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Effects of sub-lethal concentrations Diazinon on some physiological parameters of liver and kidney in the African catfish

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Research Paper

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The present study was undertaken to evaluate the influence of Diazinon on some enzyme active alkaline phosphatase (ALP), aspirate aminotransferase (AST) and alkaline phosphatase (ALT) in liver and levels urea and creatinine in kidney of African catfish (*Clarias gariepinus*). After exposure to deferent concentrations of Diazinon the LC_{50} values was 11.76 mg L^{-1} at 96 h for *Clarias gariepinus*. The results showed that long-term exposure to sub-lethel concentrations of Diazinon (1.1 mgL^{-1} and 3.3 mgL^{-1}) were highly significant ($P<0.05$) in all periods for (ALP), (AST) and (ALT) in liver and both of Urea and creatinine in kidney.All parameters were statistically significant when vitamin C and vitamin E plus Diazinon treated group compared with Diazinon-treated group ($P<0.05$).

Key words: Kidney, Liver, Enzyme, catfish

INTRODUCTION

Pesticides are known to affect all members of an ecosystem from the smallest invertebrates to humans and their toxicities in both urban and agricultural settings are responsible for the death of many birds and fishes (Khan et al., 2003).

Diazinon is one of the pesticides (Orgonophosphorus, OPIs) widely used in the world to control flies of ornamental plants and food crops, nematodes, soil insects in croplands by inhibiting acetylcholinesterase, on fruit and vegetable field crops. Although, the aquatic environment is not the target one for the use of such pesticides but find their way into water bodies (Jyothi and Narayan, 1999). Depending on form, the Environmental Protection Agency (EPA) has classified Diazinon as a toxicity class II or III pesticide, based on a scale of I to IV, I being the most toxic class (Meister, 2000). The results of a number of studies have evidenced the presence of Diazinon in surface waters (Dutta and Pan, 2000 and Adedeji, 2010).

Previous studies (Pawlowski et al., 2006; Adedeji et al., 2009 and Omitoyin, 2006) have reported that the exposure of fish to pesticides resulted in several changes in biochemical parameters. All chemical reactions in the cell are catalyzed by enzymes and introduction of foreign chemicals in the cell generally disturb and inhibit many enzymes function (Hamm and Hinton, 1998). According to Coppo et al. (2002) cells contain enzymes that are necessary to their function. When the integrity of cell is disrupted, enzymes escape into plasma/serum where their activity can be measured as useful index of cell

integrity. Modifications in enzyme activity occur by cell death, increase or decrease enzymes production, obstruction of normal execratory route, increase cell membrane permeability, or impair circulation (Kaneko, 1989).

Adedeji, (2010) reported that the African catfish (*C. gariepinus*) which exposed to 6.6 mgL^{-1} of diazinon showed a significant decrease of Alkaline phoshatase (ALP) and acid phosphatase in the experimental groups, but the values of aspartate aminotransferase (AST) and creatine kinase were similar in both the control and experimental groups. Inyang et al. (2011) studied the effect of Diazinon, on ALP in the plasma and organs in *C. gariepinus* (ranging from 1.00 to 10.0 mgL^{-1}) in 30-day. This study shown that Diazinon did not cause any significant difference in plasma ALP over the concentrations tested between experimental and control specimens. However, ALP values in all the organs (intestinal tract, kidney, muscle, gill and liver) decreased with increasing concentration of Diazinon.

MATERIALS AND METHODS

Specimens collection

One hundred and seventy three healthy fish of the Nile catfish, Clarias gariepinus (weighing 300 ± 15g) were collected from the River Nile at Assiut, Egypt. Fishes immediately were transported to the fish laboratory in the Department of Zoology, Faculty of Science, Assiut University.The experimental fishes were reared in aerated glass tanks (160 L capacity) and acclimatized for two weeks before being used in the experimental study. The experimental fish fed pellets at a rate of 2-3% of wet weight twice daily. Feces and residual food were aspirated regularly. The water temperature, pH and dissolved oxygen (DO) concentrations will be measured daily (22.5 ± 1.33°C, 6.65 ± 0.19 pH and 6.75 ± 1.40 mg/L DO).

