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Evaluation of Blood Characteristics of Grass Carp (*Ctenopharyngodon idella*) after Exposure to Organophosphate, Diazinon

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Abstract: The effect of different sublethal concentrations of diazinon at 1, 2 and 4 mg/L on some hematological and biochemical parameters of grass carp (*Ctenopharyngodon idella*) weighing 850±155g was studied after 1, 7, 15, 30 and 45 days post exposure to the toxicant as a bath for 12 hours at 18-22 °C. In one day exposure fish to the toxicant, the values of neutrophiles exposed to 2 and 4mg/L were significantly higher than control fish, while lymphocyte and monocyte counts in fish exposed to 2 and 1mg/L were significantly lower than control one ($P<0.05$). Also, no significant differences were found in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) among experimental fish and between experimental fish and control one ($P>0.05$). Seven day after exposing fish to the toxicant, values of WBC in all experimental fish were significantly higher than control fish, while level of neutrophiles in fish exposed to 1mg/L and values of lymphocytes in fish exposed to 2 and 4mg/L were significantly higher and lower than control fish, respectively. Also, values of ALT, AST and ALP in all experimental fish were significantly lower, higher and lower than control fish, respectively ($P<0.05$). Fifteen days post-exposing fish to the toxicant, values of MCH, ALT and AST in all experimental fish were significantly lower than control group, while levels of WBC and RBC were

significantly higher and lower than control fish, respectively (P.<0.05). Thirty days post-exposing fish to toxicant, the values of neutrophiles, WBC and RBC in fish exposed to 2 mg/L were lower than control fish (P<0.05). Also, levels of heterocytes and lymphocytes in all experimental fish were significantly higher and lower than control fish, respectively. Also, levels of AST, ALP and LDH in almost all experimental fish were significantly lower than control fish (P<0.05), while no significant differences was found in the level of AST among experimental fish and between experimental and control fish (P>0.05). Forty five days post-exposing fish to the toxicant, the values of WBC and heterocytes in all experimental fish were significantly higher than control one, while levels of lymphocytes, ALT and ALP were lower than control fish (P<0.05). Also, the level of AST in fish exposed to 4mg/L and level of LDH in fish exposed to 1 and 4 mg/L were significantly lower than control fish (P<0.05).

KEY WORDS : Diazinon, grass carp, haematology, serum biochemistry.

Introduction

Use of insecticides in agriculture has been more and more therefore it has caused many problems in relation with their overall effects on the environment. These chemicals are not always selective and have many adverse effects on non-target species. The leaching of long time pesticides into natural waters may lead to a serious deterioration in fish populations (Chakrabarty & Banerjee, 1988; Tsuda *et al.*,1990; Magdy *et al.*, 1992; Alam *et al.*, 1995; Hughes *et al.*, 1997; Bailey *et al.*, 1997). Toxic chemicals can affect fish at all levels of organisation from the morphological structure of organ systems to the molecular structure and activity of important enzymes (Sakr & Gabr, 1992; Keizer *et al.*, 1995; Moor & Waring, 1996). Many chemicals used for high agricultural industry, have been reported to cause marked changes in the blood parameters of fresh water fishes. Insecticides are commonly employed in agriculture and their toxicity to different groups of vertebrates has been widely studied (Chakrabarty *et al.*, 1988; Keizer *et al.*, 1990; Alam & Maughen, 1992; Sancho *et al.*, 1992; Tsuda *et al.*, 1997).

Diazinon [O-O-diethylO-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] is a widely used pesticide in the Iran and around the world. This chemical is a organophosphorus insecticide with a wide range of insecticidal activity and has been used from the early 1950s (Stone & Gradon, 1985; Eisler, 1986; Menconi & Cox, 1994; Moore & Waring, 1996). Diazinon inhibit the enzyme of acetylcholine estrase (AChE) and also it can inhibit the activity of Ca-ATPase enzyme during

nervous toxicity (Ansari *et al.*, 1987; Keizer *et al.*, 1995; Hamm *et al.*, 1998). Since there is a high possibility of long exposing of grass carp to diazinon in northern aquaculture of Iran, the present study was carried out to evaluate long effect of the toxicant chronic concentration on some blood parameters of this valuble species under suitable water quality conditions.

