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Study of Action of Sublethal Concentrations of Diazinon on Blood Indices of Male and Female *Anabas testudineus*

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ABSTRACT

Anabas testudineus is a commercially important and nutritionally rich fish. This investigation evaluated haemato-biochemical responses of Anabas testudineus associated with exposure of sublethal concentrations 3.275 ppm (1/2th of LC50) and 1.31 ppm (1/5th of LC50) of diazinon 60 EC under laboratory conditions for one week (7 days), two weeks (14 days) and four weeks (28 days). Increase exposure time and dose lead to decrease of Hemoglobin (Hb), Total Erythrocyte Count (TEC) and Hematocrit (HCT) values, but increase of Total Leucocytes Count (TLC), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Hemoglobin (MCH) values in both sexes. Significant (P<0.01) increase in blood glucose (77.44 mg/dl) and cholesterol (232.03 mg/dl) level was more noticed among female whereas total protein contents were significantly decreased. Three factors ANOVA analysis showed significant (p<0.01) variation in TLC (F=5.56), MCV (F=20.445), MCHC (F=12.427), glucose (F=4.029) and cholesterol (F=5.008). Sublethal concentrations of diazinon lead to subtle changes in blood indices of test fish. ANOVA showed that the pesticide concentrations had more influence than the duration of exposure in all cases (P<0.01). Findings of this study showed sex-related and depicted severity of diazinon was both dose and duration dependant.

Keywords: *Anabas testudineus*, Diazinon, Sublethal concentration, Blood indices

INTRODUCTION

Fisheries and aquatic resources are exceptionally valuable natural assets, which provide long-term benefits in return for minimal care and protection. Appreciation of fisheries and aquatic systems has been accompanied by increasing concern about the effects of growing human populations and human activity on aquatic life. Pesticides are one group of toxic compounds linked to human use that have a profound effect on aquatic life and water quality. Pesticides in the aquatic environment can negatively affect the ecosystem [1,2]. Among the various animal groups, fishes have been identified as being very sensitive to pollutants and have been the most popular test organism because they are presumed to be the best understood organism in the aquatic environment [3]. Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc [4]. Hence, its use is growing and becoming very important for toxicological researches. It is noted that studies on fish blood gives the possibility of knowing physiological conditions within the fish long before outward manifestation of pathological condition. The climbing perch, *Anabas testudineus,* is one of the potential cultivable air-breathing species attracting attention of the pisciculturists owing to their high production potential from paddy fields and stagnant shallow ponds, high economic value, and preference due to its tolerance to adverse environmental conditions in the derelict water bodies [5,6]. With increased use of pesticides in agriculture, it has become important to know about the toxic effect of pesticides on such non-target organisms as these test fishes.

One such widely used toxicant is diazinon, which is a type of organophosphorous pesticides [7]. Although, the aquatic environment is not the target one for the use of such pesticides the results of a number of monitoring studies have

evidenced the presence of diazinon and its metabolite, diazoxon, in surface waters [8,9]. That is why great attention has been paid to the effect of diazinon on fish organism. However, sex-related variations in various hematological values of fishes exposure to pesticide are scanty [10-12]. The aim of the present work was to assess the influence of sublethal concentration of the pesticide on the test fish and observed changes in the haematological parameters of male and female *Anabas testudineus* in order to show the toxic effects as indices of the pesticide stress.

