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*Full Length Research Paper*

# Acute effect of diazinon on blood plasma biochemistry in the African catfish (*Clarias gariepinus*)

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**The acute effect of diazinon on the African catfish (*Clarias gariepinus)* was assessed by comparing the biochemical blood plasma profiles of a control group and a group exposed to the effect of the pesticide Diazintol$^{R}$ (162 mg/ml of diazinon as the active substance). The activities of selected enzymes, metabolite and electrolytes concentrations were measured on 16 specimens of controls, and in 20 specimens, of (*C. gariepinus* of mean weight 350 ± 15 g, and mean total length 35 ± 2.0 cm) exposed for 96 h to the effects of Diazintol$^{R}$ at a concentration of 6.6 ppm. The results showed a significant decrease of cholinesterase ($p < 0.05$) lactate dehydrogenase ($p < 0.05$) alkaline phosphatase and acid phosphatase in the experimental group. The values of alanine and aspartate, aminotransferases, creatine kinase, were comparable in the experimental and control groups. A significant decrease ($p < 0.05$) was observed in the total protein, albumin globulin and lactate concentration in the experimental group compared with the control group. Glucose concentration in the plasma of the experimental group was significantly higher ($p < 0.05$) than that of the control group. A significantly higher ($p < 0.05$) concentration of plasma sodium and potassium was observed in the experimental group and a significantly lower ($p < 0.05$) concentration of plasma calcium and phosphorus, compared with those in the control group. The results of the biochemical blood plasma profile indicate a marked neurotoxic effect of diazinon and shows that this changes could be used as biomarkers for aquatic pollution.**

**Key words:** Organophosphorous pesticide, acute toxicity, enzymes, total protein, glucose, lactate, electrolytes.

## 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 (Adedeji et al., 2009).

Diazinon is a widely used toxicant in a number of organophosphorous pesticides (Rober and Hutson, 1998). Although the aquatic environment is not the target one for the use of such pesticides the results of a number of monitoring studies have showed the presence of diazinon and its metabolite, diazoxon, in surface waters (De Vlaming et al., 2000). Biochemical characteristics of blood are among the important indices of the status of internal environment of the fish (Edsall, 1999). Changes in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of these substances.

The major biochemical response to the effect of diazinon in fishes is the inhibition of enzymes. Hamm et al. (1998) also observed changes in carbohydrate metabolism in the eel, *Anguilla anguilla*. The glycogen contents in the liver and muscular tissue was significantly decreased while glucose and lactate concentrations in the blood were significantly increased. Present study aims to evaluate the acute effect of diazinon on plasma profile of the African catfish, *C. gariepinus* following the determination of $LC_{50}$ of diazinon in African catfish by Adedeji et al. (2008).

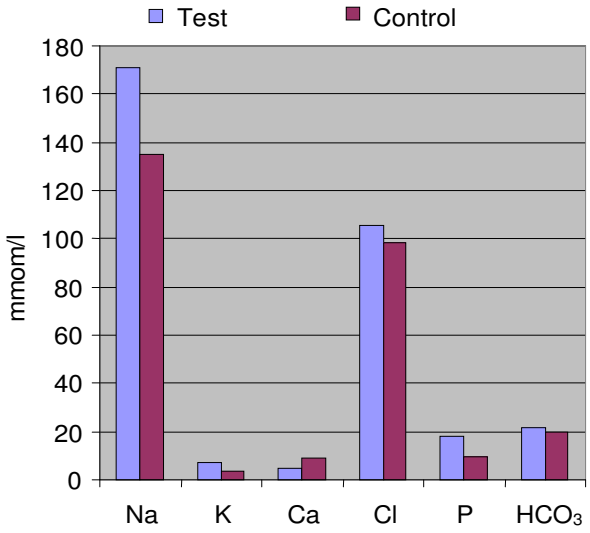


**Figure 1.** Acute effect of diazinon on plasma electrolytes

## MATERIALS AND METHODS

### Acute toxicity test and blood collection

The 96th acute toxicity test in replicates was as describe by Adedeji et al. (2008), while blood collection was as describe by Adedeji et al. (2009).