Materials and methods

1.Fish

One hundred twenty grass carps (*Ctenopharyngodon idella)* weighing 850±155 g from Mazandaran province fish farms were used for the experiments. Fish were held in 1200L tanks containing 15 fish each tank with constant water flow. The experiments were initiated after 4 days of fish acclimation to new conditions. Fish were fed with fresh vegetables consisting of lucern, clover and lettus.

2.Water quality

Water quality parameters including water temperature, dissolved oxygen and pH were 20±2°C, 7.7 mg/l and 7.5, respectively. Also, the level of NH_3, NO_2 and CO_2 were in range of acceptable levels during the trials.

3.Application of diazinon

Diazinon 60% emulsion was applied to the water of tanks at concentration of 1, 2 and 4 mg /L for 12 hours . Fish were then transferred to clean water and were kept for 45 days. Control groups were kept in clean water separately.

4. Collection and processing of samples

Samples were collected after 1,7,15,30 and 45 days post exposure to the toxicant. Five fish per treatment were used each sampling time. Blood was obtained by cutting fish tail after stunning with a sharp blow to the head and removing the scales and scraping the tail with alcohol. In order to hematological study, blood was sampled and stabilized with EDTA at 50 IU/ml blood. Smears were obtained from blood samples at the same time of blood collection. In order to biochemical study, the blood samples were obtained separately in tubes without EDTA.

5. Haematological study

The leucocytes morphology and their differentiations were studied using the smears obtained from blood samples . The smears were first air dried , fixed in

96% ethanol for 30 minutes , stained by Giemsa staining for 30 minutes and were examined under compound microscope with magnification of 400X. The stabilized blood samples were used for determination of erythrocyte count (RBC), leukocyte count (WBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin(MCH)and mean corpuscular haemoglobin concentration (MCHC) on control and experimental groups using method described by Klontz, (1994) and Harmers, (1995) .

6. Biochemical study

The no stabilizing blood samples were centrifuged for 15 min at 400 g, sera were separated, and the activity levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) of sera samples were then determined using an automatic analyzer (eppendorf, EPOS 5060) optimized by Boehringer GmBH tests.

7. Data analysis

Statistical parameters including mean, standard deviation, variance, standard error for test and control groups were calculated (SPSS version 10) and results were processed statistically by means of the analysis of variance (ANOVA) at $P<0.05$.

Results

1.Haematological study

Results of haematological profiles of both control and experimental groups at different times of sampling are shown in Table 1. One day after exposing fish to the toxicant, the values of neutrophiles in fish exposed to 2 and 4mg/L were significantly higher than control fish, while lymphocyte counts in fish exposed to 2 mg/L were significantly lower than control one ($P<0.05$) (Table 1A). Also, values of myelocytes and monocytes in fish exposed to 1 mg/L were significantly lower than control fish. Seven day after exposing fish to the toxicant, the level of Hb was significantly higher in fish exposed to 1 and 4 mg/L than control fish ($P<0.05$) (Table 1B). Also, values of WBC in all experimental fish were significantly higher than control fish, while level of neutrophiles in fish exposed to 1mg/L and values of lymphocytes in fish exposed to 2 and 4mg/L were significantly higher and lower than control fish, respectively ($P<0.05$) (Table 1B).

Table 1: Effects of various concentrations of diazinon on haematological indices of grass carp after 1, 7, 15, 30 and 45 days post-exposure