MATERIALS AND METHODS

The toxicant diazinon 60 EC had been used in this study. The commercial formulation of diazinon, Basudin 60 EC brand, with the active ingredient diazinon (O, O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate with purity of 60% and dissolved in 40% acetone, was used to prepare test solutions of diazinon [13]. Toxicity concentrations were defined based on previous studies on diazinon 60 EC toxicity to same fish species. The acute 96 hours LC50 value of diazinon in *Anabas testudineus* was 96 h 6.55 ppm [14]. Concentrations 3.275 ppm ($1/2^{th}$ of LC50) and 1.31 ppm ($1/5^{th}$ of LC50) of diazinon were selected for the test. *Anabas* captured from the Tankapani pond, Khurda District, Bhubaneswar (19° 40"N to 20° 25"N Latitude and 24° 55"E to 36°05"E Longitude; area) by using a net and transferred to cement tank (2010). Sexually matured fishes (male: 12.00 ± 0.50 cm, 30 ± 1.00 g; female: 13.50 ± 0.50 cm, 45 ± 1.00 g) were used in this investigation. Fishes were washed with 0.1% $KMnO_4$ solution to avoid dermal infection and APHA et al. [15,16] were followed for maintaining fishes. The collected fishes were acclimated to laboratory conditions in dechlorinated tap water for 30 days. The fishes were fed with commercial fish feed during acclimation period. After acclimation, fishes were separated into different groups of 10 each in similar sized tank. The water in the aerated tank was changed in every 24 hours to maintain the concentration of the diazinon 60 EC during the period of exposure. No mortality occurred in any group during the experimental period. Two hundred seventy test fishes were used for the present study and these were divided into twenty seven groups, each group consisting of ten fishes. The first twelve groups (six male and six female groups) were exposed to 3.27 ppm and the second twelve groups were exposed to 1.31 ppm diazinon for one week (7 days), two weeks (14 days) and four weeks (28 days) while the remaining three groups were maintained as control in diazinon free water.

At the end of the exposure period, fishes were caught very gently using a small dip net. Samples of 2 ml blood were taken from the caudal vein of non-anaesthetized fish by sterilize syringe containing EDTA as an anticoagulant for blood analysis [17].

Hematological analysis

Haemoglobin estimation by cynmethaemoblobin method [12,18]. Estimation of Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) as per Pal et al. [19] and Pradhan et al. [12]. The number of cells count was determined as described by Svobodova et al. and Oluyemi et al. [20,21]. The Hct was estimated by using microhemotocrit reader and expressed in percentage. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated as per method Dacie and Lewis [17] and pradhan et al. [12].

Blood biochemical analysis

Estimation of blood plasma protein was carried out as per method of Lowry et al. [22]. Bovine serum albumin was used as standard and different (0.5-5 mg/ml) solutions were prepared by mixing stock BSA solution (1 mg/ml). 0.2 ml protein solution was taken from each dilution, followed by mixing of 2 ml of alkaline copper sulphate solution, which were mixed well and incubated at room temperature for 10 min. Then, Folin Ciocalteau solution (0.2 ml) was added to each tube and incubated for 30 min. The optical density was taken at 660 nm and absorbance was plotted against protein concentration to obtain a standard calibration curve. Blood glucose level was estimated by Glucose Oxidase and Peroxidase (GOD/POD) method using the GOD/POD kit (Coral Clinical Systems), 1 ml of test reagent was added to all the test tubes. Then, 10 ml of distilled water, 10 ml of standard solution and 10 ml of plasma samples were added to the respective marked test tube. These solutions were incubated at 25°C for 30 min and optical density measured the absorbance at 505 nm. Cholesterol amount was determined by the CHOD/PAP method using Kit (Crest Biosystem, India) [23,24].

Statistical analysis

All data were analyzed using ANOVA with the aid of SPSS 17 for windows. The mean values obtained for each variable analyzed at the different experimental concentrations were compared to each other by parametric analysis of variance (ANOVA). The data were statistically analyzed using Duncan's multiple range tests to determine differences in means [25].

RESULTS AND DISUSSION

In the present study, the effect of diazinon on the test fishes in sublethal exposure revealed that pesticide had considerable impact on the different blood parameters (Table 1). Hemoglobin (Hb) concentration varied from 9.16 to 11.54 gm/dl and 7.77 to 11.21 gm/dl in male and females respectively. Hb values at one week (7 days), two weeks (14 days) and four weeks (28 days) followed decrease pattern in both sexes.

