Research Article

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Ameliorative effect of lycopene and vitamin e on some haematological and biochemical parameters of *oreochromis niloticus* against diazinon toxicity

Abstract

The present investigation aimed to determine the toxicological effects of diazinon (DZN) pesticide on hematological and biochemical parameters of the widely consumed *Oreochromis niloticus*. Adult specimens of *Oreochromis niloticus* were exposed to two sublethal concentrations (0.76 and 2.3mg/l) of DZN against the ameliorative effect of lycopene (10mg/kg) and vitamin E (50mg/kg) for 14 and 28 days. Empirical data of results obtained were subjected to statistical analysis of variance (ANOVA) to test the effects of DZN, lycopene (LYC), vitamin E (VE) and exposure periods. DZN significantly led to decline in red blood cell count (RBC's), blood haemoglobin (Hb), haematocrit value (Hct), white blood cell count (WBC's) and lymphocytes (LYM). While, the mean values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophils (NEU), monocytes (MON), eosinophils (EOS), serum protein (total protein, albumin and globulin), glucose, AST, ALT, ALP, urea and creatinine were significantly increased from the control values. Lycopene and vitamin E supplementation play appositive role in detoxification of DZN toxicity. The results suggest that DZN can negatively affect the hematology and physiology of fish. It was observed that supplementation of lycopene and vitamin E decreases the toxic effect of diazinon.

Keywords: fish, diazinon, lycopene, vitamin e, haematology, biochemistry

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Abbreviations: DZN, diazinon; ANOVA, analysis of variance; LYC, lycopene; VE, vitamin E; RBC, red blood cell; Hb, haemoglobin; Hct, haematocrit; WBC, white blood cell; LYM, lymphocytes; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; NEU, neutrophils; MON, monocytes; EOS, eosinophils; OPIs, organic phosphorus insecticides; DO, dissolved oxygen; BW, body weight; Ht, haematocrit; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase

Introduction

The exposure of fish to several types of chemical agents may induce changes in several haematological and physiological parameters, which are frequently used to evaluate fish health. Haematology has been widely used for the detection of physiological alterations following different stress conditions. Therefore, haematological techniques are the most common method to determine the sublethal effects of pollutants.[1,2] Insecticides Organic Phosphorus Insecticides (OPIs) are a major component of many pesticides with widespread use in both agricultural and domestic situations. However, approximately 85-90% of applied agricultural pesticides never reach target organisms, but disperse through the air, soil and water.[3] Diazinon (DZN) (O,O-diethyl-O-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate) is a contact OPIs with a broad range of insecticidal activity and widely used throughout the world with applications in agriculture and horticulture.[4] Various reports have been published with respect to DZN and its effects on biochemical and hematological parameters of fish.[5] The estimation of DZN free radical generations and the antioxidant defense has become an important aspect of investigation in mammals and animals especially with the natural antioxidants like carotenoids including beta-carotene and lycopene[6–8] and vitamin E (alpha-tocopherol).[7–9] These naturally occurring antioxidants play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors.[10,11] The antioxidant potential of these micronutrients could maintain the functional and structural integrity of important immune cells and in turn enhance immunity.[8,10–12] Therefore, the assessment of the relative antioxidant potency of vitamin E and carotenoids has received particular attention.[7,8,10,11,13] In fact, lycopene has a higher antioxidant potential than alpha-tocopherol and beta-carotene.[7] The mechanism ultimately responsible for the antioxidant property of carotenoids is still unknown.[14,15] The carotenoids are a family of fat-soluble pigments that are prevalent in numerous fruits and vegetables. Several studies have investigated the potential of carotenoids to ameliorate oxidative stress. Lycopene, which is a naturally occurring carotenoid that is present in tomatoes and tomato products, has attracted considerable attention as a potential chemo-preventive agent. Recently, lycopene has become a focus of interest because of its highly efficient antioxidant scavenging activity against singlet-oxygen and free radicals. Thus, lycopene may prevent oxidative damage, toxicity, and disease. Lycopene is one of the most effective antioxidants in the carotenoid family.[16–18] (Table 1).

According to Hassel,[19] biochemical changes occurs in fishes that are exposed to environmental contaminants, such changes which may include pesticides and their metabolites have necessitated a number of studies to determine their effects in aquatic environment on biochemical parameters in fish.[20] Several authors have investigated the effect of pesticide in fish.[5,21–24] The primary aim of this study was to evaluate the chronic toxicity of diazinon on the alterations in the haematological and biochemical parameters that are induced by DZN in the blood of *Oreochromis niloticus*.[7] The secondary objective was to determine the role of lycopene and Vitamin E in alleviating the negative effects of DZN.

