

SUBACUTE INTOXICATION OF A DIAZINONE60® IN MONOSEX NILE TILAPIA, OREOCHROMIS NILOTICUS L. AND ITS RELATION TO ECONOMIC LOSSES

Talaat Talaat Saad¹, Zinab khameis el-Meadawy² and Atallah ST³

1- Departments of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt

2- Departments of pharmacology, Faculty of Veterinary Medicine, Alexandria University, Egypt

3- Departments of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Egypt

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ABSTRACT: The monosex Nile tilapia, *Oreochromis niloticus* L., was exposed to subacute concentration (1.46µg/l) of an organophosphorus compounds insecticide, Diazinone60 for 28 consecutive days. The mortality incidence percent due to Diazinone60 toxicity was 0 %, 25 %, 33.34 % and 41.66 % for the periods at 7-Day, 14-Days, 21-Days and 28-Days, respectively, with overall mortality rate 100 %. While, the return losses for each /100 fish was 0 , 18 , 18 and 54 LE/ 100 fish for the periods at 7-Day, 14-Days, 21-Days and 28-Days, respectively, with overall losses about 90 LE / 100 fish. Behavioural, clinical, haematological, serum biochemical and histopathological consequences were assayed at a regular interval of 7 days. The abnormal behavioural responses and toxic symptoms were described. Exposure to Diazinone60 not only significantly decreased lymphocyte and basophile percentages, total leucocytic and total erythrocytic counts, haemoglobin percentage and packed cell volume value, but also caused serious effects in the form of hypoproteinaemia, hypoalbuminaemia, hypercholesterolaemia, hyperglycaemia and significantly increased serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities. Moreover, the histopathological results indicated that the haemobiotic organs were affected by Diazinone60, primarily liver and gills. Our data suggest that subacute exposure to Diazinone60 exerts a serious metabolic distress on the fish corresponding to the exposure period. In addition, the assayed parameters and histopathological findings can be as good biomarkers of organophosphorus compounds ecosystem pollution.

INTRODUCTION

Synthetic organophosphorus compounds have become good substitutes for organochlorine, organophosphate and carbamate insecticides due to their properties of high bioefficacy, biodegradable and comparatively lower toxicity to mammals and birds ([Kamlaveni et al., 2001](#); [Parvez and Raisuddin, 2006](#)). They are also considered as relatively non-persistent, and are therefore not expected to biomagnify through food chain. Maximum bioconcentration factors ranged from 698 X (Diazinone60) to 6090 X (bifenthrin) for whole fish ([EPA, 1999](#)). As a consequence of these beneficial qualities, organophosphorus compounds have attracted farmers and health departments to use them in pest control in households. Nevertheless, agriculture and veterinary practice leads to the exposure to their possible toxic effects of manufacturing workers, field applicators, the ecosystem and finally the public ([Smith and Stratton, 1986](#)). Organophosphorus compounds have been shown to be up to 1000 times more toxic to fish than mammals and birds at comparable concentrations ([Köprücü and Aydn, 2004](#)). Subacute toxicity data are scarce for organophosphorus compounds and aquatic organisms. They have been found to be extremely toxic to fish, Diazinone60, a synthetic type II organophosphorus compounds insecticide being one of the most toxic. Due to their lipophilicity, organophosphorus compounds have a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of the fish to aqueous organophosphorus compounds exposures. Fish seem to be deficient in the enzyme system that hydrolyzes organophosphorus compounds ([Viran et al., 2003](#)). Therefore, subacute effects of organophosphorus compounds to fish incorporate behavioral changes and tissue damage ([Smith and Stratton, 1986](#)). Generally, Diazinone60 affects the overall physiological profile in fish with particular reference to the energy metabolism, biochemical and hematological characteristics ([Kumar et al., 1999](#); [El-Sayed et al., 2007](#)). The mechanism of its neurotoxic effectiveness is the same

Corresponding Author: Talaat Talaat Saad, Departments of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt. E-mail: talaat_fish@yahoo.com

as that of other type II organophosphorus compounds: blockage of the sodium channels and inhibition of γ -aminobutyric acid receptors. Effects of Diazinone60 on nervous, respiratory and hematological systems have been reported ([Golow and Godzi, 1994](#)). Possible toxicological actions of the organophosphorus compounds include disruption of calcium and phosphate homeostasis ([Srivastav et al., 1997](#)) and abnormalities in hematopoiesis and protein synthesis ([Svobodova et al., 2003](#)). The present study aimed to evaluate the ecotoxicological hazards of Diazinone60-based preparations through assessment of its subacute intoxication consequences, on the basis of the clinical picture, blood profile and blood biochemical biomarkers of toxicity on monosex Nile tilapia. These fish occupy an important status because they can be acclimatized easily in our country due to increased marine area and they serve as stable food for the vast population. In addition, to better understand the effects of some observed changes, fish specimens had been examined histopathologically.