The total number of erythrocyte ranged from 2.36 × 10^6 to 3.05 × 10^6 per cubic mm of blood and 2.05 to 2.8 × 10^6/ mm^3 blood in male and female fish respectively. Total erythrocyte values followed decrease pattern in both the sexes when compared with the control specimens and most significant reduction was observed in sublethal (3.227 ppm) concentration on 28th day. Total Leukocyte Count (TLC) value was higher in 4th week (28 days) and TLC varied between 13.74 × 10^3 per cubic mm of blood and 21.92 × 10^3 per cubic mm of blood and 12.28 and 18.82 × 10^3 per cubic mm of blood in males and females respectively. The Hct value ranged from 22.70 to 34.18% in males and varied between 21.91 and 30.23% in females indicating that this was higher for males than that of female. MCV value ranged between 153.21 and 170.02 μ^3 for male and 144.49 and 152.65 μ^3 for female fishes. MCH value was ranged from 38.62 to 44.94 pg in male whereas in female variation range was 37.48 and 45.98 pg.

Biochemical parameters, glucose and protein were noted as slightly higher in female than males (Table 2). Glucose

Table 1: Comparative evaluation of haematological parameters of *A. testudineus* under different treatments of diazinon 60 EC

Gender	Duration	Dose		Hb	TEC	TLC	HCT	MCV	MCH	MCHC
Male	1st week	Control	Mean	11.35^{a}	2.93^{a}	13.74^{c}	34.18^{a}	153.21^{b}	39.56^{b}	31.78^{d}
			SE	0.16	0.08	0.60	0.15	0.29	0.15	0.16
		1.31 ppm	Mean	11.15^{a}	2.96^{a}	14.36^{c}	29.40^{a}	156.25^{a}	39.53^{b}	33.47^{bc}
			SE	0.15	0.06	0.49	0.43	0.12	0.28	0.40
		3.227 ppm	Mean	10.78^{a}	2.68^{a}	16.14^{c}	28.79^{a}	156.87^{a}	39.70^{b}	36.24^{a}
			SE	0.17	0.13	0.33	0.23	0.09	0.58	0.15
	2nd week	Control	Mean	11.54^{a}	2.96^{a}	14.11^{c}	33.76^{a}	153.96^{b}	40.03^{b}	34.37^{b}
			SE	0.19	0.09	0.29	0.13	0.07	0.07	0.44
		1.31 ppm	Mean	11.48^{a}	2.93^{a}	19.65^{b}	27.87^{a}	157.14^{a}	38.62^{bc}	32.30^{c}
			SE	0.07	0.19	0.86	0.43	0.12	0.20	0.10
		3.227 ppm	Mean	10.29^{a}	2.48^{a}	18.75^{ab}	24.66^{a}	159.77^{a}	39.62^{b}	34.86^{b}
			SE	0.04	0.17	0.38	0.67	0.34	1.28	0.12
	4th week	Control	Mean	10.54^{a}	3.05^{a}	14.23^{c}	31.48^{a}	156.30^{a}	40.80^{b}	35.23^{ab}
			SE	0.13	0.12	0.33	0.60	0.27	0.16	0.17
		1.31 ppm	Mean	10.45^{a}	2.94^{a}	21.92^{a}	29.49^{a}	158.55^{a}	44.94^{a}	35.97^{ab}
			SE	0.17	0.13	0.13	1.79	0.25	0.68	0.32
		3.227 ppm	Mean	9.16^{a}	2.36^{a}	20.63^{a}	22.70^{a}	170.02^{a}	41.52^{ab}	38.25^{a}
			SE	0.47	0.20	0.28	0.24	0.62	0.87	0.34
Female	1stweek	Control	Mean	11.13^{a}	2.58^{a}	13.40^{c}	30.03^{a}	144.49^{c}	41.47^{ab}	30.70^{d}
			SE	0.19	0.04	0.58	0.21	0.20	0.18	0.20
		1.31 ppm	Mean	11.08^{a}	2.80^{a}	14.24^{c}	26.75^{a}	146.83^{c}	41.19^{ab}	32.07^{c}
			SE	0.38	0.13	0.20	0.33	0.73	0.31	0.23
		3.227 ppm	Mean	9.05^{a}	2.25^{a}	14.15^{d}	25.31^{a}	147.02^{c}	45.89^{a}	38.10^{a}
			SE	0.53	0.09	0.17	0.25	0.11	1.23	0.12
	2ndweek	Control	Mean	11.16^{a}	2.46^{a}	12.28^{d}	30.23^{a}	144.34^{c}	44.66^{a}	33.24^{bc}
			SE	0.17	0.05	0.18	0.33	0.31	0.22	0.11
		1.31 ppm	Mean	10.34^{a}	2.40^{a}	18.24^{b}	24.66^{a}	147.74^{c}	39.69^{b}	31.59^{d}
			SE	0.45	0.07	0.55	0.08	0.63	0.48	0.28
		3.227 ppm	Mean	8.91^{a}	2.14^{a}	18.82^{b}	25.75^{a}	150.95^{b}	37.48^{c}	35.10^{ab}
			SE	0.23	0.05	0.42	0.84	0.51	0.39	0.21
	4th week	Control	Mean	11.21^{a}	2.43^{a}	14.17^{c}	29.30^{a}	147.29^{c}	45.98^{a}	33.36^{bc}
			SE	0.14	0.08	0.39	0.47	0.21	0.11	0.26
		1.31 ppm	Mean	9.84^{a}	2.26^{a}	18.50^{bc}	25.89^{a}	149.52^{b}	48.16^{a}	34.83^{b}
			SE	0.19	0.04	0.23	0.71	0.31	0.37	0.13
		3.227 ppm	Mean	7.77^{a}	2.05^{a}	18.57^{b}	21.91^{a}	152.65^{b}	40.30^{b}	35.63^{b}
			SE	0.18	0.12	0.45	0.61	1.11	0.30	0.36