The fish were divided into two groups. Group A which serves as the control has 16 fish while group B the experimental group has 20 *C. gariepinus*. Group B was exposed to 6.6 ppm of diazinon while A was not exposed to any toxicant. The $LC_{50}$ 6.6ppm obtained by Adedeji et al. (2008) was used in this study.

The test was carried out in a semistatic way, the bath being exchanged every 24 h to ensure constant concentration of the active substance. The physicochemical indices of diluting water used in this acute toxicity test were all within normal range free (Carbon (IV) oxide 43 mg/l, total alkalinity 12.25 mg/l NH3 (unionized) 0.04 mg/l) temperature 26 ± 1°C. Water saturation with oxygen ranged from 90 - 100%, pH 7.60 - 7.85

### Plasma collection

Blood was drawn from the posterior caudal vein as describe by Schmitt et al. (1999) and 2 ml was decanted in heparinised bottles after which the plasma was separated by centrifugation and thus separated into plasma, bufy coat and packed erythrocytes. The plasma was then decanted into labeled Ependorf tube with the aid of Pasteur pipette and stored at -20°C prior to subsequent analysis, which was conducted within 48 h of sample collection.

The plasma obtained after exposing the fish to 6.6 ppm of diazinon in the haematological experiment was analysed to evaluate the effects of diazinon on the plasma. After sampling, the blood was centrifuged for 15 min at 400 g. The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and cholinesterase (CHE) with substrate butyrylthiocholine, lactate dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP), acid phosphatase (ACP) and total protein (TP), Blood urea nitrogen (BUN) glucose (GLU) and electrolytes (Na, K, Ca, P, Cl, and $HCO_3$) concentrations were determined using the automatic analyser COBAS MIRA (Hoffmann, La Roche, Co. Switzerland) and using optimised tests of Boehringer Mannheim GmBH by means of spectrophotometer Varian DMS 200. Globulin was calculated by subtracting albumin value from total protein value. For the determination of CK activities the plasma was diluted 5 - 10 times with a physiological solution.

### 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 and Levene's tests for equality of variance (SAS, 1988). A P-value of $P < 0.05$ was taken as significant.

## RESULTS

### General effect

The experimental *C. gariepinus* after 96 h of action of the diazinon-based organophosphorous pesticide showed loss of movement co-ordination and orientation. The fish were swimming in a half-circle, and there was loss of balance; their response to external stimuli was a bouncing movement and fin tremor; there was a conspicuous darkening of the body surface. Others general effects were as described by Adedeji et al. (2009). The control *C. gariepinus* showed no clinical changes.

### Electrolytes

In the experimental group (test) there was a significantly higher ($p < 0.05$) concentration of Na, Cl and K, and significantly lower concentration ($p < 0.05$) of Ca and P, compared with the control group. The results of the effects on the electrolyte are presented in Figure 1.

### Blood urea nitrogen

There was no effect on the levels of blood urea nitrogen (Figure 2).

### Proteins

In the experimental group, there was a significantly decrease ($p < 0.05$) concentration in the amount of total plasma protein, albumin and globulin (Figure 2).

### Enzymes

The acute effect of diazinon on the plasma concentration of enzymes in the *C. gariepinus* exposed is shown in Figure 3 (CK, ALK, ALP, ACP, CHE, LDH, GOP and GPT). There was a significant decrease in plasma concentration of CHE, LDH, ALP and ACP ($p < 0.05$) in the catfish exposed to acute effect of diazinon and there was no significant difference in the concentration of other