MATERIALS AND METHODS

Fish and aquaria

A total number (80) of apparently healthy adult Nile tilapia (*Oreochromis niloticus* L.), (monosex phenotype), were obtained from the Barseek Fish Farm, Bohera Governorate. Average weights and age of the fish were 90 ± 5 g and 4 months old, respectively. Prior to the experiment, the fish were acclimatized for 15 days in four glass aquaria (90 60 50 cm/each) filled with dechlorinated tap water under laboratory conditions (natural photoperiod 11.58–12.38 hour and temperature $25.8 \pm 1.8^\circ\text{C}$). Continuous aeration was maintained in each aquarium using an electric air pump. The fish were fed daily on commercial feed containing 25% crude protein, provided daily at 3% of body weight. Dissolved oxygen, pH and electric conductivity of the tap water used in the experiment were determined by Hack Method (Sigma Laboratory) according to the [World Health Organization \(2001\)](#). The mean values for test water qualities were as follows: dissolved oxygen 6.5 mg/l, pH 7.1, electrical conductivity 219 ± 2 $\mu\text{mho/cm}$, temperature $29 \pm 2^\circ\text{C}$, alkalinity 124 mg/l, hardness 150 mg/l as CaCO_3 and no free chlorine.

Test chemical

A stock solution of Diazinone60 (Intervet Co., France) in a volumetric flask using the diluent medium was applied. Necessary dilution of the stock solution was made and described below.

Subacute toxicity procedures

The acute 96 hr LC₅₀ value of Diazinone 60® for monosex Nile tilapia was determined in our laboratory using static method bioassay and was found to be 14.6 $\mu\text{g/l}$ ([El-Sayed et al., 2007](#)). In the present study, effects of 28 days exposure of 1.46 $\mu\text{g/l}$ Diazinone60 (one-tenth of the 96 hr LC₅₀ value) to Nile tilapia were tested in a static renewal bioassay. The bioassays were carried out in glass aquaria each holding 200 l of water and 20 fish. A duplicate set of aquaria were exposed to Diazinone60 and control thereby exposing 80 fish altogether. The required concentration was maintained and renewed every 4 days by siphoning of entire aquarium water in the reservoir and replaced by the same volume of treated water. At days 7, 14, 21 and 28 of the experiment, eight fish from both control and Diazinone60- based organophosphorus compounds-exposed groups were taken for hematological, biochemical and Histopathological examinations. Mortalities, behavioral changes and clinical toxic signs of tested fish were closely followed up and recorded throughout the entire experimental period.

Haematological profiles

The body surface of monosex Nile tilapia was cleaned and blotted dry with adsorbent paper. The blood samples were collected from the caudal artery using disposable tuberculin syringe for estimation of total erythrocytic count (TEC), total leucocytic count (TLC) and package cell volume (PCV) content according to [Stoskopf, \(1993\)](#). Haemoglobin percentage (Hb%) was assessed according to [Drubkin, \(1946\)](#) and differential leucocytic count was determined according to [Lucky, \(1977\)](#) and [Schalm, \(1986\)](#). Further- more, blood was collected without anticoagulants for serum separation for clinical-biochemical determinations.

Biochemical blood serum profiles

Using commercially available kits by Pasteur Laboratory, the activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were estimated as directed by [Reitman and Frankel, \(1957\)](#) and [Kid and King, \(1954\)](#), respectively. Furthermore, serum total protein, albumin, globulin, glucose and cholesterol content were determined spectrophotometrically as indicated by the methods of [Doumas et al., \(1981\)](#); [Reinhold, \(1953\)](#); [Coles, \(1974\)](#); [Safinaz, \(2001\)](#); [Trinder, \(1969\)](#) and [Schettler et al., \(1975\)](#) respectively.

Histopathological examination of tissues

Afterwards, fresh specimens of liver, gills, kidneys and spleen were collected from both control and Diazinone60-exposed fish in ice-cold condition for Histopathological studies. The specimens were rapidly fixed in 10% neutral buffered formalin for at least 24 hr. The fixed specimens were processed through the conventional paraffin embedding technique. From the prepared paraffin blocks, 5- μ m thick sections were obtained. These sections were stained by haematoxylin and eosin according to the method described by [Culling, \(1983\)](#).

