Acute Effects Of Diazinon On Blood Parameters In The African Catfish (Clarias Gariepinus)
O Adedeji, O Adedeji, O Adeyemo, S Agbede

Citation

Abstract
The aim of this study was to assess the effect of diazinon [0,0-diethyl 0-(2-isopropyl-6-methylpyrimidin-4yl) phosphorothioate] on cultured and wild African catfish (Clarias gariepinus). The effect was assessed based on results of acute toxicity tests and on a comparison of results of haematological examination of a control and an experimental group exposed to Diazintol pesticide preparation (active substance 162mg.l⁻¹ of diazinon). The acute toxicity test lasting 96 hr was performed semistatically on the African catfish of mean weight 350±15 g, and mean total length of 35±2.0 cm. Examination of erythrocyte and leucocyte profile was performed on 16 control and 20 experimental specimens of the catfish after 96 hr of exposure to Diazintol in concentration of 9.4 mg.l⁻¹. The 96hr LC₅₀ value of Diazintol for the African catfish is 6.6 mg l⁻¹. The experimental group of the African catfish showed significantly lower values (p < 0.05) of erythrocyte count (RBC), haemoglobin content (Hb) and haematocrit (PCV) compared to the control group. Values of MCV, MCH and MCHC were comparable in both groups during the study. In contrary, there was a significant decrease in leucocyte count (p < 0.05), as well as in both the relative and absolute lymphocyte count (p < 0.05) and a significant increase in both the relative and absolute count of developmental forms of neutrophile granulocytes: myelocytes (p < 0.05) and metamyelocytes (p < 0.05) in the experimental group. Relative and absolute count of monocytes and both the band- and segmented neutrophile granulocytes was comparable in both groups during the study. Changes in values of both the erythrocyte and leucocyte profile after exposure to diazinon-based preparation may be referred to disruption of haematopoiesis as well as to a decrease on non-specific immunity of the fish.

INTRODUCTION
Pesticide use is known to cause serious environmental problems, especially in the dry season, because during this period the dilution capacity of the water systems is low, thus increasing the risk of high concentrations of toxic chemicals. Moreover, the dry season is often the critical period for many animals, especially fish and birds. Fish stocks suffer from natural mortality and high fishing pressure at the end of the dry season. Contamination of water by pesticides either directly or indirectly can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans eating these fishes.

Blood analysis is crucial in many fields of ichthyological research and fish farming and in the area of toxicology and environmental monitoring as possible indicator of physiological or pathological changes in fishery management and diseases investigation (Adedeji et al., 2000). Haematological indices are very important parameters for the evaluation of fish physiological status. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners, and diseases (Luskova, 1997; Golovina, 1996; Zhiteneva et al., 1989). In warm-blooded animals, changes in the blood parameters of fish, which occur because of injuries or infections of some tissues or organs, can be used to determine and confirm the dysfunction or injuries of the latter (organs or tissues). However in fish, these parameters are more related to the response of the whole organism, i.e. to the effect on fish survival, reproduction and growth. Although the mechanisms of fish physiological and biochemical reaction to xenobiotics has not been enough investigated, it is obvious that species differences of these mechanisms exist. Thus, a significant number of data on the changes of haematological indices of salmonid fish under the influence of various environmental factors (chemicals as well), which allow certain conclusions to be drawn, can be applied to other fish species only with much care (Folmar, 1993). On the basis of studies of warm-blooded animals, specific indices reflecting the effect of some substances on the organism (e.g. changes in the blood serum cholinesterase...
under the influence of insecticides or changes in methaemoglobin under the influence of nitrites) have been determined, however the complex of unspecified biochemical indicators of blood reveals more fully the general effect of pollutants on fish and makes possible to forecast the consequences of long-term exposure to chemical pollutants.

Many workers have assessed the effect of various pesticides on the behaviors and haematological responses of various species of fish, (Anees, 1978; Benarji and Rajendranath, 1990; Svoboda et al., 2001) and have found varying responses after exposing the fish to varying sublethal concentrations using the 96 hours acute toxicity tests. The purpose of this study is to assess and contributes to knowledge on the haematological changes in cultured and wild African catfish following the exposure of the fish to diazinon.