Table 1 The fish groups exposed to Diazinon (DZN1=0.76, DZN2=2.3mg/L), Lycopene (LYC=10mg/kg) and vitamin E (50mg/kg BW) and their combinations

Group treatment	C	LYC	VE	LYC+VE	DZN1	DZN1+LYC	DZN1+VE	DZN1+LYC+VE	DZN2	DZN2+LYC	DZN2+VE	DZN2+LYC+VE
Diazinon (mg/L)	0	0	0	0	0.76	0.76	0.76	0.76	2.3	2.3	2.3	2.3
Lycopene (mg/kg)	0	10	0	10	0	10	0	10	0	10	0	10
Vitamin E (mg/kg)	0	0	50	50	0	0	50	50	0	0	50	50

C, control; LYC, lycopene; VE, vitamin E; DZN1, low diazinon dose; DZN2, high diazinon dose

Materials and methods

Sample collection and chemicals

One hundred and twenty healthy fishes of the Nile tilapia, *Oreochromis niloticus* (157.3±21.4g in weight, 22±1.72cm in length), were caught from the fish farm of faculty of Agriculture, Assiut University, Egypt. Fishes immediately were transported to the fish laboratory in the department of Forensic Medicine and Toxicology faculty of Veterinary Medicine, Assiut University. The experimental fishes were reared in aerated glass tanks (160L capacity) and acclimatized for 2weeks before being used in the experimental study. The experimental fish fed pellets at a rate of 3% of fish body weight twice daily. Faeces and residual food were aspirated regularly. The water temperature, pH and dissolved oxygen concentrations (DO) were measured daily (22.2±1.5C, 6.9±0.2pH and 6.5±1.03mg/L DO). Light cycle was 12h light and 12h dark. The insecticide Diazinon was supplied by Bayer Company for intermediate chemicals, Egypt. Lycopene ((EC) No 1272/2008) was purchased from Sigma-Aldrich Chemical (St Louis, MO, USA) and DL-α-tocopherol (VE) acetate were obtained from Merck (Germany).

Experimental design

Fishes were weighed, measured and classified randomly into 12 groups (10 fish/tank) according to doses of DZN, lycopene, vitamin E and their combinations. The diets (maize and soybean, 15 g/kg fish) were pelleted after addition of vitamin E and lycopene doses for the treated groups and the addition of suitable amounts of molasses and water. The diets were dried at room temperature and stored in small bags for fish feeding.

Stock solution (1,000ppm) of Diazinon (O,O-diethyl-O-[2–isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate) was prepared and stored in clean glass bottles and diluted to concentrations of (0.76 and 2.3mg/l). Such low sublethal diazinon concentrations (1/10 and 3/10 of 96h LC50) were chosen according to levels monitored by Soyingbe et al.[25] Diazinon doses were prepared and added constantly to the aquarium for 4weeks. The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish diazinon levels. Lycopene was added to the diet in concentration (10mg/kg BW). Dose response of lycopene was described previously by Ural.[26] Also, vitamin E (a-tocopherol) was supplemented in 50mg/kg BW (body weight). Such vitamin E concentration was chosen according to levels monitored by Ortuno et al.[27]

Blood analysis

Haematology

After 14 and 28day periods, blood samples of the control and treated fish (5 fish/treatment) were collected from caudal vein of the fish in a small plastic tubes containing heparin solution (0.2ml/ml blood) as anticoagulant. The red blood cell (RBC) and white blood cell (WBC) counts were performed using a haemocytometer and Natt et al.,[28] solution. The haemoglobin concentration (Hb) was determined with Drabkin's reagent read at 540nm,[29] and the haematocrit (Ht) was determined by a microhaematocrit centrifugation technique. The erythrocyte indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] were calculated by standard formulas with the data of the Ht, RBC and Hb.[30,31]

Biochemistry

For biochemical analysis blood samples were collected and left to coagulate for 15–20min at 4°C prior to centrifugation for 20min at 3,000rpm to separate serum. The fresh serum was subjected to biochemical analysis. Total protein, albumin and globulin (g/100ml) content contents were determined colourimetrically using assay kits supplied by Diamond Diagnostics, Egypt. Serum glucose (mg/L), urea (mg/l) and creatinine (mg/L) were determined, using assay kits supplied by (Spectrum Diagnostics, Egypt). Activities of aspartate aminotransferase (AST, U/I), alanine aminotransferase (ALT, U/I) and Alkaline Phosphatase (ALP, U/I) were determined kinetically using assay kits (Spectrum Diagnostics, Egypt). The samples were measured by spectrophotometer (Micro Lab 200 Vital Scientific-etherland).

Statistical analysis

The results are expressed as the mean±standard error. The data were analyzed for statistical significance between the control and experimental groups with an analysis of variance (one-way ANOVA) and Duncan's test using the SPSS 16 computer program (SPSS). P-values <0.05 were considered statistically significant.

Results

Fish showed abnormal behavior during the experimental period. At the start of the exposure, fish were alert, lost swimming coordination and buoyancy control with elevation of opercular beat rate, which increased with time. After some time they tried to avoid the toxic water by fast swimming and jumping. In tanks with DZN concentrations, the fish swam unsteadily with jerky movements and hyperactive excitability. There was no mortal fish recorded in aquaria with high dose of DZN.

Haematological parameters

The effects of DZN, lycopene, vitamin E and their combination on the haematological parameters after 14 and 28days periods of *Oreochromis niloticus* are given in Table 2. RBCs, Hb and Hct showed a significant decrease after diazinon treatments. The results showed that DZN, lycopene and vitamin E main effects were significant (P>0.05) in both periods for RBCs, Hb and Hct. In addition, lycopene–vitamin E interactions were highly significant in both periods. In addition, the main effects of DZN–LYC–VE interactions were only significant in the second period. The time of exposure main effect was highly significant (P>0.0001) for previous three parameters.

The normal value of mean corpuscular value (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of *O. niloticus* in the two periods are given in Table 2. Diazinon and lycopene main effects were highly significant (P>0.0001) in the two periods for previous parameters except MCV after 14days of low dose. The main effect of vitamin E was highly significant at the 28days period for MCV, MCH and MCHC. The time of exposure main effect was highly significant (P>0.0001). Diet supplementation with lycopene and/or vitamin E improved haematological parameters in comparison with the control.