Economic losses

The economic losses of the fish due to exposure to Diazinone60 were determined from dead fish, weight of dead fish and the losses in return due to dead fish according to the following equations ([Atallah and El-Banna, 2005](#)).

a- Weight of dead fish = Number of dead fish X Average weight of the fish (gm).

b- Losses in returns (L.E) = Weight of the fish (Kg) X Price of Kg fish (L.E).

Statistical analysis

The hematological and biochemical data was statistically analyzed for the effects of groups (control versus Diazinone60- exposed) and days using two-way ANOVA that was run using the computer package of the [Statistical Analysis System, \(2001\)](#). Means were separated using least square means of the same program.

RESULTS

Behavioral changes and clinical toxic symptoms

The behavioral responses of the tested fish were recorded daily. Normal behavior with no mortality was recorded for fish of the control group throughout the entire experimental period and for Diazinone60-exposed fish during the first 2 weeks. After that, the intoxicated fish exhibited abnormal behavioral changes in the form of rapid operculum movement, swimming at the water surface and gasping from the water as a respiratory distress. Later, they showed signs of nervous manifestations like less activity, laying down on their sides or remained hanging vertically in the water and occasionally remaining motionless on the aquarium bottom until death. Mortality incidence percent was 0 %, 25 %, 33.34 % and 41.66 % for the periods at 7-Day, 14-Days, 21-Days and 28-Days, respectively, with overall mortality rate 100 % (Table, 1). The return losses for each /100 fish was 0 , 18 , 18 and 54 LE/ 100 fish for the periods at 7-Day, 14-Days, 21-Days and 28-Days, respectively, with overall losses about 90 LE / 100 fish (Table, 1). In animals exposed to Diazinone60, the clinically observed toxic signs were color darkening of the body surface, slight erosions and/or rotting of fins and tail, slimness, general loss of fish scales, eye cataract and sometimes exophthalmia. In addition, there were congestion of anal opening, anemia (enlarged head with small body) and malnutrition. At the same time, the post-mortem signs included general congestion of the liver, kidneys and gills in the first week extended to paleness of body organs by the end of the experiment.

Hematological findings

The results of the differential leucocytic count, erythrocyte profile and leucocyte profile of the control and Diazinone60- exposed monosex Nile tilapia under the study are given in table 1. In relation to the control specimens, all fish exposed to the toxicant had significant ($P < 0.05$) lower lymphocyte, basophile and TLC percentages at all weeks, as well as TEC and Hb percentages, but after the first week and PCV content at the last week of exposure. Even Diazinone60 significantly ($P < 0.05$) increased neutrophile percentage. Monocyte and eosinophile values were comparable. Concerning the effects change over time of Diazinone60 exposure, only Hb% and PCV value were significantly ($P < 0.05$) decreased at the last week; however, the other parameters were comparable at all weeks.

Table 1: Mortality in both control and 1.46 µg/l of Diazinone60-exposed monosex *Oreochromis niloticus* throughout 28 days exposure (n = 100).

	Control Number	Number of fish at each week in Diazinone60 toxicated fish	Mortality due to Diazinone60 1.46	Mortality %	Weight losses (gm)/40 fish	Return losses/40 fish/LE	Return losses/100 fish/LE
7-Days	40	40	0	0	-	-	-
14-Days	40	32	8	25	720	7.20	18
21-Days	40	24	8	33.34	720	7.20	18
28-Days	40	0	24	41.66	2160	21.60	54
Total Mortality	-	-	40	100	4500	36.00	90

Price of Kg fish in whole sale market = 10 LE

Table 2: Changes in the blood profiles in both control and 1.46 µg/l of Diazinone60-exposed monosex *Oreochromis niloticus* throughout 28 days exposure (n = 100).