* values are significant at $P<0.05$

Table 1A: After 1 day

Parameter	Treatment (ppm)	No.	Mean	Variance	Std. Error
PCV (%)	4	4	30.75	0.917	0.4787
	2	4	27.50	4.33	1.0408
	1	4	38.75	2.917	0.8539
	Control	2	32.00	32	4.0000
Hb (g/100ml)	4	4	7.60	7.33E-02	0.1354
	2	4	6.97	0.136	0.1842
	1	4	8.77	4.25E-02	0.1030
	Control	2	8.15	2.645	1.1500
MCV (m^3)	4	4	0.000173	2.5E-11	0.0000
	2	4	0.0002	2.86E-09	0.0000
	1	4	0.000223	2.5E-11	0.0000
	Control	2	0.00017	4.5E-10	0.0000
MCH (pg)	4	4	0.000043	1.66E-12	0.0000
	2	4	0.000051	2.049E-10	0.0000
	1	4	0.00005	7.5E-12	0.0000
	Control	2	0.000042	1.25E-11	0.0000
MCHC (%)	4	4	24.71	0.147	0.1915
	2	4	25.40	0.445	0.3333
	1	4	22.66	0.289	0.2687
	Control	2	25.41	0.344	0.4150
WBC (/mm^3)	4	4	9250.00	16666.667	64.5499
	2	4	8400.00	951666.67	487.7670
	1	4	9400.00	11666.667	54.0061
	Control	2	14000.00	6480000.0	1800.000
RBC($10x10^6$)	4	4	1.79	4.4403	0.0331
	2	4	1.66	1.68E-02	0.0648
	1	4	1.75	1.66E-02	0.0645
	Control	2	2.30	2.00E02	0.1000
Heterocyte (%)	4	4	12.50	1.667	0.6454
	2	4	12.00	14	1.8708
	1	4	31.75	3.58	0.94648
	Control	2	21.50	4.5	1.5000
Neutrophil (%)	4	4	20.50*	1.667	0.6454
	2	4	35.50 *	6.33	1.2583
	1	4	9.00	7.33	1.3540
	Control	2	3.00	2	1.0000
Lymphocyte (%)	4	4	63.25	.917	0.4787
	2	4	48.00 *	8.667	1.4719
	1	4	55.00 *	.667	0.4082
	Control	2	66.50	.5	0.5000
Myelocyte (%)	4	4	3.00	2	0.7071
	2	4	2.00*	4.667	1.0801
	1	4	2.25	.25	0.2500
	Control	2	5.00	0	0.0000
Monocyte (%)	4	4	0.75 *	.917	0.4787
	2	4	2.50	3.667	0.9574
	1	4	2.00 *	.667	0.4082
	Control	2	4.00	0	0.0000

Table 1B: After 7 day

Parameter	Treatment (ppm)	No.	Mean	Variance	Std. Error
PCV (%)	4	4	32.750	4.99	1.108678
	2	4	23.750	1.58	0.629153
	1	4	35.500	1.667	0.645497
	Control	2	25.500	4.5	1.500000
Hb (g/100ml)	4	4	8.0500 *	1E-02	0.05000
	2	4	5.8750	0.229	0.239357
	1	4	7.5000 *	0.167	0.204124
	Control	2	6.0000	0	0.000000
MCV (m^3)	4	4	0.0002	2.067E-02	0.0000223
	2	4	0.0001	8.25E-10	0.0000146
	1	4	0.0002	6.917E-10	0.0000135
	Control	2	0.0001	4.500E-10	0.000015
MCH (pg)	4	4	0.00004	9.667E-11	0.0000041
	2	4	0.00004	3.825E-11	0.0000030
	1	4	0.00017	5.641E-08	0.0001188
	Control	2	0.000044	7.200E-11	0.0000060
MCHC (%)	4	4	24.6575	2.315	0.760782
	2	4	24.7300	1.678	0.647740
	1	4	21.1675	2.942	0.857626
	Control	2	23.6100	3.864	1.390000
WBC (/mm^3)	4	4	13712.50*	2003958.33	707.80618
	2	4	12237.50*	533958.33	365.36226
	1	4	10550.00*	123333.33	175.59423
	Control	2	7400.000	45000	150.00000
RBC($10x10^6$)	4	4	1.525000	8.917E-02	0.149304
	2	4	1.4050	4.277E-02	0.103401
	1	4	1.38250	7.892E-03	0.04442
	Control	2	1.7000	2E-02	0.1000
Heterocyte (%)	4	4	34.250000	2.917	0.853913
	2	4	54.7500*	4.91778	1.108678
	1	4	41.250000	4.917	1.108678
	Control	2	32.500000	4.5	1.500000
Neutrophil (%)	4	4	6.750000	.917	0.478714
	2	4	8.500000	3.667	0.957427
	1	4	10.75000*	2.917	0.853913
	Control	2	4.500000	.5	0.500000
Lymphocyte (%)	4	4	54.250000	5.583	1.181454
	2	4	33.5000*	1.667	0.645497
	1	4	45.0000*	8.667	1.471960
	Control	2	60.5000	.5	0.500000
Myelocyte (%)	4	4	3.75000	6.917	1.314978
	2	4	2.0000	2	0.707107
	1	4	2.0000	0	0.000000
	Control	2	1.50000	.5	0.500000
Monocyte (%)	4	4	1.0000	1.333	0.577350
	2	4	1.7500	1.583	0.629153
	1	4	1.0000	0.667	0.408248
	Control	2	1.0000	0.00	0.000000