Mean values followed by different letters in the rows are significantly different, $p<0.05$.

concentration varied from 64.62 to 83.24 mg/dl and 63.63 to 86.78 mg/dl in male and females respectively. Blood glucose values at 1, 2 and 4 weeks followed increase pattern in both sexes and most significantly increased value was observed in 1.31 ppm in female (86.78 mg/dl) compared with the control groups. Protein values among the test fishes varied from 2.30 to 3.05 gm/dl in male and 1.96 and 2.76 gm/dl in female. Cholesterol content of fish showed variations ranging from 191.89 to 220.07 mg/dl in male and 200.78 mg/dl and 232.03 mg/dl. Blood cholesterol level ($P<0.01$) increased significantly throughout study period in both sublethal concentrations.

Correlations among blood indices showed significant negative correlation between Hb-Cholesterol ($r=-0.49$, $p<0.01$), TEC-Cholesterol ($r=-0.495$, $p<0.01$) and TLC-Hct ($r=-0.547$, $p<0.01$) (Table 3). A positive correlation found between Hb-Hct ($r=0.678$, $p<0.01$). TLC-glucose ($r=0.655$, $p<0.01$), MCV-MCH ($r=0.523$, $p<0.01$). Significant ($p<0.01$) influence of pesticide on different blood parameters was seen the basis of gender, duration and dose (Table 4). There was significant ($p<0.01$) interaction between gender and duration observed in most parameters except Hb (ANOVA, $F=0.126$ Sig.$=0.882$), TEC, glucose and cholesterol (ANOVA, $F=0.611$, Sig.$=0.542$). Gender-Dose interaction also significant ($p<0.01$) except glucose (ANOVA, $F=0.220$ Sig.$=0.803$) and cholesterol (ANOVA, $F=0.320$ Sig.$=0.728$). Interactions were also significant ($p<0.01$) between duration and dose in most parameters except protein, cholesterol and Duration-Dose had also significant ($p<0.01$) influence in most parameters except (ANOVA, $F=0.230$ Sig.$=0.920$) (Table 5). Randomized Block Design, Three-way ANOVA showed significant ($p<0.01$) gender-dose-duration interaction for TLC, MCV, MCH, MCHC, glucose and cholesterol except Hb, TEC and protein (ANOVA, $F=0.618$, Sig.$=0.652$) (Table 6). ANOVA showed that the pesticide concentrations have more influence ($P<0.01$) than the

Table 2: Comparative evaluation of blood biochemical parameters of *A. testudineus* under different treatments of diazinon 60 EC