Parameter	7 days		14 days		21 days		28 days	
	Control	Diazinone60 1.46						
Number	40	40	40	32	40	24	40	14
Lymphocyte (%)	51 ± 0.58*	43 ± 1.00†	51 ± 0.58*	44 ± 0.58†	50.5 ± 1.20*	45.6 ± 1.20†	50.0 ± 1.15*	44.7 ± 1.45†
Monocyte (%)	1.30 ± 0.3	1.00 ± 0.1†	2.00 ± 0.1	0.97 ± 0.3†	2.00 ± 0.1	1.70 ± 0.3†	2.10 ± 0.3	1.33 ± 0.3†
Basophil (%)	8.3 ± 0.30*	6.0 ± 0.20†	8.0 ± 0.30*	7.0 ± 0.40†	8.2 ± 0.58*	6.3 ± 0.30†	9.0 ± 0.58*	7.0 ± 0.20†
Eosinophil (%)	9.0 ± 0.80	9.0 ± 1.00†	8.0 ± 0.58	8.0 ± 0.58†	9.0 ± 0.58	9.3 ± 0.88†	8.0 ± 0.58	9.3 ± 0.88†
Neutrophil (%)	30.3 ± 0.67	41.0 ± 2.00*†	31.0 ± 0.58	40.3 ± 0.67*†	30.3 ± 1.20	37.0 ± 2.20*†	29.9 ± 0.33	37.7 ± 0.58*†
Total leucocyte count (TLC) (10 ³ /mm ³)	22.0 ± 0.58*	19.5 ± 0.50†	22.0 ± 0.58*	20.0 ± 0.58†	22.0 ± 0.20*	20.3 ± 0.33†	21.0 ± 0.58*	19.3 ± 0.33†
Total erythrocyte count (TEC) (10 ⁶ /mm ³)	1.77 ± 0.07	1.55 ± 0.05†	1.70 ± 0.06*	1.40 ± 0.06†	1.70 ± 0.06*	1.30 ± 0.06†	1.80 ± 0.06*	1.40 ± 0.17†
Haemoglobin (g %)	8.3 ± 0.33	7.3 ± 0.88†	7.7 ± 0.33*	6.0 ± 0.60†‡	8.0 ± 0.58*	6.0 ± 0.58†‡	8.3 ± 0.33*	5.0 ± 0.58‡
Packaged cell volume (PCV) (%)	24.0 ± 0.58	25.0 ± 1.53†	24.3 ± 0.88	23.5 ± 0.50†‡	23.0 ± 0.58	21.7 ± 1.45†‡	24.3 ± 0.67*	18.0 ± 1.73‡

*Means of control and Diazinone60-exposed fish within various blood profiles within days having star superscript are significantly different (P < 0.05).

†,‡ Means of Diazinone60-exposed fish within various serum blood profiles having different letters are significantly different (P < 0.05).

Table 3: Changes in the serum biochemical profiles in both control and 1.46 µg/l of Diazinone60-exposed monosex *Oreochromis niloticus* L throughout 28 days exposure (n = 100).

Parameter	7 days		14 days		21 days		28 days	
	Control	Diazinone60 1.46	Control	Diazinone60 1.46	Control	Diazinone60 1.46	Control	Diazinone60 1.46
Number	40	40	40	32	40	24	40	14
AST (IU/dl)	56.5 ± 0.58	66.0 ± 1.00*†	59.4 ± 0.58	67.0 ± 0.58*†	56.7 ± 0.88	64.7 ± 0.33*†	56.7 ± 0.67	63.0 ± 0.20*†
ALT (IU/dl)	56.0 ± 0.58	61.5 ± 1.50*†	56.3 ± 0.88	60.3 ± 1.20*†	57.0 ± 0.58	61.0 ± 0.58*†	56.0 ± 1.00	59.3 ± 0.33*†
ALP (IU/dl)	7.3 ± 0.33	8.5 ± 0.50†	8.0 ± 0.10	8.7 ± 1.20†	8.0 ± 0.58	9.33 ± 1.86*†	8.0 ± 0.58	11.3 ± 0.33*†
Albumin (g/dl)	3.70 ± 0.06	3.55 ± 0.15†	3.77 ± 0.07*	3.03 ± 0.07‡	3.60 ± 0.12*	2.27 ± 0.09§	3.67 ± 0.09*	2.50 ± 0.21§
Globulin (g/dl)	2.0 ± 0.32	1.80 ± 0.10†	1.83 ± 0.09*	1.17 ± 0.09†	1.97 ± 0.20	1.63 ± 0.13†	2.27 ± 0.17*	1.33 ± 0.20†
Total protein (g/dl)	5.7 ± 0.31	5.35 ± 0.25†	5.60 ± 0.06*	4.20 ± 0.15‡	5.57 ± 0.32*	3.90 ± 0.06‡	5.93 ± 0.09*	3.83 ± 0.15‡
Glucose (mg/dl)	71.3 ± 0.88	77.7 ± 0.88*‡	70.7 ± 0.33	77.0 ± 1.50*‡	70.3 ± 0.67	78.7 ± 1.33*‡	64.3 ± 1.20	82.5 ± 0.50*†
Cholesterol (mg/dl)	194.7 ± 2.7	208.5 ± 0.5*†	197.0 ± 3.5	202.7 ± 0.9*†	198.7 ± 1.9	203.3 ± 0.3*†	190.0 ± 2.5	201 ± 1.2*†