Fifteen days post-exposing fish to the toxicant, values of MCH in all experimental fish were significantly lower than control group ($P<0.05$) (Table 1C). Levels of WBC and RBC in all experimental fish were significantly higher and lower than control fish, respectively ($P<0.05$) (Table 1C). Also, heterocyte count in fish exposed to 2mg/L and neutrophile count in fish exposed to 4 mg/L were higher than control fish. Furthermore, lymphocyte count in fish exposed to 2 mg/L was lower than control fish ($P<0.05$)(Table 1C). Thirty days post-exposing fish to diazinin, the values of WBC and RBC in fish exposed to 2 mg/L were lower than control fish (Table 1D) ($P<0.05$). Also, levels of heterocytes and lymphocytes in all experimental fish were significantly higher and lower than control fish, respectively, while level of neutrophiles in fish exposed to 2 mg/L was significantly lower than control fish (Table 1D) ($P<0.05$). Forty five days post-exposing fish to the toxicant, the values of WBC and heterocytes in all experimental fish were significantly higher than control one, while levels of lymphocytes in all experimental fish were lower than control fish (Table 1E) ($P<0.05$).

2. Biochemical study

Results of biochemical features are shown in Table 2. No significant differences were found in levels of ALT, AST, ALP and LDH among experimental fish and between experimental and control fish one day after exposing fish to the toxicant (Table 2A) ($P<0.05$). Values of ALT, AST and ALP in all experimental fish were significantly lower, higher and lower than control fish 7 days post-exposing fish to toxicant (Table 2B) ($P<0.05$). Furthermore, level of LDH in fish exposed to 2mg/L was significantly lower than control fish (Table 2B) ($P<0.05$). Levels of ALT and AST in all experimental fish were lower than control fish 15 days post-exposure to the toxicant (Table 2B)($P<0.05$), while there was no significant difference in level of LDH between experimental and control fish (Table 2C) ($P>0.05$). Also, level of ALP in fish exposed to 4mg/L was significantly lower than control one (Table 2C) ($P<0.05$). Levels of AST, ALP and LDH in almost all experimental fish were significantly lower than control fish 30 days post-exposing fish to toxicant (Table 2D) ($P<0.05$), while no significant difference was found in the level of AST among experimental fish and between experimental and control fish (Table 2D) ($P>0.05$). The values of ALT and ALP in all experimental fish were significantly lower than control one 45 days post-exposing fish to toxicant (Table 2E) ($P<0.05$). Also, the level of AST in fish exposed to 4mg/L and level of LDH in fish exposed to 1 and 4 mg/L were significantly lower than control fish (Table 2E) ($P<0.05$).

