* over 24, 48 and 96 hours. In blood parameters notable declines were observed statistically at dimethoate exposure during the same time compared with control group (Figure 1-4) and the letters a, b and c show significant differences (p < 0.05). But, Hct contents were increase in dimethoate exposure (58%). The maximum decrease of 40% Hb and 25% RBCc were observed in 24 hours and 26% RBCc at 96 hours.

The blood Hb, RBCc and WBCc levels, except of Hct levels, decreased dimethoate exposed fish at both exposure periods (Figure 1-4). In the compared with control group the exposures of dimethoate did not cause any significant changes in RBCc and WBCc levels of fish at 24 hours while they caused an decrease in its levels at the end of the exposure period. Similarly, Thangavel, *et al.* [20] reported similar results when exposing the fish *Sarotherodon mossambicus* to dimecron. While Singh and Srivastava [21] showed that another organophosphate insecticide Formothion gave a significant increase in the total erythrocyte count and hemoglobin content in fish.

**Figure 1:** Hemoglobin levels (Hb) in *O. niloticus* were evaluated statistically by exposure to dimethoate during the same time. The letters a, b and c show significant differences (p < 0.05).

**Figure 2:** Hematocrit levels (Hct) in *O. niloticus* were evaluated statistically by exposure to dimethoate during the same time. The letters a, b and c show significant differences (p < 0.05).
Dimethoate was classified as a toxic substance for fish. Changes in the erythrocyte profile suggest a compensation of oxygen deficit in the body due to liver damage. Hematological indices are commonly used as indicators of pollution in fish [22,23]. This index reflects respiratory status of animals. Toxicants in hematological parameters of fish generally occur due to the osmotic changes resulting in hemodilution or hemoconcentrations [24]. Significantly depressed blood hematological parameters were observed in blood (RBCc and WBCc) (P < 0.005) of fish at exposure of dimethoate in this study.

The present study showed that exposure of dimethoate in the alterations in serum ion levels of *O. niloticus* (Table 1-3). Na, Cl and Ca levels decreased, but K levels increased in dimethoate exposed fish at exposure times. Martinez and Colus [25] shows that in the hydromineral balance may be a consequence of the action of pollutants on organs involved in osmoregulation, on endocrine system, on metabolism, or on active transport processes. McDonald., et al. [26] show that plasma Na and Cl similarly affected by toxic chemicals as
similar shown of our work. Serum Na and Cl ions levels decreased in fish *Prochilodus scrofa* [27] and *Cyprinion mhalensis* [28]. Klyszejko and Lyczywek [29] shown that Na levels decreased in *C. carpio*, although K level was increased by pesticide as similarity of our work.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na (mmol/L)</th>
<th>Cl (mmol/L)</th>
<th>Ca (mmol/L)</th>
<th>K (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>161.1 ± 0.03 a</td>
<td>144.1 ± 1.20 a</td>
<td>13.4 ± 0.10 a</td>
<td>4.54 ± 0.11 a</td>
</tr>
<tr>
<td>T1</td>
<td>165.2 ± 0.08 b</td>
<td>148.2 ± 0.01 b</td>
<td>12.1 ± 0.57 b</td>
<td>5.31 ± 0.12 b</td>
</tr>
<tr>
<td>T2</td>
<td>162.4 ± 0.13 a</td>
<td>145.2 ± 0.10 a</td>
<td>11.5 ± 0.10 b</td>
<td>5.45 ± 0.17 b</td>
</tr>
<tr>
<td>T3</td>
<td>160.1 ± 0.30 a</td>
<td>142.1 ± 0.65 a</td>
<td>11.1 ± 0.10 b</td>
<td>5.95 ± 0.66 b</td>
</tr>
</tbody>
</table>

Letters a, b and c show the differences between groups (P < 0.05).