MATERIALS AND METHODS

ACUTE TOXICITY TEST AND BLOOD COLLECTION

The acute toxicity tests lasting 96 hr was performed semistatically on the African catfish of mean weight 350±15 g, and mean total length of 35±2.0cm according to Adedeji, 2006 unpublished. At the end of 96 hr blood samples were collected from the remnant fish. Examination of erythrocyte and leucocytes profile was carried out on 16 catfish (control) and 20 catfish (experimental). For blood collection 0.2mg benzocaine dissolved in 5ml acetone in 4 liters of water was used as an anesthetic agent. Blood was drawn from the posterior caudal vein according to Schmit et al, (1999) with a 22 G hypodermic needle and 2ml was decanted into plastic tubes containing the sodium salt of ethylene diamine tetra acetic acid (Na-EDTA) as an anticoagulant. Whole blood (50µl) was stained for enumeration of red blood cells (Shaw, 1930). Blood smears were air dried for 5 min, fixed in absolute methanol, and stained for 60 seconds in giemsa stain.

HAEMATOLOGICAL ANALYSES

Red blood cell (RBC), total white blood cell (WBC) and platelet counts were done using the Neubauer haemocytometer. The haematocrit or packed cell volume (PCV) and haemoglobin (Hb) concentration values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods (Hesser, 1960), respectively. The mean corpuscular volume haemoglobin (MCV), mean corpuscular haemoglobin concentration (MCHC) were calculated from the data using standard formulae:

Blood smears made and stained with Giemsa, were used to determine WBC, thrombocyte count and differential WBC counts. Percentage of RBC, WBC and thrombocytes were determined by counting 1,500 cells. The WBC and thrombocyte percentage was multiplied by the RBC count from the haemocytometer to determine the WBC and thrombocyte absolute count. For the differential count, WBC and thrombocytes were counted until 200 WBC were enumerated on blood smears, and the percentage of each WBC type and of thrombocyte were multiplied by the total WBC and thrombocyte count to obtain absolute differential cell counts. This method of manually determining total WBC and differential count has been recommended for avian (Zinkl, 1986) and fish (Stoskopf, 1993) blood, because nucleated RBC prevent accurate enumeration using automated analysis (Huffman, Arkoosh, & Casillas, 1997). The majority of blood values determined for fishes have been reported as mean ± SEM (Hrubec et al., 2000), the data generated in this study are thus presented as mean ± SEM.

STATISTICAL ANALYSES OF DATA

The data obtained from this study were subjected to various statistical tools. The differences in the means (± SEM) between groups were assessed using Students’t-test, Pearson’s Correlation & Levene’s test for Equality of Variance (SAS, 1988). A P value of P < 0.05 was taken as significant.

RESULTS

Mean haematological profile of both control and test (cultured and wild catfish)

RED BLOOD CELLS

Fish after an acute exposure to diazinon had significantly lower erythrocyte count (p < 0.05) when compared with the control specimens (cultured and wild catfish), as shown in Table 1 and figure1.

WHITE BLOOD CELLS

There was a significant decreased (p < 0.05) in WBC count in the test groups (cultured and wild catfish) following the exposure to acute effect of diazinon (Table 1, and figure 4).

PACKED CELL VOLUME

The PCV value was significantly (p < 0.05) decreased in the test group (cultured and wild catfish) exposed to the acute effect of the diazinon. (Table 1 and figure 2).
HAEMOGLOBIN CONTENT

At (p < 0.05) there was a significant decrease in the haemoglobin content of the exposed group (cultured and wild catfish) compared with the control groups. (Table 1 and figure 3)

DIFFERENTIAL WHITE BLOOD CELL COUNTS

The absolute and relative values of the differential WBC counts are as shown in Table 1. There was a decrease in both the absolute and the relative lymphocyte counts in the experimental group (p<0.05) following the acute effect of diazinon on the experimental groups.

THROMBOCYTE COUNT

There was a significant increase in thrombocyte count in the experimental group following exposure to acute effect of diazinon (p>0.05). Table 1 and figure 5.

Table 1: Mean values of Haematological Analysis Control and Exposed Group

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBC E10 6</td>
<td>RBC E10 6</td>
</tr>
<tr>
<td></td>
<td>Values</td>
<td>Values</td>
</tr>
<tr>
<td>Erythro</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Hematocrit</td>
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<td>30.0</td>
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<tr>
<td>Hemoglobin</td>
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<td>9.0</td>
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<tr>
<td>Mean corpuscular volume (MCV)</td>
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<td>62.0</td>
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<tr>
<td>Mean corpuscular hemoglobin (MCH)</td>
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<td>18.0</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (MCHC)</td>
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<tr>
<td>Red cell distribution width (RDW)</td>
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<td>12.0</td>
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<tr>
<td>Platelets</td>
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</table>