The normal values of white blood cells (WBC) count, lymphocytes (LYM), monocytes (MONO), neutrophils (NEU), eosinophils (EOS) and basophils (BAS) of *Oreochromis niloticus* in both periods given in Table 3. Diazinon main effect, was highly significant (P<0.0001) in all exposure periods for the all previous parameters except BAS. Lycopene's main effect was significant in all exposure periods in the above parameters. Vitamin E's main effect was significant in all exposure periods for WBC, NEU, LYM and MONO. DZN-LYC-VE interaction was significant in WBC, LYM, NEU and MONO and not significant in BAS. The time of exposure main effect was highly significant for the previous parameters. LYC-VE-time of exposure interactions effect was significant in all parameters expect BAS. In the present work, lycopene and/or vitamin E improved the haematological parameters of *Oreochromis niloticus* exposed to DZN (Table 3).

Biochemical parameters

Proteins content: The total protein, albumin and globulin contents of *O. niloticus* for 14 and 28days periods are given in Table 4. Diazinon increases reflects highly significant decrease in the three previous parameters at both periods (P<0.0001). The main effect of LYC and/or vitamin E in the two periods was also highly significant (P>0.0001). No significant interactions between DZN, LYC and VE were recorded in the two periods. The time of exposure main effect was highly significant (P>0.0001). In the present study, diet supplementation with lycopene and/or vitamin E in both periods showed a marked elevation in the contents of total protein, albumin and globulin in DZN-exposed fish (P>0.0001). Lycopene beneficial effect in increasing total protein, albumin and globulin of DZN-treated fish superimposes the effect of vitamin E alone or in combination with LYC in comparison with the reduced DZN-induced parameters (Table 4).

Glucose level: The normal glucose level of *O. niloticus* for 14 and 28days, LYC-DZN, DZN- VE, VE and LYC 14days periods are given in Table 4. Diazinon main effect was significantly increased in both periods (P<0.0001). In addition, the main effects of DZN–LYC and DZN–VE interactions in the two periods were significant (P<0.0001). The time of exposure main effect was highly significant (P<0.0001). Diet supplementation with vitamin E and/or LYC improved DZN-induced hyperglycemia in comparison with the control.

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP): The present study, showed significant increase in AST, ALT and ALP activities in serum of DZN-exposed fish in comparison with the other groups (Table 5). Diazinon induced highly significant (P<0.001) increase in these parameters activities in the two periods. The main effects of LYC, VE and their interactions were highly significant (P<0.001) in the two periods. The time of exposure main effect was also highly significant (P<0.0001). The diet supplementation with VE and/or LYC to DZN-treated fish decreased significantly the activities of AST, ALT and ALP serum (P<0.0001) to the control level.

Urea and creatinine: Urea and creatinine results as kidney functions are presented in Table 5. The main effects of DZN, LYC, VE and their interactions were highly significant (P<0.0001) in the two periods. Similarly, the time of exposure main effect was highly significant (P<0.0001). Levels of urea and creatinine were significantly (P<0.0001) decreased in DZN-exposed fishes fed diets supplemented with VE and/or LYC. The increases of DZN showed a significant increase in previous parameters.

Discussion

In the present work, more or less nervous manifestation of DZN-treated fish in the form of jerky uncoordinated movement, fins stretching and scale loss symptoms was recorded. Changes in colour and loss of appetite were also observed for some fishes. Similar observations were recorded by Koprucu et al.,[32] Far et al.,[33] Banaee and Ahmadi,[34] Banaee et al.,[35] in fishes exposed to diazinon. Also, similar behavioral responses determined in this study have been observed with the guppy[36] freshwater catfish, *Heteropneustes fossilis*[37] and young mirror carp, *Cyprinus carpio*[38] exposed to various concentrations of the synthetic pyrethroids cypermethrin and deltamethrin. Diet supplementation with LYC and/or VE for 14 and 28days showed improvement in behavioral changes and the fishes were noticed in better conditions. Similar results for vitamin E and lycopene were observed by Mekkawy et al.,[7] Mekkawy et al.,[8] Ohaida,[9] Ural,[26] Soltan et al.[39]

The DZN-exposed fish showed a significant reduction in their RBCs, Hb and Hct in comparison with those exposed to DZN with supplementation of LYC and vitamin E. These results are in agreement with Banaee et al.,[23] Koprucu et al.,[32] who found a significant reduction in the RBCs, Hb and Hct in European catfish, *Silurus glanis* and common carp, *Cyprinus carpio*, respectively. The reduction in these parameters at sublethal levels of Diazinon might be due to the destruction of mature RBCs and the inhibition of erythrocyte production.[40,41] These DZN-induced decreases in haematological parameters may be also due to disequilibrium of the osmotic pressure inside and outside the blood cell.[42] A low RBCs or haemoglobin count indicates anemia, or severe bleeding. Low haemoglobin usually means the animal has anemia. Anemia results from conditions that decrease the number or size of red cells, such as excessive bleeding, a dietary deficiency, destruction of cells because of a transfusion reaction or mechanical heart valve, or abnormally formed haemoglobin.[43] Decreases in the number or size of red cells also decrease the amount of space they occupy, resulting in a lower haematocrit. A low haematocrit, combined with abnormal blood tests, confirms the diagnosis.[23] The decrease in the RBC count and the Ht and Hb levels may be due to the inhibition of erythropoiesis, haemosynthesis, or osmoregulatory dysfunction or due to an increased rate of erythrocyte destruction in the hematopoietic organ.[44]