Chemicals

The present study used diazinon (chemical product identification: Product Name: Diazinon, Molecular Formula: C12H21N2O3PS, Molecular Weight: 304.35, Chemical Name: O,O-diethyl 0-2-isopropyl-6-methyl (pyrimidine-4-yl) phosphorothioate Form: Liquid, Colour: Dark brown, Odour: Organophosphate odour (RAYFULL HOLDING CO., LTD. CHINA), Ascorbic acetate (VC) and DL-α- tocopherol (VE) acetate were obtained from Merck (Germany).Stock solution (1000 ppm) of Diazinon was prepared and stored in clean glass bottles and diluted to concentrations of 1.1 and 3.3 mgL^{1}. Dose was prepared and added constantly to the aquarium for 4 weeks. The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish Diazinon levels.

Experiments

1. Determination of sub-lethal concentrations Estimation of LC_{50}: Groups of fish were exposed to different concentrations of Diazinon as (0.00, 4.8, 6.6, 7.8, 9.0, 12.0, 13.5, 15.0, 16.5, 18.0 and 20.0 ppm) (7 fish / tank) in static systems (160 L capacity). The first group was used as control. Fish were not fed during the experimental period as recommended by Reish and Oshida (1987). According to Parrish (1985) was calculated from the data obtained in acute toxicity bioassays.

2.After determining LC_{50} 96 h value, two sub-lethal concentrations of Diazinon (1.1 and 3.3 mgL^{-1}) were taken (Table 1). Water was changed every day in the control and the treated groups, the concentrations of Diazinon remained as the same during the experimental period. A facility for oxygenation of the test solution was provided. *C. gariepinus* specimens were exposed to 1.1 and 3.3 mgL^{-1} of Diazinon for 28 days (four weeks).

Table 1. Mean values of lethal concentrations (LC_{50}) of Diazinon to *Clarias gariepinus*.

Time (HR)	LC_{50} (mgL^{-1})
24	17.4
48	14.23
72	12.33
96	11.76

Experimental procedure

After one weak of acclimation, eight experimental fish each was introduced individually into the aquaria containing each of treatment levels and control. Each treatment and control classified into three replicated, according to doses of Diazinon (Table 2).

Biochemical parameters

At 14 and 28 day periods, study tissues samples of the control and treated fish (4 fish/group) were dissected. The tissues were washed in ice-cold physiological saline solution and stored at -70°C until analysis. Then 0.5 g each of organs were macerated with pestle and mortar. To each of macerated samples, 5 mL of percloric acid was added and centrifuge at the rate of 3000 rpm for 10 min. the supernatants were removed and stored in plain bottles at -20°C for analysis.

Liver functions Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphates

Table 2. The fish groups exposed to Diazinon doses (D_1=1.1 and D_2=3.3 mgL^{-1}, VC = Vitamin C 50 mg/kg and VE= vitamin E = 100 mg/kg).

Groups	Experimental		
	Diazinon doses (mgL^{-1})		Vitamin doses (mg/kg)
	D_1	D_2	VC and VE
control	-	-	-
D1	1.1	-	-
D1 + VC + VE	1.1	-	50 + 100
D2	-	3.3	-
D2 + VC + VE	-	3.3	50 + 100

(ALP), were determined colorimetrically using assay kit supplied by Diamond Diagnostics, Egypt. In addition, kidney functions (Creatinine and Urea) were estimated using kits supplied from Biomerieux (France). The samples were measured by spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd).

Statistical analysis

The data were analyzed using SPSS. Statistically significant differences between treatment and control groups were determined by analysis of variance one-way ANOVA. Significant differences ($P<0.05$) were reanalyzed by LSD method to determine which one of the individual groups was significantly different from the control group. All parameters were expressed as mean ± standard error.