Table 1C: After 15 days

Parameters	Treatments (ppm)	No.	Mean	Variance	Std. Error
PCV (%)	4	4	34.0000	1.667	0.408248
	2	4	25.0000	7.583	1.581139
	1	4	24.7500	8	0.629153
	Control	2	30.5000	.5	0.500000
Hb (g/100ml)	4	4	8.8750	5.667E-02	0.06292
	2	4	6.67500	0.167	0.268871
	1	4	6.97500	1.583E-02	0.06292
	Control	2	8.85000	.125	0.05000
MCV (m^3)	4	4	0.000194	1.641E-09	0.0000022
	2	4	0.000148	4.905E-09	0.0000091
	1	4	0.000163	7.27E-10	0.0000074
	Control	2	0.000197	0	0.0000030
MCH (pg)	4	4	0.00005.*	8.867E-11	0.0000006
	2	4	0.000035*	5.700E-11	0.0000015
	1	4	0.000046*	1.043E-10	0.0000020
	Control	2	0.000057	5E-13	0.0000015
MCHC (%)	4	4	26.10750	0.571	0.139127
	2	4	26.82250	1.720	0.655380
	1	4	28.22250	6.677	0.547211
	Control	2	29.02000	.490	0.310000
WBC (mm^3)	4	4	7925.00 *	40625	47.871355
	2	4	9950.00 *	594400	542.37134
	1	4	8742.500*	102291.667	2529.9650
	Control	2	5050.0000	1250	50.000000
RBC($10X10^6$)	4	4	1.6625 *	1.789E-02	0.02887
	2	4	1.600 *	5E-03	0.02799
	1	4	1.1975 *	6.069E02	007773
	Control	2	3.850	5E-03	0.05000
Heterocyte (%)	4	4	25.25000	4.33	0.629153
	2	4	45.75000*	9.667	2.015564
	1	4	33.50000	0.917	1.322876
	Control	2	10.50000	4.5	0.500000
Neutrophil (%)	4	4	3.25000*	2.25	0.250000
	2	4	1.50000	1.667	0.957427
	1	4	1.0000	2.917	0.000000
	Control	2	1.50000	0	0.500000
Lymphocyte (%)	4	4	69.25000	3.33	0.478714
	2	4	51.7500*	11.667	2.393568
	1	4	65.2500	1.667	1.250000
	Control	2	85.5000	2	0.500000
Myelocyte (%)	4	4	1.0000	0.333	0.000000
	2	4	0.2500	1	0.250000
	1	4	0.0000	0	0.000000
	Control	2	1.0000	0	0.138675
Monocyte (%)	4	4	1.0000	0.25	0.000000
	2	4	0.75000	0.25	478714
	1	4	1.2500	0.333	0.250000
	Control	2	1.5000	.5	**0.500000**

Table 1D: After 30 days

Parameters	Treatments (ppm)	No.	Mean	Variance	Std. Error
PCV (%)	4	4	34.250000	0.917	0.478714
	2	4	27.500000	15	1.936492
	1	4	28.250000	11.583	1.701715
	Control	2	34.000000	0.00	0.000000
Hb (g/100ml)	4	4	9.02500	2.5E-03	0.02500
	2	4	8.375000	0.676	0.411045
	1	4	8.200000	0.600	0.387298
	Control	2	10.000000	0.00	0.000000
MCV (m^3)	4	4	0.00018	1.825E-11	0.0000021
	2	4	0.0001735	4.510E-10	0.0000106
	1	4	0.0001353	1.366E-09	0.0000188
	Control	2	0.0001435	1.25E-11	0.0000025
MCH (pg)	4	4	0.0000499	2.250E-14	0.0000000
	2	4	0.000052*	9.307E-12	0.0000012
	1	4	0.0000390	9.267E-11	0.0000048
	Control	2	0.0000	5.00E-13	0.0000005
MCHC (%)	4	4	26.3625*	0.464	0.340621
	2	4	30.630000	6.144	1.239314
	1	4	29.117500	3.101	0.880477
	Control	2	30.440000	4.5E-02	0.150000
WBC (/mm^3)	4	4	7900.0000	6666.667	40.824829
	2	4	5087.500*	443958.33	333.15099
	1	4	8420.0000	89266.667	149.38764
	Control	2	7825.0000	1250	25.000000
RBC($10X10^6$)	4	4	1.802500	9.167E-05	0.004787
	2	4	1.587500*	1.889E-02	0.06872
	1	4	2.162500	0.146	0.190979
	Control	2	2.365000	2.450E-03	0.03500
Heterocyte (%)	4	4	46.00000*	0.667	0.408248
	2	4	47.50000*	27.667	2.629956
	1	4	49.5000*	17	2.061553
	Control	2	25.000000	2	1.000000
Neutrophil (%)	4	4	3.000000	0.667	0.408248
	2	4	1.00000*	1.333	0.577350
	1	4	4.000000	16	2.000000
	Control	2	4.000000	0.00	0.000000
Lymphocyte (%)	4	4	48.7500*	0.917	0.478714
	2	4	50.0000*	56.00	3.741657
	1	4	37.0000*	35.333	2.972092
	Control	2	68.00000	2.000	1.000000
Myelocyte (%)	4	4	1.250000	0.25	0.250000
	2	4	0.750000	0.917	0.478714
	1	4	8.250000	12.25	1.750000
	Control	2	2.000000	0.00	0.000000
Monocyte (%)	4	4	1.000000	0.00	0.000000
	2	4	0.750000	0.917	0.478714
	1	4	1.250000	0.25	0.250000
	Control	2	1.000000	0.00	0.000000