*Means of control and Diazinone60-exposed fish within various biochemical profiles within days having star superscript are significantly different (P < 0.05). †,‡,§Means of Diazinone60-exposed fish within various serum biochemical profiles having different letters are significantly different (P < 0.05). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

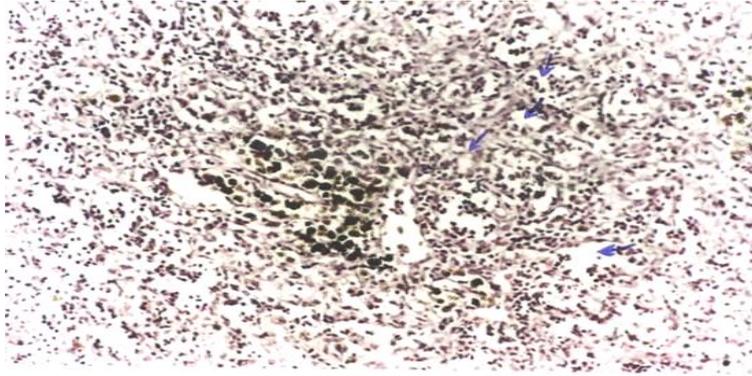


Figure 1: Hepatopancrease of monosex Nile tilapia exposed to Diazinone60® (1.46 µg/l) shows diffuse to moderate fatty vacuolation of the hepatocytes in comparison with control after 1 week of exposure. Haematoxylin and eosin staining (magnification x160).

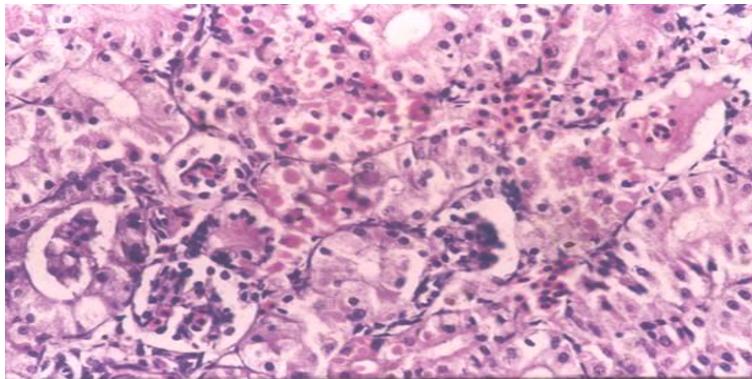


Figure 2: Posterior kidney of monosex Nile tilapia exposed to Diazinone60® (1.46 µg/l) shows moderate hyper-activation of melano- macrophage centres (arrows) in comparison with control after 1 week of exposure. Haematoxylin and eosin staining (magnification x160).

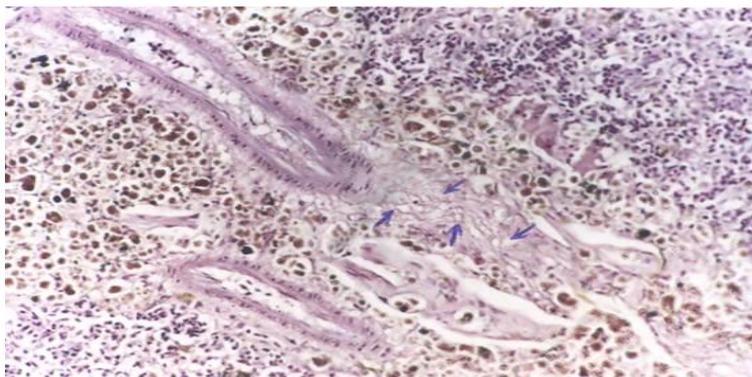


Figure 3: Spleen of monosex Nile tilapia exposed to Diazinone60 (1.46 µg/l) showed hyper-activation of melanomacrophage centres (arrow) in comparison with control after 2 weeks of exposure. Haematoxylin and eosin staining (magnification x160).

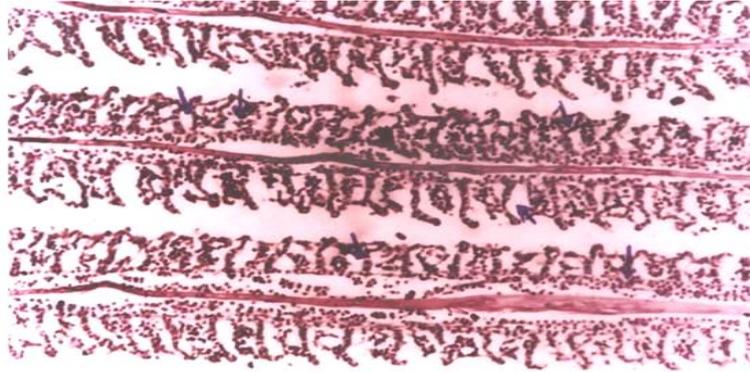


Figure 4: Gills of monosex Nile tilapia exposed to Diazinone60 (1.46 µg/l) show moderate epithelial hyperplasia at the base of secondary lamellae (arrow) in comparison with control after 3 weeks of exposure. Haematoxylin and eosin staining (magnification x160).