Gender	Duration	Dose		Glucose	Protein	Cholesterol
Male	1st Week	Control	Mean	64.95[b]	3.05[a]	195.27[b]
			SE	0.45	0.04	1.47
		1.31 ppm	Mean	71.18[a]	2.59[a]	210.94[ab]
			SE	0.37	0.13	4.48
		3.227 ppm	Mean	72.91[a]	2.43[a]	222.07[a]
			SE	0.51	0.06	5.34
	2nd Week	Control	Mean	67.62[a]	2.92[a]	193.47[b]
			SE	0.33	0.03	2.23
		1.31 ppm	Mean	75.24[a]	2.36[a]	205.43[ab]
			SE	0.54	0.12	4.26
		3.227 ppm	Mean	83.24[a]	2.36[a]	215.77[a]
			SE	1.57	0.03	7.13
	4th Week	Control	Mean	65.25[a]	2.92[a]	191.89[b]
			SE	0.33	0.02	0.73
		1.31 ppm	Mean	73.85[a]	2.53[a]	206.43[ab]
			SE	0.57	0.04	5.98
		3.227 ppm	Mean	73.24[a]	2.30[a]	214.30[ab]
			SE	0.49	0.05	6.54
Female	1st Week	Control	Mean	62.98[b]	2.48[a]	210.87[ab]
			SE	0.17	0.02	1.74
		1.31 ppm	Mean	69.60[a]	2.18[a]	226.48[a]
			SE	0.30	0.04	3.13
		3.227 ppm	Mean	71.94[a]	1.96[a]	231.63[a]
			SE	0.19	0.05	3.26
	2nd Week	Control	Mean	64.18[b]	2.44[a]	202.27[ab]
			SE	0.25	0.05	1.90
		1.31 ppm	Mean	73.78[a]	2.26[a]	216.60[a]
			SE	0.52	0.03	6.73
		3.227 ppm	Mean	76.84[a]	2.19[a]	221.20[a]
			SE	0.14	0.12	9.17
	4th Week	Control	Mean	63.63[b]	2.76[a]	200.78[a]
			SE	0.27	0.04	0.76
		1.31 ppm	Mean	72.28[a]	2.56[a]	226.47[a]
			SE	0.46	0.10	11.73
		3.227 ppm	Mean	77.44[a]	2.21[a]	232.03[a]
			SE	6.84	0.08	9.58

Mean values followed by different letters in the rows are significantly different, $p<0.05$.

Table 3: Correlation analysis of blood parameters of *A. testudineus*

Parameters	Hb	TEC	TLC	HCT	MCV	MCH	MCHC	Glucose	Protein	Cholesterol
Hb	1									
TEC	.689**	1								
TLC	-.307*	-.106	1							
HCT	.687**	.578**	-.547**	1						
MCV	-.027	.251	.671**	-.164	1					
MCH	-.158	-.180	-.152	-.017	-.302*	1				
MCHC	-.494**	-.217	.307*	-.387**	.523**	.235	1			
Glucose	-.326*	-.250	.655**	-.600**	.433**	-.251	.382**	1		
Protein	.454**	.517**	-.258	.743**	.100	.007	-.256	-.498**	1	
Cholesterol	-.490**	-.495**	.167	-.648**	-.121	.090	.269*	.426**	-.747**	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 4: Analysis of Variance of blood parameters of *A. testiudineus* on exposure to diazinon 60 EC toxicity