Table 1E: After 45 day

Parameters	Treatments (ppm)	No.	Mean	Variance	Std. Error
MCHC (%)	4	4	26.107500	7.742E-02	0.139127
	2	4	26.822500	1.718	0.655380
	1	4	28.222500	1.198	0.547211
	Control	2	29.020000	0.192	0.310000
WBC (/mm³)	4	4	7925.000*	9166.667	47.871355
	2	4	9950.00*	1176666.67	542.37134
	1	4	8742.50*	25602891.7	2529.9650
	Control	2	5050.0000	5000.00	50.000000
RBC(10X10⁶)	4	4	1.750000	3.333E-03	0.02887
	2	4	1.680000	3.133E-03	0.02799
	1	4	1.525000	2.417E-02	0.07773
	Control	2	1.550000	5.000E-03	0.05000
Heterocyte (%)	4	4	25.2500*	1.583	0.629153
	2	4	45.7500*	16.25	2.015564
	1	4	33.5000*	7.00	1.322876
	Control	2	10.500000	0.5	0.500000
Neutrophil (%)	4	4	3.250000	0.25	0.250000
	2	4	1.500000	3.667	0.957427
	1	4	0.000000	0	0.000000
	Control	2	1.500000	0.5	0.500000
Lymphocyte (%)	4	4	69.2500*	.917	0.478714
	2	4	51.7500*	22.917	2.393568
	1	4	65.2500*	6.25	1.250000
	Control	2	85.5000	.5	0.500000
Myelocyte (%)	4	4	1.0000	0	0.000000
	2	4	0.25000	0.25	0.250000
	1	4	0.25000	0	0.000000
	Control	2	1.0000	0	0.000000
Monocyte (%)	4	4	1.0000	0	0.000000
	2	4	0.75000	0.917	0.478714
	1	4	1.2500	0.25	0.250000
	Control	2	1.500000	0.5	0.500000

Table 2: Effects of various concentrations of diazinon on biochemical indices of grass carp after 1, 7, 15, 30 and 45 days post-exposure
* values are significant at $p<0.05$

Table 2A: after 1 day

Parameter	Treatment (ppm)	No.	Mean	Variance	Std. Error
ALT (u/l)	4	4	7.00	.667	0.4082
	2	4	6.50	4.33	1.0408
	1	4	14.50	4.33	1.0408
	Control	2	17.50	4.5	1.5000
AST (u/l)	4	4	147.75	1392.91	18.6608
	2	4	355.25	221384	235.2574
	1	4	135.25	364917	9.5513
	Control	2	15.00	61747.744	48.0000
ALP(u/l)	4	4	100.50	7	1.3228
	2	4	108.75	29.58	2.7195
	1	4	206.50	316.33	8.8928
	Control	2	172.00	2450	35.0000
LDH(u/l)	4	4	1767.00	64444	40.1372
	2	4	960.250	144396.92	189.9979
	1	4	827.750	68080.5	105.1866
	Control	2	1334.50	212241.94	184.5000