Figure 1: Red blood cell count (RBC) of control and experimental catfish exposed to acute effect of diazinon. 1 Cultured catfish, 2 Wild catfish

All bars are mean ± SEM (Standard error of mean)

Bars within the same groups (Control or Test) with different letters are significantly different (p<0.05)

Figure 2: Packed cell volume (PCV) of control and experimental catfish exposed following acute effect of diazinon. 1 Cultured catfish, 2 Wild catfish

All bars are mean ± SEM (Standard error of mean)

Bars within the same groups (Control or Test) having different letters are significantly different (p<0.05)
**Acute Effects Of Diazinon On Blood Parameters In The African Catfish (Clarias Gariepinus)**

**Figure 4**

Figure 3: Haemoglobin concentration (HB) of control and experimental catfish exposed to acute effect of diazinon. 1 Cultured catfish, 2 Wild catfish. All bars are mean ± SEM (Standard error of mean)

Bars within the same groups (Control or Test) having different letters are significantly different (p<0.05)

**Figure 5**

Figure 4: White blood cell count (WBC) of control and experimental catfish exposed to acute effect of diazinon. 1 Cultured catfish, 2 Wild catfish

All bars are mean ± SEM (Standard error of mean)

Bars within the same groups (Control or Test) having different letters are significantly different (p<0.05)

**Figure 6**

Figure 5: thrombocyte count of control and experimental catfish exposed to acute effect of diazinon. 1 Cultured catfish, 2 Wild catfish. All bars are mean ± SEM (Standard error of mean) Bars within the same groups (Control or Test) having different letters are significantly different (p<0.05)

**MEAN CORPUSCULAR VOLUME**

The values of MCV in the experimental groups (cultured and wild catfish) showed significant decrease (p<0.05) following the acute effect of diazinon. Table 2 and figure 6, in the test treatment.

**MEAN CORPUSCULAR HAEMOGLOBIN (MCH)**

MCH values in the experimental groups (cultured and wild catfish) showed significant decrease (p<0.05) following the acute effect of diazinon. (Table 2 and figure 7)

**Figure 7**

Table 2: Derived Haematological Parameters Wild and Cultured Catfish (Control & Test)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (NO=8)</th>
<th>Test (NO=10)</th>
<th>Control (NO=8)</th>
<th>Test (NO=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV (fl)</td>
<td>146.9 ± 35</td>
<td>152.6 ± 33</td>
<td>143.5 ± 37</td>
<td>151.3 ± 35</td>
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<tr>
<td>MCH (pg)</td>
<td>3.93 ± 0.51</td>
<td>4.13 ± 0.24</td>
<td>3.69 ± 0.24</td>
<td>4.02 ± 0.12</td>
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<tr>
<td>MCH (g/l)</td>
<td>27.6 ± 16.4</td>
<td>32.0 ± 19.2</td>
<td>24.8 ± 8.6</td>
<td>30.5 ± 12.3</td>
</tr>
<tr>
<td>MCH (g/l)</td>
<td>183.5 ± 65</td>
<td>178.1 ± 50</td>
<td>180.3 ± 78</td>
<td>175.2 ± 60</td>
</tr>
<tr>
<td>MCH (g/l)</td>
<td>4.84 ± 1.09</td>
<td>4.13 ± 0.52</td>
<td>4.61 ± 0.24</td>
<td>4.05 ± 0.12</td>
</tr>
</tbody>
</table>

**Table 2:** Derived Haematological Parameters Wild and Cultured Catfish (Control & Test)
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DISCUSSION

Haematological indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999). Haematology and clinical chemistry analysis, although not often used in fish medicine, can provide substantial diagnostic information. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of haematological parameters (Van Vuren, 1986). Thus, water quality is one of the major factors, responsible for individual variations in fish haematology, since they are sensitive to slight fluctuation that may occur within their internal milieu (Fernades & Mazon, 2003). On the basis of haematological studies, it would be possible to predict the physiological state of fish in natural water bodies.