Table 2 The basic data (N=5) of blood constituent parameters of Oreochromis niloticus exposed to Diazinon (DZN), Lycopene (LYC), Vitamin E (VE) and their combinations for 14 and 28days

RBC ($10^6/mm^3$)		Hb(g/100 ml)		Hct (%)		MCV (μ^3)		MCH (pg)		MCHC (g/dl)	
	14days	28days	14days	28days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
C	1.56±0.21^{E}	1.58±0.07^{F}	8.02±0.87^{F}	8.13±0.73^{E}	25.06±1.22^{E}	25.39±3.21^{F}	16.06±1.27^{AB}	16.07±1.04^{BC}	51.41±0.89^{A}	51.46±2.07^{B}	32.00±2.22^{A}	32.02±1.29^{A}
VE	1.64±0.13^{E}	1.82±0.17^{G}	8.26±0.37^{F}	8.89±0.36^{F}	25.78±2.07^{E}	27.67±2.11^{FG}	15.72±1.08^{A}	15.20±0.87^{B}	50.37±1.44^{A}	48.85±2.88^{A}	32.04±1.62^{A}	32.13±2.13^{A}
Lycopene	1.61±0.33^{E}	1.79±0.12^{G}	8.31±0.53^{F}	9.01±0.23^{F}	25.93±1.89^{E}	28.03±1.74^{G}	16.11±1.18^{AB}	15.66±0.46^{B}	51.61±4.74^{A}	50.34±1.98^{AB}	32.05±2.81^{A}	32.14±1.09^{A}
VE+LYC	1.64±0.12^{E}	1.81±0.23^{G}	8.22±0.63^{F}	9.12±0.61^{F}	25.66±2.01^{E}	28.36±1.58^{G}	15.65±1.05^{A}	15.67±0.39^{B}	50.12±2.64^{A}	50.39±4.26^{AB}	32.03±1.35^{A}	32.16±3.02^{A}
DZN	1.20±0.22^{C}	1.01±0.08^{D}	6.92±0.43^{D}	4.83±0.22^{B}	19.76±1.03^{C}	13.49±1.21^{B}	16.47±1.01^{AB}	13.36±0.84^{A}	57.67±2.52^{BC}	61.26±2.81^{C}	35.02±2.08^{C}	35.80±2.14^{C}
DZN+VE	1.31±0.09^{CD}	1.03±0.12^{D}	7.46±0.72^{E}	6.31±0.14^{C}	22.48±1.23^{D}	19.03±1.26^{D}	17.16±1.61^{B}	18.48±0.09^{D}	56.95±5.13^{B}	47.82±3.23^{A}	33.19±3.04^{B}	33.17±1.18^{B}
DZN+LYC	1.41±0.11^{D}	1.09±0.11^{D}	7.32±0.19^{E}	6.56±0.22^{CD}	22.06±1.11^{D}	19.78±1.21^{D}	15.65±1.05^{A}	18.15±1.02^{D}	51.91±3.48^{A}	60.18±2.73^{C}	33.18±2.17^{B}	33.16±1.32^{B}
DZN+VE+LYC	1.36±0.21^{CD}	1.17±0.12^{E}	7.55±0.24^{E}	7.00±0.45^{D}	22.75±1.34^{D}	21.1±2.07^{E}	16.73±1.07^{AB}	18.03±1.04^{D}	55.51±2.61^{B}	59.83±1.64^{C}	33.12±1.88^{B}	33.18±2.31^{B}
DZN2	0.84±0.15^{A}	0.52±0.09^{A}	5.42±0.37^{A}	3.24±0.09^{A}	15.26±0.98^{A}	10.72±0.78^{A}	18.17±1.18^{C}	16.77±0.58^{C}	64.52±1.66^{D}	75.40±6.92^{F}	35.55±2.66^{C}	37.16±3.08^{D}
DZN2+VE	0.94±0.08^{B}	0.63±0.07^{B}	5.89±0.21^{B}	4.75±0.52^{B}	17.57±1.04^{B}	14.15±0.87^{C}	18.69±1.33^{C}	22.46±0.49^{F}	62.66±3.17^{D}	62.31±3.79^{CD}	33.59±1.94^{B}	33.57±2.88^{B}
DZN2+LYC	0.98±0.04^{B}	0.65±0.08^{B}	5.84±0.42^{B}	4.62±0.31^{B}	17.42±1.17^{B}	13.76±1.07^{B}	17.78±1.23^{B}	21.17±0.96^{EF}	59.59±2.45^{C}	71.08±2.61^{E}	33.51±1.76^{B}	33.58±1.76^{B}
DZN2+VE+LYC	1.02±0.05^{B}	0.72±0.12^{C}	6.09±0.18^{C}	4.94±0.28^{B}	18.17±2.01^{BC}	14.72±1.22^{C}	17.81±1.42^{B}	20.44±0.59^{F}	59.71±4.92^{C}	68.61±1.87^{D}	33.52±0.93^{B}	33.56±2.08^{B}

The data are presented as Means±S.E. Different letters indicate significant difference at $p<0.05$ RBC, red blood cells; Hb, haemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration

Table 3 The basic data (N =5) of blood constituent parameters of Oreochromis niloticus exposed to Diazinon (DZN), Lycopene (LYC), Vitamin E (VE) and their combinations for 14 and 28days.