Table 2B: after 7 days

Parameter	Treatment (ppm)	No.	Mean	Variance	Std. Error
AIT (u/l)	4	4	5.2500	.25	0.250000
	2	4	4.0000	0	0.000000
	1	4	4.5000*	.333	0.288675
	Control	2	9.5000	.5	0.500000
AST (u/l)	4	4	123.250*	16.25	2.015564
	2	4	74.5000*	99.667	4.991660
	1	4	79.7500*	7.5837	1.376893
	Control	2	16.5000	4.5	1.500000
ALP(u/l)	4	4	126.750*	5.583	1.181454
	2	4	113.350*	28.25	2.657536
	1	4	125.2500*	16.25	2.015564
	Control	2	148.5000	4.5	1.500000
LDH(u/l)	4	4	1618.000	11818	54.355313
	2	4	1117.25*	7351.583	42.870687
	1	4	1181.500	1353.667	18.396105
	Control	2	1388.000	1425	27.000000

Table 2C: after 15 days

Parameter	Treatment (ppm)	No.	Mean	Variance	Std. Error
AIT (u/l)	4	4	5.00000*	.25	0.408248
	2	4	6.25000*	.667	0.478714
	1	4	8.00000*	.25	0.408248
	Control	2	12.5000	.5	0.500000
AST (u/l)	4	4	87.5000*	18.25	2.500000
	2	4	98.5000*	13.667	1.040833
	1	4	104.000*	9.583	2.738613
	Control	2	138.5000	.5	4.500000
ALP(u/l)	4	4	71.5000*	99.33	1.848423
	2	4	85.7500	28.25	4.534589
	1	4	82.5000	10.25	2.533114
	Control	2	140.5000	32	3.500000
LDH(u/l)	4	4	727.7500	2744.33	11.932973
	2	4	939.2500	742.917	8.730932
	1	4	879.7500	3277.667	16.178046
	Control	2	1076.500	4512.5	21.500000

Table 2D: after 30 days

Parameter	Treatment (ppm)	No.	Mean	Variance	Std. Error
AlT (u/l)	4	4	5.500000	4.4240	0.707107
	2	4	5.000000	1.33	0.577350
	1	4	5.000000	1.667	0.645497
	Control	2	10.000000	2	1.000000
AST (u/l)	4	4	102.5000*	127	5.634714
	2	4	113.7500*	120	5.498106
	1	4	107.250*	132.917	5.764475
	Control	2	147.50000	4.5	1.500000
ALP(u/l)	4	4	39.2500*	16.25	2.015564
	2	4	53.5000*	188.33	6.861730
	1	4	84.2500*	23.583	2.428134
	Control	2	117.00000	72	6.000000
LDH(u/l)	4	4	741.0000*	5612.667	37.458866
	2	4	823.000*	7608.667	43.613836
	1	4	930.000*	1151.333	16.965652
	Control	2	1255.0000	1058	23.000000

Table 2E: after 45 days

Parameter	Treatment (ppm)	No.	Mean	Variance	Std. Error
AlT (u/l)	4	4	5.0000*	.667	0.408248
	2	4	6.2500*	.917	0.478714
	1	4	8.0000*	.667	0.408248
	Control	2	12.500000	.5	0.500000
AST (u/l)	4	4	87.5000*	25	2.500000
	2	4	98.5000*	4.33	1.040833
	1	4	104.0000	30	2.738613
	Control	2	138.5000	40.50	4.500000
ALP(u/l)	4	4	71.5000*	13.667	1.848423
	2	4	85.7500*	82.25	4.534589
	1	4	82.5000*	25.667	2.533114
	Control	2	140.50000	24.5	3.500000
LDH(u/l)	4	4	727.750*	569.583	11.932973
	2	4	939.25000	304.917	8.730932
	1	4	879.750*	1046.91	16.178046
	Control	2	1076.5000	924.50	21.500000

Discussion

The examination of haematological and biochemical parameters of grass carp shows that effect of the organophosphorous insecticide diazinon, at sub-lethal levels elicit a response in the blood picture .The insignificantly decrease of RBC counts and Hb values except the dose of 1 mg/L, and increase of MCH values (Table 1A) after 24 hours post exposure to diazinon indicate that the toxicant caused an effect similar to the production of anemia. Similar results have been reported by Eisler (1967) for fish exposed to methyl parathion and by Aness