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Gender	Hb	12.098	1	12.098	59.645	.000**
	TEC	2.973	1	2.973	76.286	.000**
	TLC	56.222	1	56.222	105.166	.000**
	HCT	84.275	1	84.275	75.778	.000**
	MCV	1387.456	1	1387.456	2.394E3	.000**
	MCH	69.996	1	69.996	74.051	.000**
	MCHC	10.288	1	10.288	54.496	.000**
	Glucose	36.457	1	36.457	4.225	.047
	Protein	.963	1	.963	71.861	.000**
	Cholesterol	2119.387	1	2119.387	21.547	.000**
Duration	Hb	11.717	2	5.859	28.883	.000**
	TEC	.179	2	.090	2.299	.115
	TLC	215.152	2	107.576	201.225	.000**
	HCT	47.052	2	23.526	21.154	.000**
	MCV	230.438	2	115.219	198.780	.000**
	MCH	120.872	2	60.436	63.937	.000**
	MCHC	43.253	2	21.627	114.559	.000**
	Glucose	187.808	2	93.904	10.882	.000**
	Protein	.163	2	.082	6.087	.005**
	Cholesterol	457.573	2	228.786	2.326	.112
Dose	Hb	24.405	2	12.203	60.159	.000**
	TEC	1.691	2	.845	21.692	.000**
	TLC	251.652	2	125.826	235.362	.000**
	HCT	405.289	2	202.645	182.212	.000**
	MCV	357.182	2	178.591	308.112	.000**
	MCH	20.278	2	10.139	10.726	.000**
	MCHC	117.452	2	58.726	311.080	.000**
	Glucose	1185.498	2	592.749	68.689	.000**
	Protein	2.517	2	1.258	93.938	.000**
	Cholesterol	5309.805	2	2654.902	26.991	.000**

All significant values are designated with asterisks.

duration of exposure and gender in all cases.

Study based on the effect induced by sevin on haematological indices of *Clarias batrachus* showed reduction in the number of Hb, red blood cells, packed cell volume indicating anemia [26]. Significant reduction in erythrocytes and haemoglobin has been observed at sublethal concentrations as observed in this experiment is in agreement with [27,28] who reported a decrease in Hb, RBC count and Hct content in malathion exposed freshwater carp, *Cyprinus carpio*. The significant decrease in the Hb concentration may be due to either an increase in the rate at which the Hb is destroyed or to a decrease in the rate of Hb synthesis. Ambient-toxicants might have caused disintegration of RBC, which in turn has caused reduction of hemoglobin and hematocrit count. It is observed that erythrocyte and haemoglobin concentrations follow a direct and physiological interrelationship as remarked by Chatterjee and

Table 5: Evaluation of the variables (ANOVA) to determine whether blood parameters of *A. testiudineus* is dependent on gender, duration or dose

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Gender * Duration	Hb	.051	2	.025	.126	.882
	TEC	.036	2	.018	.467	.631
	TLC	6.006	2	3.003	5.617	.003**
	HCT	6.002	2	3.001	2.698	.081
	MCV	18.730	2	9.365	16.157	.000**
	MCH	9.710	2	4.855	5.136	.01**
	MCHC	7.077	2	3.538	18.744	.000**
	Glucose	37.996	2	18.998	2.202	.125
	Protein	.384	2	.192	14.334	.000**
	Cholesterol	120.248	2	60.124	.611	.548
Gender * Dose	Hb	2.108	2	1.054	5.196	.010
	TEC	.120	2	.060	1.543	.227
	TLC	11.382	2	5.691	10.645	.000**
	HCT	14.032	2	7.016	6.308	.002**
	MCV	23.804	2	11.902	20.534	.000**
	MCH	20.321	2	10.161	10.749	.000**
	MCHC	3.470	2	1.735	9.190	.001**
	Glucose	3.802	2	1.901	.220	.803
	Protein	.141	2	.071	5.279	.010
	Cholesterol	62.975	2	31.488	.320	.728
Duration* Dose	Hb	2.096	4	.524	2.584	.053
	TEC	.347	4	.087	2.229	.085
	TLC	111.733	4	27.933	52.250	.000**
	HCT	43.612	4	10.903	9.804	.000**
	MCV	91.283	4	22.821	39.371	.000**
	MCH	147.846	4	36.961	39.103	.000**
	MCHC	45.627	4	11.407	60.423	.000**
	Glucose	54.014	4	13.503	1.565	.205
	Protein	.112	4	.028	2.092	.102
	Cholesterol	90.521	4	22.630	.230	.920

All significant values are designated with asterisks.