Treatments Parameters	WBC ($10^3/mm^3$)		Lymphocyte (%)		Monocytes (%)		Neutrophils (%)		Eosinophils (%)		Basophils (%)	
	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
C	27.45±2.67^{F}	27.46±3.03^{E}	57.66±3.91^{D}	55.32±3.73^{D}	1.62±0.07^{AB}	1.62±0.23^{A}	29.34±1.56^{A}	30.82±1.89^{A}	2.81±0.04^{B}	3.03±0.15^{A}	0.85±0.04^{A}	1.00±0.02^{B}
VE	28.65±1.78^{F}	27.19±2.07^{E}	56.88±1.23^{D}	55.84±3.11^{D}	1.52±0.05^{A}	1.63±0.41^{A}	28.89±2.13^{A}	29.49±2.19^{A}	2.74±0.08^{AB}	3.00±0.23^{A}	0.93±0.06^{B}	0.92±0.03^{B}
Lycopene	27.25±1.33^{F}	26.98±3.41^{E}	54.92±2.98^{D}	54.99±1.38^{D}	1.66±0.61^{AB}	1.57±0.12^{A}	29.03±1.32^{A}	31.12±2.06^{A}	2.59±0.11^{A}	2.83±0.13^{A}	0.86±0.06^{A}	0.94±0.02^{B}
VE+LYC	27.12±0.45^{F}	28.01±3.09^{E}	55.43±2.67^{D}	56.72±2.32^{D}	1.58±0.09^{A}	1.62±0.41^{A}	29.13±0.87^{A}	30.23±1.21^{A}	2.65±0.54^{AB}	2.91±0.31^{A}	0.87±0.11^{A}	0.91±0.05^{B}
DZN	20.49±3.85^{B}	10.59±2.89^{A}	48.82±4.23^{BC}	40.31±0.87^{B}	1.71±0.09^{B}	1.86±0.21^{C}	37.88±2.12^{C}	45.48±1.18^{D}	3.18±0.42^{D}	3.45±0.26^{C}	0.86±0.12^{A}	0.89±0.09^{AB}
DZN+VE	22.79±1.45^{C}	14.52±2.21^{BC}	52.42±2.89^{C}	46.34±3.76^{C}	1.68±0.05^{B}	1.74±0.42^{B}	34.42±1.31^{B}	43.27±2.98^{C}	2.83±0.07^{B}	3.12±0.41^{AB}	0.91±0.11^{B}	0.88±0.09^{AB}
DZN+LYC	23.89±2.76^{D}	15.91±1.63^{C}	51.63±3.43^{C}	46.21±4.98^{C}	1.63±0.31^{AB}	1.71±0.32^{AB}	33.94±3.92^{B}	40.18±4.01^{B}	2.85±0.18^{B}	3.10±0.45^{AB}	0.88±0.07^{AB}	0.84±0.05^{A}
DZN+VE+LYC	25.42±0.91^{E}	19.85±2.51^{D}	52.66±0.98^{C}	45.32±4.73^{C}	1.63±0.16^{AB}	1.70±0.33^{AB}	31.39±2.74^{A}	40.31±1.18^{B}	2.80±0.05^{B}	3.06±0.42^{AB}	0.89±0.08^{B}	0.86±0.08^{A}
DZN2	18.85±2.42^{A}	9.74±1.56^{A}	39.21±0.87^{A}	35.71±3.71^{A}	1.89±0.04^{D}	1.99±0.12^{D}	44.37±3.94^{E}	49.76±2.91^{E}	3.42±0.01^{E}	4.10±0.17^{D}	0.91±0.04^{B}	0.85±0.04^{A}
DZN2+VE	21.65±1.29^{BC}	13.79±1.95^{B}	43.36±0.69^{AB}	38.67±1.18^{AB}	1.80±0.08^{D}	1.95±0.18^{ED}	41.68±1.89^{D}	43.33±3.82^{C}	3.09±0.12^{CD}	3.61±0.35^{C}	0.83±0.05^{A}	0.88±0.12^{AB}
DZN2+LYC	22.27±0.58^{C}	14.79±0.72^{BC}	47.22±2.74^{B}	41.33±1.34^{B}	1.77±0.05^{C}	1.91±0.15^{D}	42.18±1.78^{E}	43.09±1.34^{C}	3.00±0.21^{C}	3.47±0.21^{C}	0.90±0.08^{B}	0.86±0.08^{A}
DZN2+VE+LYC	24.15±1.31^{D}	18.84±0.32^{D}	42.77±1.22^{B}	40.25±2.65^{B}	1.72±0.04^{C}	1.90±0.07^{D}	39.87±0.87^{CD}	41.32±2.12^{BC}	2.93±0.11^{C}	3.28±0.45^{B}	0.88±0.09^{B}	0.87±0.07^{A}

The data are presented as Means±S.E. different letters indicate significant difference at $p<0.05$ WBC, white blood cells; Lym, lymphocytes; Mono, monocytes; Neutro, neutrophils; Eos, eosenophils; Baso, basophils

Table 4 The basic data (N = 5) of blood constituent parameters of Oreochromis niloticus exposed to Diazinon (DZN), Lycopene (LYC), Vitamin E (VE) and their combinations for 14 and 28days