(1978_b) for fish exposed to three organophosphorus insecticides consisting of diazinon, methyl parathion and dimethoate. In addition, a significant ($P<0.05$) decrease of WBC counts and lymphocytes values and significant increase of neutrophil value have been observed in this stage. Similar results have been reported in different fishes by other authors (e.g. Pandy *et al.*, 1979; Mahajan & Juneja, 1979; Chouhan *et al.*, 1983; Dabral & Chaturvedi, 1983; Magdy *et al.*, 1992; Svoboda *et al.*, 2001). Taking into consideration that organophosphates may induce hypoxic stress in fish and the fact that teleosts generally respond to low environmental oxygen by an increase in the concentration of blood Anticholinesterases are known to alter the erythrocyte membrane permeability to Na^- and K^- ions (Greig *et al.*, 1963). Therefore, diazinon probably trigged some metabolic disturbances by effect on the membrane permeability and decrease the oxygen consumption of erythrocytes and often seems associated with hypoxic conditions produced either directly by decrease of the water PO_2 or following treatments which damage the ability of the fish to transfer oxygen across the gill water/ blood barrier (Hughes *et al.*, 1997). Histological observations of the gills indicated that the lamellar epittalium thickens after treatment with malachite green and this thickening hinders the exchange of gases between the gill lamella and the water (Magdy *et al.*, 1992). Another possible mechanism that is the role of peritubular tissue of the kidney to perform the haematopoietic function in *Channa punctatus* (Aness, 1978_b). This may offer another possible explanation for the hematological abnormalities in the present study. In addition , Svobodo *et al.*, (2001) has discussed that changes in values of both the erythrocyte and leukocyte profile after exposure to diazinon, may be refered to disruption of hematopoiesis as well as to a decrease on non specific immunity of the fish as was mentioned by other workers as well (Camerson,1970; Aness,1978a). However, it was expected that blood values after this stage would change accordingly. Generally, there was a fluctuation in blood parameters of all experimental fish sampled in different times post exposure. Such fluctuations indicate that fish homatopoietic tissues were in stress and were constant by struggling to maintain normal condition.

In this study a decrease in values of lymphocyte and neutrophiles in fish exposed to 1 and 2 mg/L of diazinon was observed after 15 and/or 30 post-exposure, while an increase in heterocyte counts was obtained almost throughout the expriment

time up to 45 days post-exposing. Similar results have been reported by other workers (Alkahem, 1994; Chakrabarty & Banerjee, 1988; Svoboda *et al.*, 2001). The mechanism inducing such a lymphopenia might be a decrease in delivery of lymphocytes to the circulatory system through a reduced lymphcyte production, alternatively a rapid destruction of cells or an increased rate of perpheral removal of lymphocytes. The most probable explanation is that there is a failur in lymphocyte production together with the dissolution of cells (Alkahem, 1994). Ellis (1981) suggested that lymphopenia in fishes is accompanied by neutrophilia as seen in the present study.

Also, generally a significant decrease in values of ALT, ALP, AST and LDH in experimental fish are similar to results found in *Channa punctata* and common carp exposed to acute level of diazinon 96 hour (Sastry & Sharma, 1980; Luskova *et al.*, 2002). Significant reduction in the concentration of LDH in the blood plasma of experimental grass carp , compared with the control group , indicates a decrease in the glycolytic process due to the lower observed similar results of the effect of diazinon as Luskova *et al.*, (2002) also observed similar results when common carp were exposed to acute level of diazinon. However, other authors reported an increase in plasma LDH level in various fish species (e.g. Gill *et al.*, 1990; Ceron *et al.*, 1997). It has been suggested that ALP is inhibited after 96-hour of the effect and then it reduces its normal values or even an increased activity is observed (Sasrty & Sharma, 1980). A reduction in level of ALP support the assumption that the liver tissues of the experimental grass carp was remarkably affected by of diazinon as necrosis of the hepatocytes was shown by microscopic examination (data not shown).

Also, decreased levels of ALT and AST in experimental fish indicate that diazinon may damages paranchymatous tissues or skeletal musculature. However ALT was increased in experimental groups of grass carp following exposing to diazinon compared to control one.

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