Table 6: Three-way ANOVA (Randomized Block Design) evaluating the influence of gender, duration and dose on various blood indices of *A. testiudineus*

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Gender * Duration * Dose	Hb	1.510	4	.377	1.861^{NS}	.139
	TEC	.217	4	.054	1.393^{NS}	.256
	TLC	11.889	4	2.972	5.560**	.001**
	HCT	13.465	4	3.366	3.027^{NS}	.030
	MCV	47.402	4	11.851	20.445**	.000**
	MCH	65.780	4	16.445	17.398**	.000**
	MCHC	9.384	4	2.346	12.427**	.000**
	Glucose	139.070	4	74.767	4.029**	.003**
	Protein	.033	4	.008	.618^{NS}	.652
	Cholesterol	2001.911	4	500.478	5.088**	.000**

All significant values are designated with asterisks.

Ganguly, and Reddy and Bashamohideen [29,30]. Increase TLC perhaps a typical immune response of the fish against a toxic invasion and may be due to leukemia during which the number of WBC increase. This is in agreement with the findings of Sampat et al. [31] when they exposed the *O. niloticus* to a toxic environment. The toxicant induced severe physiological stress that possibly disturbed the neurohormonal axis leading to alteration in the leukocyte count. Similar findings in fishes exposed to environmental pollutants were also reported [32]. The decrease in Hct in fish

exposed to the pesticide was maximum in higher sublethal exposures may be due to decreased erythrocyte numbers. Increase MCH and MCHC also noted [33] following a short-term exposure of tench *(Tinca tinca)* to lead. In addition, increase in MCV, MCH and decreased MCHC values indicate that the anemia was of a macrocytic type. Similar result was observed in acute effect of diazinon on carp [13].

Exposure to different concentrations of pesticides caused an increase in the blood glucose levels, which could be attributed to differences in respiration, physiological activities and an imbalance between the hepatic output of glucose and the peripheral uptake of the sugar. It is also noted by previous workers [34] that stressful stimuli elicit rapid secretion of both glucocorticoid and catecholamines hormones from the adrenal tissue. The adrenergic effects may result in the increase of blood sugar within minutes after the onset of stress due to pesticides. This investigation showed decrease protein level when test fish exposed to sublethal concentrations in different exposure periods. Similar findings also noted by Das and Mukherjee [35]. The experiments conducted [36] *C. punctatus* exposed to sublethal concentrations of carbamate lead to decrease in blood proteins. Fall in protein level may be due to impaired function of kidney or due to reduced protein synthesis owing to liver cirrhosis [37]. The present work also supports the observations of Sastry and others [38] in this regard who opined that such an interference results in the depletion of total protein in the plasma of fish when exposed to quinalphos. Pesticide has also been induces blood cholesterol level, which might be utilized in the conversion of bile salt and in the synthesis of steroid hormone. Hypercholesterolemia may enhances denovo cholesterogenesis in liver, which is responsible for increased blood cholesterol [39,40].

CONCLUSIONS

Finally, it is concluded that the fish hematological parameters such as HB, RBC and MCHC are decreased showing anemia conditions. The plausible explanation of all these altered forms of blood cells could be attributed to the severity of "toxic stress" induced by diazinon, which can be due to abnormal erythropoiesis, inadequate haemoglobin formation, damage to the red cells after they leave bone marrow or increased erythropoiesis by bone marrow to compensate for anaemic conditions. Toxicants caused hypoproteinaemia, hypoglycemia and hypocholesterolemia. Further investigations for the quantitative and qualitative parameters are required to mark the erythrocytes as direct pollution indicators.

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REFERENCES

[1] Saeed, M., et al., *J Fish and Marine Sciences*, **2012**. 4(4): p. 369-375.

[2] Shankar, K.M., Kiran, B.R., Venkateshwarlu, M., *J Scientific Research*, **2013**. 1(1): 15-36.

[3] Bindubhaskaran, A.B., *Thesis submitted to Cochin University of Science And Technology, Cochin, India*, **2011**. p. 1-314.

[4] Satheeshkumar, K.P., et al., *Comparative Clinical Pathology*, **2011**. p. 204-210.

[5] Mukhopaday, P.K., Dehadrai, P.V., *Environmental Pollution*, **1980**. 22: p. 149-158.

[6] Pethiyagoda, R., *Fresh water fishes of Sri Lanka, Wildlife Heritage Trust of Sri Lanka*, **1991**. p. 1-362.