Treatments Parameters	TP (g/100 ml)		Albumin (g/100 ml)		Globulin (g/100 ml)		Glucose (mg/l)	
	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
C	3.71± 0.32^{F}	3.88±0.42^{E}	1.67±0.05^{F}	1.79±0.13^{E}	2.18±0.34^{E}	2.15±0.21^{E}	63.56±4.31^{AB}	54.06±4.08^{A}
VE	4.13±0.41^{G}	4.35±0.25^{F}	1.82±0.04^{G}	1.85±0.16^{EF}	2.42±0.27^{F}	2.41±0.19^{F}	58.79 ±3.83^{A}	59.24±2.63^{A}
Lycopene	4.04±0.01^{G}	4.24±0.17^{F}	1.76±0.12^{F}	1.91±0.15^{F}	2.24±0.09^{EF}	2.35±0.31^{E}	55.37 ±2.78^{A}	55.11±3.48^{A}
VE+LYC	3.76± 0.42^{F}	4.27± 0.18^{F}	1.55±0.43^{EF}	1.94±0.07^{F}	2.03±0.19^{E}	2.37±0.07^{EF}	53.87±2.72^{A}	52.82±5.18^{A}
DZN	2.41± 0.21^{B}	1.45±0.43^{A}	0.92±0.03^{A}	0.76±0.02^{A}	1.20±0.05^{B}	0.69±0.01^{A}	96.91± 3.85^{E}	120.90 ±7.86^{E}
DZN+VE	2.75±0.19^{C}	2.74±0.12^{C}	1.31±0.12^{D}	1.05±0.01^{BC}	1.44±0.08^{C}	1.37±0.08^{C}	75.38±6.78^{BC}	83.26 ±4.52^{B}
DZN+LYC	3.11±0.09^{E}	2.53±0.19^{BC}	1.43±0.14^{E}	1.13±0.07^{C}	1.72±0.12^{D}	1.59±0.08^{D}	70.03±5.35^{B}	79.02±2.51^{B}
DZN+VE+LYC	2.73± 0.01^{C}	2.43± 0.27^{B}	1.12±0.06^{B}	1.21±0.04^{D}	1.46±0.16^{C}	1.33±0.04^{C}	68.09± 2.43^{B}	80.71±3.38^{B}
DZN2	2.18±0.41^{A}	1.33±0.19^{A}	0.85±0.11^{A}	0.70±0.02^{A}	1.10±0.06^{A}	0.64±0.07^{A}	166.68±6.22^{F}	207.95±7.88^{F}
DZN2+VE	2.61±0.23^{BC}	2.52±0.15^{BC}	1.24±0.08^{C}	1.00±0.06^{B}	1.37±0.09^{C}	1.30±0.02^{BC}	86.68±9.18^{D}	104.29±4.92^{D}
DZN2+LYC	2.89±0.16^{D}	2.81±0.21^{D}	1.33±0.04^{D}	1.05±0.02^{BC}	1.60±0.13^{D}	1.54±0.11^{D}	80.53±6.71^{C}	97.87±2.84^{C}
DZN2+VE+LYC	2.49±0.34^{B}	2.40±0.17^{B}	1.06±0.09^{B}	1.00±0.03^{B}	1.38±0.17^{F}	1.26±0.21^{B}	78.30±5.82^{CD}	93.26±6.91^{C}

The data are presented as Means±S.E. Different letters indicate significant difference at $p<0.05$ Total Protein (TP), Albumin, Globulin and Glucose

Table 5 The basic data (N =5) of blood constituent parameters of Oreochromis niloticus exposed to Diazinon (DZN), Lycopene (LYC), Vitamin E (VE) and their combinations for 14 and 28days.

Treatments Parameters	AST (IU/l)		ALT (IU/l)		ALP		Urea		Creatinine	
	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days	14 days	28 days
C	78.45±0.40^{A}	73.08±0.75^{A}	32.12±0.11^{B}	30.88±0.43^{B}	25.66±1.36^{A}	27.23±0.93^{A}	45.68±3.76^{B}	46.04±0.75^{B}	0.30±0.03^{B}	0.30±0.43^{B}
VE	81.02±0.28^{A}	78.19±0.15^{A}	29.11±0.38^{B}	25.49±0.34^{A}	24.19±1.84^{A}	25.98±1.27^{A}	44.35±2.86^{B}	42.47±0.15^{A}	0.29±0.01^{AB}	0.28± 0.34^{AB}
Lycopene	77.09±0.67^{A}	75.30±0.31^{A}	24.01±0.72^{A}	27.95±0.29^{AB}	25.97±2.83^{A}	25.96±0.85^{A}	39.46±1.87^{A}	40.36± 0.31^{A}	0.27±0.01^{A}	0.26±0.29^{A}
VE+LYC	76.48±0.37^{A}	79.92± 0.31^{A}	26.36±0.43^{A}	25.2± 0.31^{A}	26.84±3.12^{A}	26.31±0.97^{A}	41.87±2.76^{AB}	43.74± 0.31^{AB}	0.26±0.02^{A}	0.27± 0.31^{A}
DZN	121.04±0.72^{D}	148.22±1.32^{D}	52.46±0.58^{E}	83.26±0.22^{G}	49.15±2.81^{D}	61.33±2.86^{C}	71.74±5.82^{F}	108.20±1.32^{F}	0.52±0.04^{F}	0.83±0.22^{F}
DZN+VE	95.08±0.98^{B}	104.79±0.12^{B}	39.73±0.51^{D}	48.70±0.51^{CD}	43.55±0.97^{C}	52.74±3.87^{B}	57.53±3.81^{D}	66.43± 0.12^{D}	0.39±0.06^{CD}	0.48±0.51^{D}
DZN+LYC	92.22±0.77^{B}	109.09±0.21^{B}	36.76±0.30^{C}	45.46±0.30^{C}	39.04±2.33^{B}	50.22±1.98^{B}	53.22±3.79^{C}	58.73± 0.21^{C}	0.36±0.02^{C}	0.45± 0.30^{CD}
DZN+VE+LYC	91.13±1.07^{B}	105.51±0.29^{B}	36.29±0.62^{C}	43.1±0.23^{C}	38.71±1.72^{B}	53.87±2.08^{B}	55.36±2.69^{CD}	59.12± 0.29^{C}	0.36±0.04^{C}	0.43± 0.23^{C}
DZN2	166.42±23.14^{E}	237.15±14.32^{E}	87.93±2.15^{F}	74.78±4.89^{F}	72.65±6.45^{F}	87.39±6.95^{E}	93.98±9.07^{G}	141.74±4.82^{F}	0.83±0.03^{G}	1.33±0.06^{G}
DZN2+VE	114.22±8.47^{C}	115.14±9.52^{C}	50.45±1.45^{E}	61.83±3.87^{E}	62.22±4.91^{E}	71.94±4.87^{D}	69.03±5.09^{F}	79.52±4.19^{E}	0.47±0.02^{E}	0.56±0.10^{E}
DZN2+LYC	111.80±9.75^{C}	120.95±2.76^{CD}	42.27±0.98^{D}	58.43±5.81^{E}	51.12±2.65^{CD}	75.44±2.89^{D}	61.21±4.74^{E}	76.39±2.17^{E}	0.42±0.04^{D}	0.60±0.03^{E}
DZN2+VE+LYC	116.29±1.65^{CD}	123.83±13.25^{CD}	41.73±2.66^{D}	50.04±2.33^{D}	55.09±3.72^{D}	59.36±3.74^{C}	66.99±2.89^{F}	74.61±3.31^{E}	0.42±0.01^{D}	0.59±0.07^{E}