Lethal test acute toxicity

The acute toxicity results at different time intervals are shown in (Table 1). After different times in terms of fish mortality, the results showed that 50% of fish tested were died at 17.4, 14.23, 12.33 and 11.76 mg L^{-1} Diazinon after 24, 48, 72 and 96 h respectively (Table 1). Also, correlation between the concentration of Diazinon and fish mortality rate under acute toxicity test was significant ($p<0.05$) and with increasing of Diazinon concentrations, mortality increased.

RESULTS

Behavior

Normal behavior (vigorous activity, static equilibrium, active swimming, normal gill movement and hanging horizontally in the water of fish and natural color with darkness brown) was observed for fish in control group and group exposed to less dose at initial experiment. The abnormal behavior responses were observed at all concentrations higher than 4.8 mgL^{-1}, such as restlessness, sudden quick movements, air gulping, rolling movements, swimming on the back. A neural paralytic syndrome was typical for fish poisoned with Diazinon. Strong restlessness started when fish came into contact with the poisoning bath and they tried to jump out of the water. Fish excitation was reflected by an increased reaction to exogenous stimuli and by cramp movements of fins and mouth. Also orientation in water the fish were swimming in half-circle, continuously lying on one side; their response to external stimuli was a bouncing movement. Reaction to excitation was manifested by sudden movement and fin tremor. The affected fish became very weak, settled at bottom and died in increasing numbers at higher doses (20.00 mgL^{-1}). Normal color and behavior were observed in the control group. However, the color body surface became pale progressively with slightly increased amount of mucus and with extensive pigmentation mainly on the dorsal part through higher doses at the end of 96 h of exposure time. Mortality pattern for 24, 48, 72, and 96 h are as presented in (Tables 1). The LC_{50} (96 h) was calculated to be 11.77 mgL^{-1}.

Biochemical parameters

Liver functions

The basic data of alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino transferase (AST) of *C. gariepinus* exposed to Diazinon (1.1 and 3.3 mgL^{-1}) and Diazinon doses plus vitamin C and vitamin E for 14 and 28 days are given in (Tables 3). Long-term exposure to Diazinon causes an increase in AST, ALP and ALT ($P<0.05$) in all exposure periods for the three previous parameters. Diazinon plus vitamin C and vitamin E interaction was significant for AST and ALT.

Kidney functions

The basic data of urea and creatinine of *C. gariepinus* exposed to Diazinon dose (1.1 and 3.3 mgL^{-1}) and Diazinon doses plus vitamin C and vitamin E for 14 and

Table 3. ALP, AST and ALT in the liver of *C. gariepinus* exposed to various concentrations of Diazinon, Vitamin C and Vitamin E for 14 and 28 days.

Treatment	Parameter after 14 days			Parameter after 28 days		
	ALP(U/l)	AST(U/l)	ALT(U/l)	ALP(U/l)	AST(U/l)	ALT(U/l)
Control	79.6±0.35^{A} (78.9-80)	219.04±17.14^{A} (199.04-53.17)	46.17±3.36^{D} (39.49-0.24)	78.19±7.48^{A} (65.98-91.8)	232.81±7.47^{A} (225.23-247.75)	43.03±1.33^{C} (40.36-44.39)
D1	79.16±0.07^{A} (79.04-9.29)	112.33±6.63^{AB} (99.66-122.09)	97.74±6.01^{CD} (89.27-09.36)	79.46±0.65^{A} (78.15-80.18)	98.14±3.81^{B} (93.15-105.64)	109.95±3.40^{BC} (103.17-113.97)
D+VC+VE	78.04±0.12^{A} (77.82-78.27)	192.37±3.75^{CD} (185.87-198.88)	51.76±1.01BC (50.02-53.53)	80.85±0.98A (78.89-81.9)	143.12-1.69BC (140.15-146.02)	69.88±0.88BC (68.14-70.99)
D2	79.04±0.07^{A} (78.91-79.17)	109.99±21.04^{E} (87.92-152.06)	101.71±16.35AB (69.051-19.42)	78.66±0.07A (78.53-78.79)	60.52±10.66BC (40.158--76.18)	186.76±37.94B (137.81-61.46)
D2+VC+VE	80.30±0.07^{A} (80.18-80.43)	132.85±10.79^{E} (111.74-147.3)	77.11±6.72AB (68.56-90.38)	79.54±0.07A (79.42-79.67)	118.45±3.86C (112.53-125.71)	93.90±3.01A (88.29-98.63)