[7] Roberts, T.R., Hutson, D.H., *The Royal Society of Chemistry*, **1998**. p. 1-475.

[8] De Vlaming, V., et al., *Environmental Toxicology and Chemistry*, **2000**. 19: p. 42-62.

[9] Mansingh, A., Wilson, A., *Pollution Bulletin*, **1996.** 30: p. 640-645.

[10] Mulcahy, M.F., *J Fish Biology*, **1970**. 21: p. 203-209.

[11] Poston, H.A., *Fish Research Bulletin*, 1**966**. 29: p. 28-29.

[12] Pradhan, S.C., Patra, A.K., Pal, A., *J Applied Ichthyology*, **2013**. p. 1-7.

[13] Rauf, A., Arain, N., *Turkish Journal of Veterinary and Animal Sciences*, **2013**. 37: p. 535-540.

[14] Rahman, M.Z., et al., *Naga*, **2002.** 25(2): p. 8-12.

[15] A.P.H.A. (American Public Health Association), 20th edn. APHA, Washington, DC**, 1998.**

[16] Deanovic, BHC., et al., *Environmental Toxicology and Chemistry*, **2000**. 19: p. 82-87.

[17] Dacie, J.V., Lewis, S.M., Practical Haematology, 6th Edition, New York, United States, **1984.**

[18] Lavanya, S., et al., *Chemosphere*, **2011**. 7: p. 977-985.

[19] Pal, A., Parida, S.P., Swain, M.M., *Russian J Herpetology*, **2008**. 15 (2): p. 110-116.

[20] Oluyemi, K.G., Adecparusi, E.A., Olanrewage, J., *Research J Animal Sciences*, **2008**. 2: p. 17-21.

[21] Svobodova, Z., Pravoda, D., Palackova, J., *Research Unit of Fish Culture and Hydrobiology*, **1991**. p. 1- 3.

[22] Lowry, O.H., et al., *J Biological Chemistry*, **1951**. 193: p. 265-275.

[23] Pradhan, S.C., et al., *Comparative Clinical Pathology*, **2014**. 23(3): p. 509-518.

[24] Triander, P., *Annals of Clinical Biochemistry*, **1969**. 6: p. 24-27.

[25] Duncan, DB., *Biometrics*, **1955**. 11: 1- 42.

[26] Patnaik, L., Patra, A.K., *J Applied Science and Environmental Management***, 2006**. 3: p. 5-7.

[27] Ramesh, M., et al., *J Environmental Protection*, **1993**. 13: p. 124-127.

[28] Singh, N., Srivastava, A.K., *J for Nature Conservation*, **1991**. 3: 121-125.

[29] Chatterjee, S., Ganguly, S., *Environment and Ecology*, **1993**. 11: p. 889-891.

[30] Reddy, P.M., Bashamohideen, M., *Acta Hydrochimica et Hydrobiologica*, **1989**. 17: p. 101-107.

[31] Sampath, K., James, R., Akbarali, K.M., *Indian J Fisheries*, **1998**. 45: 129-139.

[32] Thakur, N., Sahani, S.; *Environment and Ecology*, **1993**. 11(4): p. 875-878.

[33] Shah, S.L., *J Applied Toxicology*, **2006**. 26(3): p. 223-228.

[34] Banerji, C.J., Oommen, A.V., *Indian Journal of Experimental Biology*, **2003**. 41: p. 242-247.

[35] Das, B.K., Mukherjee, S.C., *Comparative Biochemistry and Physiology - Part C*, **2003**. 134: p. 109-121.

[36] Sharma, S., Herborg, M.L., Therriault, T.T., *Diversity and Distributions*, **2009**. 15: p. 831-840.

[37] Kumari, A.S., Kumar, S.R.N., *J Zoology*, **1995**. 15: p. 124-126.

[38] Sastry, K.V., Siddiqui, A.A., Singh, S.K., *Chemosphere*, **1982**. p. 11-211.

[39] Gill, T.S., Pande, J., Tewari, H., *Biochemistry and Physiology*, **1990.** 36: p. 290-299.

[40] Koley, S.K., Kumar, R., *Aquacult*, **2012**. 13: p. 93-96.