BLOOD CELL COUNTS

PCV, HB, RBC

Haematology studies in teleosts have indicated that haematocrit values might be useful as a general indicator of fish health, since fish given iron deficient diets, or those exhibiting anaemia, all possess reduced haematocrit (PCV) values (Gatlin & Wilson, 1986). Previous haematological studies of nutritional effects (Rehulka, 1989, 2000), infectious diseases (Rehulka, 2002a) and pollutants (Rehulka, 2002b) brought knowledge that erythrocytes are a
major and reliable indicator of various sources of stress (Rainza-Paiva et al., 2000). Erythrocytes reflect the state of the organism over a prolonged period of time (Sniezsko, 1961) and Haley & Weiser (1985) High concentration of pesticides or long term exposure of fish to their sublethal concentration usually decreases erythrocyte indices. In this study, the main haematological response of catfish (cultured and wild) to the acute exposure to diazinon based organophosphorous pesticide LC$_{50}$ 6.6ppm was a significant decrease (p < 0.05) of erythrocyte count, haematocrit value and haemoglobin content compared to the control groups. Table 1 and figures 1, 2 & 3.

Decreased erythrocyte count and haemoglobin content in freshwater fish Cyprinus carpio after acute exposure to diazinon was also reported by Svoboda et al., (2001). Other effective substances of organophosphorous pesticides also induce changes which give evidence for decreased haematopoiesis followed by anemia induction in fish. Changes in erythrocyte profile induced by acute effect of dichlorvos in Clarias batrachus (Benarji and Rajendranath, 1990), trichlorphon in Piaractus mesopotamicus (Tavares et al., 1999), malathion in Cyprinion wabsoni (Khattak and Hafeez, 1996), forrnition in Heteropneustes fossilis (Singh and Srivastava, 1994) and of Ekolux organophosphorous preparation in Oreochromis mossambicus (Sampath et al., 1993). 

**WBC AND DIFFERENTIAL WBC COUNT**

There was a significant decreased in WBC count as well as in both the relative and absolute lymphocyte counts in the test group cultured and wild catfish (p<0.05). Table 1 and figures 4 and 5. Significant decrease of white blood cell count and absolute lymphopenia and granulocytosis characterize the white blood cell profile of common carp after the acute exposure to diazinon-based pesticide. Lymphopenia as a consequence of methylparathion based pesticide was reported by Nath and Banerjee (1996) in Heteropneustes fossilis and also by Siwicki et al., (1990) in common carp after an acute effect of trichlorfon. Ghosh and Banerjee (1993) reported lymphopenia and both neutrophile and eosinophile granulocytosis in Heteropneustes fossilis after an effect of dimethoate in 96hr LC$_{50}$ concentration. A decreased non-specific immunity in fish can be expected after acute exposure to organophosphorous pesticides due to decreased white blood cell count, lymphopenia and granulocytosis. Lymphopenia and granulocytosis have been reported after exposure to many pollutants (Svobodova et al., 1996). These changes in differential WBC count also give evidence for decreased level of nonspecific immunity in fish after acute exposure to toxic substances.

**THROMBOCYTES**

Diazinon induced a significant increase in thrombocyte count in the experimental group. Thrombocytes are comparable to mammalian blood platelets and play an important role in blood clotting, which prevents blood loss from hemorrhage. A high number of thrombocytes reduce clotting time (Srivastava, 1969); by as much as 50% in cases where the number of circulating thrombocytes was found to be one to two times higher than normal (Cassilas & Smith, 1977).

**ERYTHROCYTE INDICES**

MCV and MCH values showed significant decrease in the experimental group following acute effect of diazinon. Table 2, figures 6 and 7.

Another type of haematological response to the effect of organophosphorous compounds was a significant increment of MCV associated with increment of PCV value and drop of MHC. This response was reported in Common carp after acute effect of phenitrothion, imidan and dichlorvos (Svobodova 1971, 1975).

**CONCLUSION**

The role of blood parameters in the assessment of the health status of fish is emphasized by the observations of Cyriac, et al. (1989) and Omoriege (1998) who noted the possibility that changes in the blood will reveal conditions within the body of the fish long before any outward manifestation of diseases. However detailed studies of the diversity of pathological changes in blood indices is required in order to find general regulations of transformation in the blood system of fish caused by various pollutants as well as pesticides with the possibility of finding critical point, below which irreversible changes in the blood circulation, blood formation and immune system, which lead to the increased mortality of fish, will go a long way in validating the use of haematological parameters as a useful biomarker to measure the exposure of fish to pesticides in natural waters.
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This study has enhanced knowledge of physiological values of RBC and WBC indices including differential counts in the African catfish (Clarias gariepinus) cultured and wild exposed to various concentrations of diazinon. It has also been able to contribute to the applied and basic research needs of aquatic toxicology. It is important to know that erythropoiesis is influenced by a number of environmental factors and must be taken into account when haematological indices are evaluated.

References


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