The data are presented as Means ± S.E. (Min-Max). Different letters indicate significant difference at p<0.05. Alkaline phosphates (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Vitamin C (VC), vitamin E (VE), Diazinon (D).

Table 4. Urea and creatinine in the kidney of *C. gariepinus* exposed to various concentrations of Diazinon, Vitamin C and Vitamin E for 14 and 28 days.

Treatment	Parameter after 14 days		Parameter after 28 days	
	Urea(mg/dl)	CREAT(mg/dl)	Urea(mg/dl)	CREAT(mg/dl)
Control	8.58±1.11^{D} (6.75-0.60)	0.57±0.07^{EFG} (0.45-0.70)	8.06±1.26^{DE} (5.64-9.9)	0.53±0.08^{DEF} (0.37-0.66)
D1	40.18±1.52^{C} (38.34-43.2)	1.38±0.04^{DEF} (1.3-1.44)	66.40±2.50^{CD} (62.97-71.27)	2.21±0.08^{CDE} (2.09-2.37)
D1+VC+VE	20.31±0.20^{B} (20.01-20.7)	0.88±0.00^{BC} (0.87-0.9)	23.38±0.73^{C} (22.54-24.84)	1.016±0.03^{CD} (0.98-1.08)
D2	70.14±4.03^{B} (64.74-78.03)	2.33±0.13^{B} (2.15-2.601)	113.4±8.37^{B} (96.76-123.40)	3.78±0.27^{B} (3.22-4.11)
D2+VC+VE	32.38±1.57^{A} (29.64-35.1)	1.19±0.05^{A} (1.09-1.3)	32.36±1.90^{A} (29.05-35.64)	1.198±0.07^{A} (1.07-1.32)

Different letters indicate significant difference at p<0.05. Urea, Creatinine (Creat), Vitamin C (VC), vitamin E (VE), Diazinon doses (D1 and D2). The data are presented as Means ± S.E. (Min-Max).

28 days are given in (Tables 4). Diazinon main effect was highly significant (P<0.05) in the two exposure periods for both of urea and creatinine. Diazinon-VC-VE interaction effect was not significant for the two parameters.Generally in this study showed that, vitamin C and vitamin E causes improve on kidney tissue of experimental fish (urea and creatinine).

DISCUSSION

In this work, it was noticed that Diazinon exposed-fishes showed some behavioral changes like hyperactivity, air gulping and surface erratic swimming. Similar behavioral changes were observed by El-Khateib and Afifi (1993); Pan and Dutta (1998); Adedeji et al. (2008); Benli and Özkul (2010) and Banaee et al. (2011) in fishes exposed to pesticides.

The present study revealed increase in ALT, AST and ALP enzymes due to Diazinon exposure. Similar results were observed by Singh and Srivastava (1990), who noticed that along with prolonged exposure time of *Heteropneuses fossilis* to copper the activity of ALP, AST and ALT increased. Jyothi and Narayan (1999) observed increase of ALP after *Clarias batrachus* exposed to carbamate and phorate, and Gabriel et al. (2011) found increase in ALT, AST and ALP enzymes due to cypermethrin exposure.