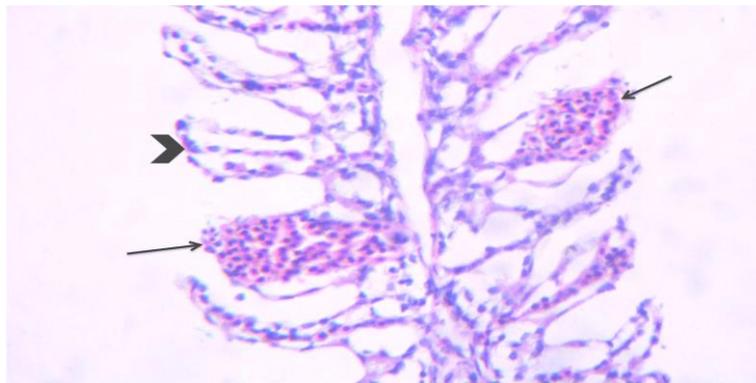


Figure 5: Gills of monosex Nile tilapia exposed to Diazinone60 (1.46 µg/l) show severe lamellar necrosis (arrow) in comparison with control after 4 weeks of exposure. Haematoxylin and eosin staining (magnification x250).

Serum biochemical findings

Results of biochemical blood serum profile of the control and Diazinone60-exposed monosex Nile tilapia are given in table 2. The data revealed that Diazinone60 significantly ($P < 0.05$) elevated serum AST and ALT enzyme activities at all weeks and ALP after the second week of the experiment in relation to the control values. However, there is non-significant ($P < 0.05$) differences in-between treated fish concerning the relevant enzyme activities at all weeks of exposure. The serum albumin and total protein content are significantly decreased starting from the second week compared to the control group.

Furthermore, significant ($P < 0.05$) differences concerning the relevant enzyme activities (ALT and AST) and levels of albumin and protein were observed, which were more pronounced at the last 2 weeks of the experimental period. The globulin content in serum of Diazinone60-exposed fish was significantly ($P < 0.05$) decreased at the second and fourth weeks compared to control ones, without any significant ($P < 0.05$) differences in-between Diazinone60-exposed fish as over time exposure. The values of glucose and cholesterol were significantly ($P < 0.05$) increased in all fish exposed to the toxicant when compared to the control throughout the entire experimental period.

Histopathological findings

In relation to control specimens (not presented), the histopathological results indicated that the haemobiotic tissues were affected by Diazinone60, primarily liver and gills. The results revealed diffuse to moderate fatty vacuolation of the hepatocytes (Figure 1), hyperactivation of melanomacrophage centres of posterior kidneys (Figure 2) and spleen (Figure 3), and moderate epithelial hyperplasia at the base of secondary lamellae to severe lamellar necrosis (Figures 4 and 5) in monosex Nile tilapia exposed to Diazinone60.

This study concluded that, the monosex Nile tilapia, *Oreochromis niloticus* L., that was exposed to subacute concentration (1.46 µg/l) of an organophosphorus compounds insecticide, Diazinone60 for 28 consecutive days suffered from severe mortality with a higher economic losses in returns and biochemical and histological changes in the serum constituents of the fish.

DISCUSSION

The higher economic losses of organophosphorous compounds especially Diazinone60 in Nile tilapia fish attributed to the decreasing fish weight, with higher mortality rate and great economic losses in returns in fish production farms ([Datta and Kaviraj, 2003](#); [Pimpão et al., 2007](#)).

A fundamental goal of ecotoxicology and hazard assessment is to determine the ecological effects of toxic chemicals on natural communities and ecosystems. Little information exists on subacute toxicity of organophosphorus compounds to fish. However, fish gills can adsorb even minute concentrations of the pesticide because of the lipophilic nature of organophosphorus compounds. Diazinone60 is highly toxic to fish; even minute concentration (0.01 mg/l) of Diazinone60 could cause 50% mortality of *Clarias gariepinus* in 24 hr ([Datta and Kaviraj, 2003](#)). These in turn can influence the whole-animal performance in the fish, which could result in stress-induced alterations in fish populations. The initial stress response is considered as an adaptive measure, designed to help the fish overcome the disturbance and regain its normal homeostatic state. If the stressor is severe or long-lasting, the fish may no longer be able to handle with it and, as a result, enter a maladaptive or distressed state leading to decreased performance, a pathological condition, or possibly death ([Vijayavel and Balasubramanian, 2007](#)). The present results indicate that long-term exposure of monosex Nile tilapia to 1.46 µg/l Diazinone60 for 28 consecutive days is not only capable of inducing stress to fish, but also is lethal.