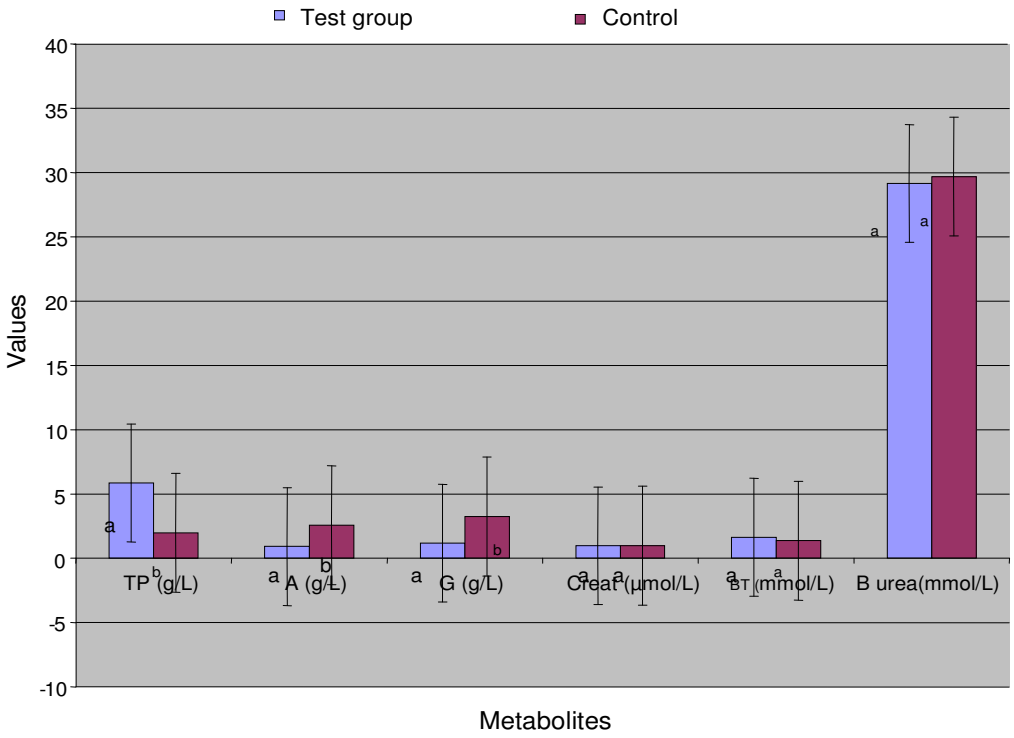


**Figure 2.** Acute effect of diazinon on plasma metabolites. All bars are mean ± SEM (Standard error of mean). Bars within the same treatment (Control or Test) with different letters are significantly different ($p < 0.05$).