Increase in the levels of ALT and AST has been shown to reflect liver damage, while increase in the ALP level may be indicator for renal and liver damage (Gill et al., 1990; Bhattacharya et al., 2005 and 2008; Ogueji and Auta, 2007). Also, It has been reported that alterations in enzymes activities in the serum directly indicates major pathologic changes in cell membrane permeability or hepatic cell rupture (Benjamin, 1978), a signal of underlying pathological process (Hayes et al., 2002 and Rao, 2006). The above observations seem to confirm the

results obtained in the present study that diazinon had effect on the cell integrity. Increase in serum level of ALP is due to increased synthesis of the enzyme in the presence of increasing biliary pressure. Significant elevation of serum ALP is an indication of cholestasis (Van Hoof and De Broe, 1994). This confirms the findings of Wannang et al. (2007); in rats that increase in the serum levels of ALP indicates the extent of cellullar damage on the liver. Ochmanski and Barabasz, (2000) reported that the increase in the activity of ALP in blood might be due to the necrosis of liver and kidney.

Urea, creatinine are biomarkers for kidney function. The present study revealed an elevation of serum urea and creatinine. Similar findings were reported by Zaki et al*.* (2010) who found an increase in the previous parameters in *Oreochromis niloticus* due to cadmium exposure. This elevation in the previous parameters may be attributed to kidney dysfunction. Kidney dysfunction may be explaining the increase in serum urea and creatinine (Alwan et al., 2009 and Zaki et al*.,* 2010). Alwan et al. (2009) revealed that the increase of creatinine level might be induced by glomerular insufficiency, increased muscle tissue catabolism or the impairment of carbohydrates metabolism. Thus this study aimed to estimate of the effects of Diazinon toxicity on some biochemical parameters and the interaction of two antioxidants to decrease the toxicity of Diazinon. of *C. gariepinus*.

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REFERENCES

Adedeji OB (2010). Acute effect of diazinon on blood plasma biochemistry in the African catfish (*Clarias gariepinus*). J. Clini. Med. Resea. 2(1): 001-006.

Adedeji OB, Adedeji AO, Adeyemo OK, Agbede SA (2008). Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*). Afric. J. Biotech., 7 (5): 651-654.

Adedeji OB, Adeyemo OK, Agbede SA (2009). Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*). Afric. J. Biotech., 8(16):1684-5315.

Alwan SF, Hadi AA, Shokr AE (2009). Alterations in haematological parameter of fresh water fish. *Tilapia Zilli* exposed to Aluminium. J. Scie. Applic., 3(1): 12-19.

Banaee M Ahmadi K (2011): Sublethal toxicity impacts of endosulfan on some biological parameters of the frish water Crayfish (*Astacus leptodacty*). J. Enviro. Scie. Issn 1819-3412 / DOI: 10. 3923/rajs.

Benjamin MM (1978). Outline of veterinary clinical pathology. 2nd edition, Iowa state University Press, Iowa, U.S.A., pp.35-105.

Bhattacharya B, Dilworth HP, Iacobuzio-Donahue C, Ricci F, Weber K, Furlong MA (2005). Nuclear beta-catenin expression distinguishes deep fibromatosis from other benign and malignant fibroblastic and myofibroblastic lesions. Am J. Surg. Pathol., 29: 653-900.

Coppo JA, Mussart NB, Fioranelli SA (2002) Physiological variation of enzymatic activities in blood of Bullforg, *rana catesbeina* (shaw, 1802). J. Rev. Vet., 12(13): 22-27.

Dutta PG,Pan G (2000). Diazinon Induced Changes in the Serum Proteins of Large Mouth Bass, *Micropterus salmoides*. J. Bull. Environ. Contam. Toxicol., Vol. 64: 287-293.

El-Khateib T, Afifi SH (1993). Inspection of *Tilapia nilotica* Nile fish exposed to organophorus compound (Diazinon). J. Assiut Vet. Med., Vol. 29: 119-124.

Gabriel UU, Jack IR, Egobueze E, Edori OS (2011). Impact of cypermethrin on selected enzymes in tissues of *Heterobranchus bidorsalis*. West African journal of Applied Ecology., Vol. 18: 122-126.