Stress responses are identified via various changes, including behavioural responses, clinical toxic symptoms, hematological variables and primarily the biochemical indicators. The abnormal behavioral responses were predominantly respiratory and nervous manifestations, ascribed to the neurotoxic effect of the Diazinone60 ([Golow and Godzi, 1994](#)). Respiratory distress is one of the early symptoms of organophosphorus compounds poisoning. The clinically observed toxic signs and post-mortem changes on fish may be attributed to the irritant and inflammatory effects of Diazinone60 ([El-Sayed et al., 2007](#); [WHO, 1999](#)). Comparable changes, clinical signs and post-mortem lesions were observed in Nile tilapia chronically exposed to phenol ([WHO, 1999](#)) and diazinon ([El-Khateib and Afifi, 1993](#)).

In view of the fact that hematological parameters reflect the poor condition of fish more quickly than other commonly measured parameters, they are widely used for the description of healthy fish and for monitoring stress responses ([Thrall, 2004](#); [Pimpão et al., 2007](#)). The subacute exposure of monosex Nile tilapia to Diazinone60 significantly decreased lymphocyte and basophile percentages and subsequently TLC count at all weeks. Such response suggests that Diazinone60 causes tissue damage and severe disturbance of the non-specific immune system leading to decreased production of leucocytes. A decrease was reported in the whole blood cell count in the blood of *Ctenopharyngodon idella* ([Shakoori et al., 1996](#)) and *Oncorhynchus mykiss* ([Atamanalp and Yanik, 2003](#)) exposed to sublethal doses of organophosphorus compounds fenvalerate and mancozeb, respectively. Conversely, a significant increase in TLC was demonstrated in catfish ([Kumar et al., 1999](#)) and Indian major carp ([Das and Mukherjee, 2003](#); [Adhikari et al., 2004](#)) exposed to sub-lethal doses of organophosphorus compounds Diazinone60.

Reduction of TEC may be indicative of an appreciable decline in the hematopoiesis. A decline in red blood cells (RBC) was observed in long-term exposure of *L. rohita* to sublethal concentrations of cypermethrin ([Das and Mukherjee, 2003](#); [Adhikari et al., 2004](#)). The Hb% was also reduced. The decreased Hb% may be due to the suggested capacity for preventing the incorporation of body iron stores in erythropoiesis and haemoglobin synthesis ([Abidi and Srivastava, 1988](#); [Adhikari et al., 2004](#)). Similar reduction of Hb% was reported in catfish ([Kumar et al., 1999](#)), rainbow trout ([Atamanalp and Yanik, 2003](#)) and Indian major carp ([Adhikari et al., 2004](#); [Nayak et al., 2004](#)). Nevertheless, considering the leakage of carboxyfluorescein from egg-yolk liposomes with allethrin added ([Moya-Quiles et al., 1994](#)) and of haemoglobin from human RBCs with allethrin incorporated ([Moya-Quiles et al., 1995](#)), the possibility remains that the decreased Hb% comes from the increased permeability of the surface membrane of erythrocytes exposed to Diazinone60 and further release of haemoglobin.

A decrease in PCV content is observed only during the fourth week of exposure showing that the toxic effect of Diazinone60 may interfere with the normal function of RBCs. Exposure to fenvalerate in *C. idella* ([Shakoori et al., 1996](#)), mancozeb in *O. mykiss* ([Atamanalp and Yanik, 2003](#)) and cypermethrin in *L. rohita* ([Adhikari et al., 2004](#)) caused a reduction in the haematocrit value. Subsequently, monosex Nile tilapia exposed to subacute concentration of Diazinone60 suffered from anaemia and malnutrition.

Furthermore, Diazinone60 significantly elevated serum AST and ALT enzyme activities at all weeks in relation to control values. These toxic effects on the cellular metabolism suggest a possible change in protein metabolism in the tissues as utilization of amino acids for the oxidation or for gluconeogenesis following to chemical stress. The increase in serum AST and ALT activities is in harmony with the findings of [Vijayavel and Balasubramanian, \(2007\)](#); [Nayak et al., \(2004\)](#) and [Rao, \(2006\)](#). The significant increment in the activities of these enzymes not only suggests a possible change in protein metabolism in the tissues consequent to chemical stress, but also is indicative for liver, kidney and gill tissue damage ([Vijayavel and Balasubramanian, 2007](#); [Rao, 2006](#)).