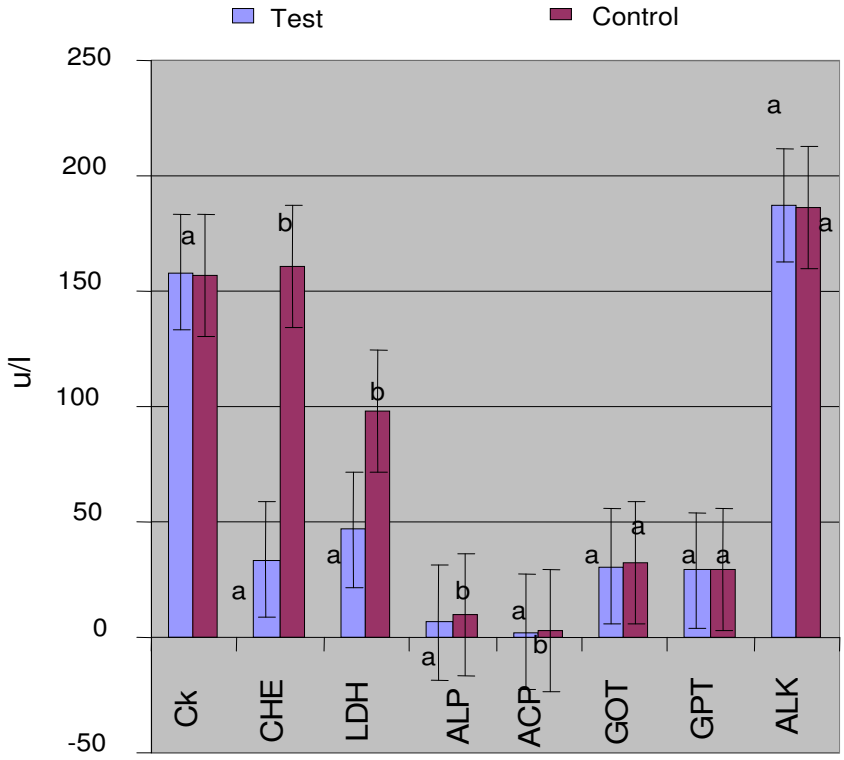


**Figure 3.** Acute effect of diazinon on plasma enzymes. All bars are mean ± SEM (Standard error of mean). Bars within the same groups (Control or Test) having different letters are significantly different ($p < 0.05$).

**Table 1**. Plasma biochemical enzymes profile of control and experimental catfish.

| V-2 | | N | Mean | Std. deviation | Std. error mean |
|---|---|---|---|---|---|
| CK | Control | 16 | 156.7500 | 9.63674 | 2.40918 |
| | Experimental | 20 | 158.250 | 10.49248 | 2.34619 |
| CHE | Control | 16 | 160.8125 | 4.40028 | 1.10007 |
| | Experimental | 20 | 33.7000 | 6.16527 | 1.37860 |
| LDH | Control | 16 | 98.2500 | 4.66905 | 1.16726 |
| | Experimental | 20 | 46.8000 | 6.97816 | 1.56036 |
| ALP | Control | 16 | 9.6063 | 1.34187 | .33547 |
| | Experimental | 20 | 6.7100 | .75804 | .16950 |
| ACP | Control | 16 | 3.0875 | .28018 | .07004 |
| | Experimental | 20 | 2.1800 | .31556 | .07056 |
| | | | 32.3750 | 4.66011 | 1.16503 |
| GOT | Control | 16 | | | |
| | Experimental | 20 | 30.7000 | 2.65766 | .59427 |
| GPT | Control | 16 | 29.3750 | 2.82548 | .70637 |
| | Experimental | 20 | 29.2500 | 2.29129 | .51235 |
| ALK | Control | 16 | 186.5625 | 24.24863 | 6.06216 |
| | Experimental | 20 | 187.2000 | 20.33302 | 4.54660 |

enzymes.(Table 1)

## DISCUSSION

The acute effect of diazinon, (in form of diazintol$^{R}$ at a concentration of 162 mg/1) $LC_{50}$ 6.6 ppm on catfish caused in cholinesterase with substrate butyrylthiocholine the activity drop by 80%, compared with the control group ($p < 0.05$). This result agrees with data on the 71% inhibition of acetylcholinesterase in the brain of *Cyprinodon variegates* (Goodman et al., 1979), 80% inhibition of that enzyme in the neural tissue of *Brachydanio rerio* (Ansari et al., 1987) and 85% inhibition of acetylcholinesterase in carp *Cyprinus carpio* (Luskova et al., 2002) following an acute effect of diazinon.

Diazinon alone is no inhibitor of cholinesterase. However, in animal bodies, it is converted to diazoxon, which is a strong inhibitor of ACHE enzyme (Gallo and Lawryk, 1991). Inactivation of CHE causes a blockage of the cholinergic transfer of nerve signals, paralysis and death due to asphyxia (Voet and Voetová 1990) of *Channa punctata* following an action of diazinon lasting 96 h. The assumed decrease in lactate dehydrogenase activity in the brain was mirrored even in the significantly decreased activity of this enzyme in the blood plasma of catfish, *C. gariepinus* exposed to diazinon ($p < 0.05$). There was a significant decrease ($p < 0.05$) in the plasma concentration of alkaline and acid phospatases in the test treatment following the acute effect of diazinon. Sastry and Sharma (1980) reported a decrease of activities in alkaline and acid phosphatases in the brain of *Channa punctatus* following the effect of diazinon. According to these authors, the alkaline phosphatase activity is inhibited after 96 h of the effect and then it resumes its normal values or even an increased activity is observed. Goel et al. (1982) reported serum alkaline and acid phosphatases decreased by 15% in *Heteropneustes fossilis*, resulting from the effect of the organophosphate malathion, the activities of alkaline and acid phosphatases in blood plasma of *Cyprinus carpio* were almost identical in the control and test treatment following exposure to acute effect of diazinon (Luskova et al., 2002).