The data are presented as Means±S.E. different letters indicate significant difference at $p<0.05$.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphates; urea and creatini

Mean corpuscular haemoglobin (MCH) measures the average amount of haemoglobin within a red cell. A similar measurement, mean corpuscular haemoglobin concentration (MCHC), expresses the average concentration of haemoglobin in the red blood cells. In contrary, values of MCV, MCH and MCHC registered in during exposure to diazinon based pesticide in 60 and 120µg/L concentrations to common carp were comparable with the control group.[23] Alteration in values of MCV, MCH and MCHC in *Cyprinus carpio* was reported.[45] Similar findings were recorded by Koprucu et al.[32] Adeyemo[46] under the influence of DZN stress in different fish species. These chemicals-induced alterations in MCV, MCH and MCHC were attributed to direct or feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress-related release of RBCs from the spleen and hypoxia.[47,48]

Leucocytes play an important role in the nonspecific or innate immunity, and the leukocyte count/activity can indicate the health status of a fish.[49] The present study indicates that the WBC count and lymphocytes were significantly decreased. However, monocytes, neutrophils and eosinophils were significantly increased in the groups that were exposed to DZN. Simillar results were obtained by Far et al. and Koprucu et al.,[32,33] in fishes that exposed to diazinon. Banaee et al.,[23] observed significant decrease of leucocyte count of common carp in during exposure to sub-lethal concentration of diazinon. Perhaps these changes have direct toxic effects on the kidney and spleen (hematopoietic tissue). Probable cause of neutrophilia can be induced by the phagocytic cells in host defense.[20,33,50] Therefore, the results of this study and other studies have showed that diazinon can weaken the immune system in nonspecific salmon. This could be due to the destruction of hematopoietic tissue and decrease in non-specific immune system due to increased concentrations of defensive poison diazinon.[33] However, the haematological values of the groups treated with lycopene and/or vitamin E were near to the control group and significantly different from the groups that were exposed to only DZN. The findings of our study suggest that lycopene and/or Vitamin E might be helpful in reducing the harmful effect of DZN by maintaining optimum haematological values. Similar observations were noted by Mekkawy et al.,[7] Mekkawy et al.,[8] Ural.[26]

Decrease in serum total protein level (hypoproteinemia), albumin and globulin were recorded in DZN-treated *O. niloticus*. Such decrease of total protein may be due to destruction of protein-synthesizing subcellular structures and inhibition of hepatic synthesis of blood protein.[51] Loss of protein from damaged kidney could contribute further to the observed hypoproteinemia.[52] Decreased total protein levels may be due to starvation, malnutrition and chronic liver diseases.[34] Other authors also found that the levels of total protein and albumin are decreased in the fish exposed to different pollutants and insecticides.[53,54] Decreased globulin levels have been reported in immune deficiency. In fact, the effect of diazinon on the immune system of tilapia (*Oreochromis niloticus*) and beluga sturgeon (*Huso huso*) as immunomodulator has been studied by other authors.[55,56] Diazinon-induced tissue destruction and hepatocyte apoptosis might be the most important agent responsible for reducing the synthesis of total protein, albumin and immunoglobulin by the liver.[56]

Decreased globulin levels have been reported in immune deficiency. In fact, the effect of diazinon on the immune system of tilapia (*Oreochromis niloticus*) and beluga sturgeon (*Huso huso*) as immune-modulator has been studied by other authors Khoshbavar-Rostami et al.[55] Significant decrease in globulins levels in plasma of fish exposed to diazinon could be due to a disruption in protein biosynthesis.[34] In the other word, diazinon induced tissue destruction and hepatocyte apoptosis might be the most important agent responsible of reducing the synthesis of total protein, albumin and immunoglobulin by the liver.[34]

In the present study, diet supplementation with lycopene and/or vitamin E for 14 and 28days led to a marked elevation in total protein, albumin and globulin of DZN-exposed fish. Elkomy et al.,[57] observed an increase in the total protein level after stress (thioacetamide) in male rat fed lycopene as supplemented diets. These findings emphasize on the antioxidant role of lycopene.[58] On the other hand, Kalender et al.,[59] Ogur et al.,[60] recorded an increase in total protein level after diazinon-induced and nitrate-induced stress in male rats fed vitamin E as supplemented diets.