**Table 1:** Ion Parameter levels (Mean ± SE) (N = 6) of the fish *O. niloticus* blood exposed to dimethoate over 24 hours.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na (mmol/L)</th>
<th>Cl (mmol/L)</th>
<th>Ca (mmol/L)</th>
<th>K (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>162.1 ± 1.10 a</td>
<td>143.1 ± 1.11 a</td>
<td>12.2 ± 0.11 a</td>
<td>4.55 ± 0.11 a</td>
</tr>
<tr>
<td>T1</td>
<td>159.2 ± 2.11 b</td>
<td>144.2 ± 0.01 a</td>
<td>13.1 ± 0.13 a</td>
<td>4.76 ± 0.03 a</td>
</tr>
<tr>
<td>T2</td>
<td>157.2 ± 1.13 b</td>
<td>143.2 ± 0.22 a</td>
<td>14.5 ± 0.10 a</td>
<td>5.44 ± 0.22 b</td>
</tr>
<tr>
<td>T3</td>
<td>156.1 ± 0.30 b</td>
<td>142.0 ± 0.11 a</td>
<td>17.1 ± 0.22 b</td>
<td>5.85 ± 0.52 b</td>
</tr>
</tbody>
</table>

Letters a, b and c show the differences between groups (P < 0.05).

**Table 2:** Ion Parameter levels (Mean ± SE) (N = 6) of the fish *O. niloticus* blood exposed to dimethoate over 48 hours.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na (mmol/L)</th>
<th>Cl (mmol/L)</th>
<th>Ca (mmol/L)</th>
<th>K (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>166.1 ± 1.20 a</td>
<td>146.1 ± 0.11 a</td>
<td>13.2 ± 0.12 a</td>
<td>4.66 ± 0.11 a</td>
</tr>
<tr>
<td>T1</td>
<td>163.2 ± 0.11 b</td>
<td>145.2 ± 0.22 a</td>
<td>12.1 ± 0.22 a</td>
<td>4.96 ± 0.23 a</td>
</tr>
<tr>
<td>T2</td>
<td>160.2 ± 0.13 b</td>
<td>144.2 ± 0.12 a</td>
<td>11.1 ± 0.11 a</td>
<td>5.46 ± 0.13 b</td>
</tr>
<tr>
<td>T3</td>
<td>159.1 ± 0.11 b</td>
<td>142.0 ± 0.30 a</td>
<td>11.0 ± 0.11 a</td>
<td>6.15 ± 0.52 c</td>
</tr>
</tbody>
</table>

Letters a, b and c show the differences between groups (P < 0.05).

**Table 3:** Ion Parameter levels (Mean ± SE) (N = 6) of the fish *O. niloticus* blood exposed to dimethoate over 96 hours.

Changes in enzymatic parameters (ALT and AST) due to dimethoate exposure in *O. niloticus* are given in figure 5 and 6. Changes in enzymatic parameters were significantly increased in dimethoate exposure compared to the control group. These changes were statistically significant.

ALT and AST activities are used for diagnosis of fish tissues damage such as liver [15]. Soufy, *et al.* [30] shown that ALT and AST activities increased significantly with carbofuran pesticide. Dogan and Can [31] determined that ALT and AST activities of *O. mykiss* were increased by dimethoate. These researchers concluded that the necrosis of liver caused leakage of this enzyme into blood stream.

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Figure 5: Serum ALT levels in O. niloticus were evaluated statistically by exposure to dimethoate during the same time. The letters a, b and c show significant differences (p < 0.05).

Figure 6: Serum AST levels in O. niloticus were evaluated statistically by exposure to dimethoate during the same time. The letters a, b and c show significant differences (p < 0.05).

Conclusion

It is concluded from the present study on the dimethoate toxicity that O. niloticus is very sensitive to hematological, biochemical and ionic parameters. In these serum parameters were observed significantly decreases or increases. Such studies will help in finding out the take precaution in pollutions.

Acknowledgements

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Bibliography


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