The resulting activity values of alkaline and acid phosphatases support the assumption that the liver tissue of the experimental fish was markedly affected. Similarly, the practically identical activity of alanine and aspartate aminotransferases and creatine kinase, observed in the control and experimental groups, indicate that diazinon damages neither parenchymatous tissues nor skeletal musculature nor disturb the permeability and integrity of cell membranes (Masopust, 1998).

The significant differences between the control and experimental fish following the action of diazinon, measured especially in glucose concentration ($p < 0.05$) may be considered to be the manifestation of stress. Ceron et al. (1997) similarly reported significant glucose increase in common eel (*Anguilla anguilla*) following a 96h

action of sublethal concentrations of diazinon.

Glucose increase is a general response of fish to acute pollutant effects, including organophosphates (Sancho et al., 1997).

The significant drop ($p < 0.05$), in lactate dehydrogenase activity in the blood plasma of the experimental fish, compared with the control, indicates a decrease in the glycolytic process due to the lower metabolic rate as a result of the effect of diazinon. On the contrary, some authors reported increased plasma lactate concentration in various fishes following acute effects of organophosphorous pesticides including diazinon (Ceron et al., 1997; Sancho et al., 1997).

After 96 hr diazinon produced a significant decrease ($p < 0.05$) in protein concentration in the blood plasma of the test catfish, as compared with the control.

A significant decrease protein concentration following an acute effect of fenitrothion was obtained by Sancho et al. (1997) and Khattak and Hafeez (1996) in eel and *Cyprinion watsoni* exposed to the effect of Malathion. Kori-Siakpere et al. (2007) observed a decrease in plasma protein, glucose and triglyceride with elevated levels of cholesterol follow-ing exposure of *C. gariepinus* fingerlings to paraquat.

The basic function of electrolytes in the body lies in controlling fluid distribution, intra and extracellular acidobasic equilibrium, maintaining osmotic pressure of body fluids and normal neuro-muscular irritability (Harper, 1977).

The increase in the concentration of Na+ found in the blood plasma of the experimental catfish shows no practical effect on the ion functions mentioned above. On the other hand, the K+ concentration in the plasma was significantly increased, which in combination with the decrease in cholinesterase indicates inhibition of the heart function and a neurotoxic damage to the CNS of the experimental catfish.

Also, the $Ca^{2}$+ and P functionally participate in maintaining normal irritability of the heart, muscles and nerves, as well as the selective permeability of cell membranes.

Therefore, the significant decrease ($p < 0.05$) in the concentrations of the above ions in the experimental fish fits in the diagnosis caused by the toxic effect of diazinon.

The decrease of concentration in phosphorus ions, and decrease of lactate dehydrogenase activity, as of products and activator of glucose metabolism, indicated the decrease of this process intensity in the test treatment due to toxic effects of diazinon.

## Conclusion

Changes in the biochemical blood profile reflect changes in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of these substances.

The more biochemical response to the effect of diazinon in fishes is the inhibition of a number of enzymes,above all acetylcholinesterase (Hamm et al., 1998). Ceron et al. (1997) observed changes in carbohydrate metabolism in the eel, *Anguilla anguilla*, during short-term exposure to diazinon (Ceron et al., 1997).

The glycogen contents in the liver and muscular tissue was significantly decreased, glucose and lactate concentrations in the blood were significantly increased. Luskova et al. (2002) in their examinations of the biochemical blood plasma profile of carp *Cyprinus carpio* indicate a marked neurotoxic effect of diazinon in fishes. From the above it can be concluded that the acute effect of diazinon on plasma biochemistry of catfish varies.

It causes significant increase in some enzymes, (CHE, LDH etc.) significant increase in blood glucose and significant decrease in plasma protein. This aspect of the study has also confirmed that changes in plasma biochemical profile can be used to monitor pesticides toxicity in fish.

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