The significant differences in glucose concentrations in plasma between the control and treatment fish, following the action of different insecticides, may be considered to be the manifestation of stress.[34] DZN-induced hyperglycemia was revealed in *O. niloticus*. The source of such hyperglycemia seems to be due to the liver glycogenolysis, resulting from the increased plasma catecholamines and corticosteroid hormones as emphasized by Mazeaud et al.[61] Pickering[62] as well as amino acids through the activation of gluconeogenesis process.[63] In agreement with our results, Ceron et al.,[64] report significant glucose increase in common eel (*Anguilla anguilla*) following a 96h action of sub-lethal concentrations of diazinon. Increases in blood glucose levels have been reported in *Heteropneustes fossilis*[65] and *Cyprinus carpio*,[23] O. mykiss[66] after exposure to cypermethrin and diazinon, respectively. Supplementation with vitamin E and/or LYC treated DZN-induced hyperglycemia in comparison with control. Martins et al.,[67] reported a reduced blood glucose level after stress (heat shock proteins) in Atlantic halibut (*Hippoglossus hippoglossus*L.) fed vitamin E supplemented diets.

The liver is the primary organ involved in xenobiotics metabolism and is a major target organ for chemicals and drugs. Hepatotoxicity is therefore an important endpoint in the evaluation of the effect of particular xenobiotics. Our results showed that DZN induced significant increase in the activities of AST, ALT and ALP as compared to other groups. ALP, ALT and AST are important indicators of liver damage in clinic findings. These enzymes were secreted into the blood in hepatocellular injury and their levels increase. Changes in these enzyme levels might differ dependent on exposure time and dose. As a result, liver enzymes including ALP, AST and ALT are released into plasma. In this sense, if the cellular injury is chronic AST and ALT levels will remain elevated.[68,69] Increased activities of AST and ALT were observed in plasma of Channa punctatus[70] exposed to organophosphorus pesticides. Banaee et al.,[23] have reported increased levels of AST and ALT followed by the exposure of common carp to diazinon. The current DZN-induced increase in ALP, AST and ALT activities showed similar results as reported by Ahmed et al.,[71] who found elevation of transaminases (AST, ALT) in rats treated with 1/30 LD_{50} diazinon for 3weeks. Kalender et al.,[59] recorded an elevation in ALT, AST and ALP levels in rats treated with diazinon. Transaminases were considered to be a more sensitive measure in evaluating liver function and damage.[72] The diet supplementation with Vitamin E and/or LYC to DZN-treated fish diet led to a significant decreases in the activity of ALP, AST and ALT serum. These results are in agreement with those of Elkomy et al.,[57] who observed decrease in the activity of ALT, AST and ALP levels of thioacetamide-treated male rat fed tomato-juice (source of lycopene) as supplemented diet. Mekkawy et al.,[7] Kalender et al.,[59] Ogur et al.,[60] also recorded decrease in activity of AST and ALT in male rats fed lycopene and vitamin E as supplemented diets.

The noticed increase in the levels of aminotransferase (ALT and AST) and the level of ALP as well as the decrease in the levels of total protein and albumin in the serum, are the major diagnostic symptoms of liver diseases.[73] Previous studies[74–76] showed that OPIs (e.g., diazinon) caused an increase in activities of ALP, ALT and AST enzymes.

Our results showed that DZN induced significant increase in urea and creatinine concentrations. Simillar results were obtained by El-Shenawy et al.,[77] Hariri et al.,[78] who found elevation of urea and creatinine in rats treated with diazinon. Also, he reported the ability of Vitamin E to decrease these parameters. Al-Attar[79] confirmed the ability of lycopene to decrease serum urea and creatinine after exposing rats to DZN. These results indicated that diazinon metabolites caused toxicity in renal system; and the immune system makes a good role for defending against foreign particles. The effect of diazinon on kidney was studied in different animals. Oral administration of diazinon for 2months to male albino rats showed degeneration of the renal tubules.[80] El-Shenawy et al.,[77] reported that exposing mice to diazinon caused degeneration of renal tubules, atrophy of glomeruli and interstitial inflammatory cells infiltrations. Jyothi et al.,[81] reported the increase in urea and creatinine in the catfish, *Clarias batrachus* exposed to sublethal concentration of two pesticides, carbaryl, a carbamate and phorate, an organophosphorus insecticide for 7days. The present results revealed also a significant increase in serum creatinine and urea in response to diazinon toxicity. Diazinon (1/30 LD_{50}) markedly decreased serum urea but did not affect creatinine level.[71] The increase in creatinine recorded in this work might be due to impaired kidney function by the used DZN. This view was supported by Kluwe[82] who indicated that an elevation of creatinine level in the blood is an indicative of impaired kidney function.

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Conflict of interet

The author declares no conflict of interest.

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