Gill GA, Bruland KW (1990). Mercury speciation in surface freshwater systems in California and other areas. Goldfrank's Toxicologic Emergencies (24): Pp.1392-1400.

Hamm JT, Hinton DE (1998). The role of development and duration of exposure to the embryotoxicity of diazinon. J. Toxic., Vol. 48(4): 403-418.

Hayes PC, Simpson KJ Garden OJ (2002). Liver and biliary tract disease. *In*: Davidson's principles and practice of medicine. Edition., Vol. 18: 832-837.

Inyang IR, Daka ER, Ogamba EN (2011). Effect of Diazinon on Acid and Alkaline Phosphatase Activities in Plasma and Organs of *Clarias gariepinus*. J. Biol. Sci. Vol. 3(3): 191-194.

Jyothi B, Narayan G (1999). Certain pesticideinduced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus* (Linn.). J. Food. Chem. Toxicol., Vol. 37: 417-421.

Kaneko JJ (1989): Clinical Biochemistry of Domestic Animals, 4th Ed. Academic Press, San Diego, P. 823.

Khanm MZ, Tabassum R, Shah EZ, Tabassum F, Ahmad I, Fatima F Khan MF (2003). Effect of Cypermethrin a,nd Permethrin on Cholinesterase Activity and Protein Contents in Rana tigrina (Amphibia). Turk. J. Zool. Vol. 27: 43-246.

Meister R (2000). Farm Chemicals Handbook. Meister publishing Company, Willoughby, OH.

Ochmanski W, Barabasz W (2000). Aluminum occurrence and toxicity for organisms. Przegl. Lek., Vol. 57: 665-668.

Ogueji EO, Auta J (2007). Investigation of biochemical effects of acute consentrations of lambda-cyhalothrin on Africa catfish *Clarias gariepinus* – Teugels., Vol. 2(1): 86-90.

Omitoyin BO (2006). Haematological changes in the blood of *Clarias gariepinus* (Burchell 1822) juveniles fed poultry litter. Livestock Res Rural Develop.,18(11): 200.

Pan G, Dutta HM (1998). The inhibition of brain acetylcholinesterase activity of juvenile largemouth bass Micropterus salmoides by sublethal concentrations of diazinon. J. Environ Res. 79(2):133-137.

Parrish PR (1985). Acute toxicity tests. Fundamentals of Aquatic Toxicology. *Eds:* Rand GM, P. S. Washington, DC, New York, Hemisphere. Pp.659.

Pawloski PH, Burzynska B, Zielenkiewicz P (2006). Theoretical model of reticulocyte to erythrocyte shape transformation. Journal Theoretical Biology. Vol. 243:24-38.

Rao JV (2006). Toxic effects of novel organophosphorous insecticide (RPR– v) on certain biochemical parameters of euryhaline fish *Oreochromis mossambicus*. J. Pest. Biochem. Physiol., Vol. 86 (2): 78 – 84.

Singh N, Srivastava AK (1990). Formothion induced hematological changes in the freshwater Indian cathfish, *Heteropneustes fossilis*. J. Ecotoxicol. Environ. Monitoring, Vol. 4: 137-140.

SPSS (1998). SPSS for Windows. SPSS Inc.

Van Hoof VO, De Broe ME (1994). Interpretation and clinical significance of alkaline phosphatase isoenzyme patterns. Crit Rev Clin Lab Sci. Vol. 31: 197-293.

Wannang NN, Jimam SN, Omale S, Dapar LMP, Gyang SS, Aguiyi JC (2007). Effects of *Cucumis metiluferus* (cucurbitaceae) fruits on enzymes and haematological parameters in albino rats. African J. Biotech. Vol. 6. (22): 2515-2518.

Zaki MS, Mostafa SO, Nasr S, Ata NS (2010). Biochemical, clinicophathlogical and microbial changes in *Clarias Gariepinus* exposed to pesticide malathion and climate change.Report and opinion. Vol. 2 (10): 70-76.