Alkaline phosphatase is an important and critical enzyme in biological processes; it is responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in this enzyme leads to biochemical impairment, lesions of the tissue and cellular dysfunction ([Rao, 2006](#)). The results show an increase in the activity of ALP in serum of monosex Nile tilapia exposed to Diazinone60, which is in accordance with the findings of [Rao, \(2006\)](#) in *O. mossambicus*. [Yousef et al., \(2006\)](#) reported that the increase in the phosphatases and transaminases activities in blood might be due to the necrosis of liver, kidney and lung and the leakage of these enzymes to the blood stream corresponding to the stress condition of the treated animals. Thus, the measurement of these enzymes in the circulating fluid is frequently used as a diagnostic tool in water pollution studies ([Palanivelu et al., 2005](#)).

Alternatively, serum total protein, particularly albumin content significantly decreased that is in concurrence with the results' of [Nayak et al., \(2004\)](#) in *L. lochia* and [Yousef et al., \(2006\)](#) in rats. The observed reduction in serum protein could be attributed in part to the damaging effect of Diazinone60 on liver cells as evidenced by the increase in the activities of serum AST, ALT and ALP and the further Histopathological findings. These changes might be indicative of the immunotoxic consequence of the Diazinone60 ([Nayak et al., 2004](#); [El-Sayed et al., 2007](#)).

A hyperglycemic effect was observed in all fish exposed to Diazinone60 when compared to the control at all weeks especially the last one. Similar observation has been reported in Indian major carp exposed to sublethal concentrations of OFC ([Das and Mukherjee, 2003](#)) for 45 days. The hyperglycemic effect after organophosphorus compounds treatment suggests effects on the glycogenesis and glycolytic pathways. [Nayak et al., \(2004\)](#) have also correlated hyperglycemia with gluconeogenesis. Wherein, the long-lasting stress caused by Diazinone60 resulted in an increase in the synthesis of adrenocorticotrophic hormone and glucagon and decrease in the synthesis of insulin. Thereby, hepatic glycogen is rapidly converted into glucose and passes into systemic circulation ever-increasing the blood glucose level ([Datta and Kaviraj, 2003](#)). This response to Diazinone60 might reflect a stress hormone-mediated response.

Cholesterol is an essential structural component of cell membranes and the outer layer of plasma lipoproteins. Thus, the increase observed in cholesterol levels may be related to the effect of Diazinone60 on lipid metabolism and the permeability of intoxicated cells ([El-Sayed et al., 2007](#); [Eraslan et al., 2007](#)). In general, the assessment of serum AST, ALT and ALP activities and proteins, glucose and cholesterol levels provide significance with respect to the evaluation of the effects of pesticides on monosex Nile tilapia, therefore their toxicity.

Regarding the Histopathological findings, it has been shown that exposure to low concentration of Diazinone60 caused destructive effects in the tissues of *O. niloticus* L., primarily liver and gills, the main targets for toxic action of chemical pollutants ([Heath, 1987](#)). Diffuse to moderate fatty vacuolation of the hepatocytes, hyper-activation of melanomacrophage centers of posterior kidneys and spleen, and moderate epithelial hyperplasia at the base of secondary lamellae to severe lamellar necrosis were seen in monosex Nile tilapia-exposed fish. Similar alterations in the mosquitofish, *Gambusia affinis*, exposed to two sublethal concentrations of Diazinone60 (0.25–0.50 µg/l) for periods of 10, 20 and 30 days, have been observed by [Cengiz and Unlu, \(2006\)](#), who attributed the commonly reported lesions into either the direct deleterious and irritant effects of Diazinone60 or the defense response of fish, ultimately the respiratory system. Histopathological inspection indicates that the exposure to low concentrations of Diazinone60® caused destructive

effect in the tissues of *O. niloticus* which results in severe physiological and biochemical problems. In summary, this study shows that Diazinone60, in subacute dose, elicits serious metabolic disorders in monosex *O. niloticus* that increased with time of exposure. As a result, Diazinone60 produced clinical, hematological, biochemical and Histopathological alterations mainly related to the immunologic system that could aggravate any diseased condition and lead to extrapolation problems to aquatic species. Consequently, the assayed parameters and Histopathological findings can be used as good biomarkers of organophosphorus compounds ecosystem pollution. Further work with toxicity testing methods in fish will be very useful in assessing possible ecotoxicological risk assessments of this pesticide.

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