



Diafenthuron Adversely Affects the Complete Blood Count, Serum Biochemical Profile and Antioxidants in Vital Organs of *Ctenopharyngodon idella*

Asher Azeem¹, Misbah Javed², Muhammad Riaz ul Haq¹, Maria Shahzeen¹, Muhammad Asif¹, Gul Muhammad¹, Khalid Hussain³, Ahmad Ali³, Muhammad Latif⁴, Noreen Samad² and Furhan Iqbal^{1*}

¹Institute of Zoology, Bahauddin Zakariya University, Multan, 60800, Pakistan

²Department of Biochemistry, Bahauddin Zakariya University, Multan, 60800, Pakistan

³Department of Zoology, Islamia University Bahawalpur, Bahawalpur, Pakistan

⁴Department of Zoology, Division of Science and Technology, University of Education, Lahore 54770, Pakistan

Asher Azeem and Misbah Javed contributed equally to this article.

ABSTRACT

Diafenthuron is the most common used pesticide in Pakistan that causes insect pest mortality by mitochondrial function inhibition. It has been reported in local news papers that this pesticide is entering in our fresh water bodies affecting aquatic life. The objective of this investigation was to report the LC₅₀ value of diafenthuron for grass carp, *Ctenopharyngodon idella* and to report the effect of various doses such as 0.0038 (T1), 0.05 (T2), 0.5 (T3), 1 (T4), 5 (T5) and 6.67 mg/L (T6) on complete blood count, serum biochemical profile and antioxidant parameters in kidney and liver of this fish following 96 h exposure. An untreated control group was maintained in parallel. Our results indicated that 96 h LC₅₀ value of diafenthuron for juvenile *C. idella* was 5.67 mg/L. Analysis of the hematological profile revealed that red blood cell (RBC) count, haematocrit, mean corpuscular hemoglobin concentration, hemoglobin, white blood cells, monocytes, granulocytes and lymphocytes significantly increased while RBC distribution width, mean corpuscular haemoglobin, mean corpuscular volume and platelet distribution width significantly decreased in diafenthuron exposed treatments than control group. Significant increase in serum triglycerides level was observed in *C. idella* treatments exposed to diafenthuron. Concentrations of catalase, superoxide dismutase and malondialdehyde also varied significantly in liver and kidney when compared between diafenthuron treated and untreated *C. idella*. In conclusion, we are reporting that diafenthuron can adversely affect the hematological, serum and antioxidant parameters of non-target organism like *C. idella* and its entry in water bodies must be prevented in order to conserve our fresh water ecosystems and food web.

Article Information

Received 13 July 2022

Revised 04 October 2022

Accepted 26 October 2022

Available online 08 September 2023
(early access)

Published 15 January 2025

Authors' Contribution

FI and NS designed and supervised the study, and revised the manuscript. AA, MJ and MRH reared the animals and performed lab experiments. MS, MA and GM collected the blood samples and recorded the data. A Azeem, KH, ML and A Ali performed the hematobiochemical and antioxidant analysis. All authors contributed in the manuscript write up and approved the manuscript.

Key words

C. idella, Diafenthuron, LC₅₀, Complete blood count, Antioxidant

INTRODUCTION

Efficient pest control systems has led us toward a significant increase in agricultural productivity since the middle of last century (Riaz ul Haq *et al.*, 2018). Approximately 2.5 million tons of pesticides are being

used in agriculture sector annually throughout the world (Qadir *et al.*, 2017). It has been estimated that only less than 1% of the applied pesticides reach to their main targets while most of the remaining directly or indirectly reaches the food chains in our aquatic ecosystems and causing morbidity and mortality life existing there (Tilak *et al.*, 2007). Aquatic animals, including fish, are exposed to pesticides by three major routes: (1) They are directly absorbed through the skin while animals are swim in water contaminated by pesticides, (2) pesticides can be up taken through the gills during respiration, and (3) they can enter the body via oral routes when animals drink pesticide-contaminated water or they feed on prey the body of whom was contaminated by pesticides (Velisek *et al.*, 2009; Crab *et al.*, 2012). Diafenthuron [1-tert-butyl-3-(2,

* Corresponding author: furhan.iqbal@bzu.edu.pk
0030-9923/2025/0001-0420 \$ 9.00/00



Copyright 2025 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

6-diisopropyl thiourea)] is a non-organofluorine benzyl urea material that used as insecticide as well as an acaricide extensively in Pakistan (Asifa *et al.*, 2010). Diafenthiuron targets the respiratory system of insect and it prevents the oxidative phosphorylation and inhibits the ATP synthesis process in mitochondria. Hence, the pest gets immobilized get it dies within few days. It has been documented that diafenthiuron is persistent in aquatic systems and it is moderate to highly toxic for aquatic life, worms and bees (Ruder and Kayser, 1993). There are reports that this pesticide is very effective against *Plutella xylostella* L. (chewing pest) (Ishaaya, 1993), *Conogethes punctiferalis* Guenee and *Bemisia tabaci* (Gennadius), sucking pest, and *Amrasca biguttula biguttula* (Ishida) (Stanley *et al.*, 2016). It is very commonly used in brinjal, tomato, tea, cardamom fields as well as it is used against many sucking pests in cotton (Kranthi *et al.*, 2004). In previous studies, diafenthiuron is reported to be toxic for honey bees, coccinellid grubs and silk worm. Among fish, it has been documented that application of doses that were ten times lower than those of reported in literature caused mortality in common carp, *Cyprinus carpio* L. (Stanley *et al.*, 2016).

Recently, we have reported the effect of sub lethal concentration of diafenthiuron on the physiology of *Labeo rohita*, and we have reported that this pesticide can affect the hematological, serum and elemental concentrations in muscles of this non target fish species (Riaz ul Haq *et al.*, 2018). This project was aimed to document the diafenthiuron's LC_{50} value for *C. idella* and to report the effects of various concentrations of this most extensively used insecticide in Pakistan on selected hematological parameters, serum biochemical profile and antioxidant metabolites in kidney and liver of *C. idella*.

MATERIALS AND METHODS

Fish sample collection

Freshwater grass carp (N = 140) of both sexes (average body weight 86.97 ± 18.15 g, body length 20.0 ± 2.37 cm) were used as experimental subjects and acclimatized to laboratory conditions for two weeks. Semi-static system was maintained during the experiments and water was renewed after every 24 h. Experimental conditions were maintained following Qadir *et al.* (2014).

LC_{50} determination

For the determination of 96h diafenthiuron LC_{50} values, group of 20 grass carp juveniles were exposed to four concentrations; 0.0038 (T1), 0.05 (T2), 0.5 (T3), 1 (T4), 5 (T5) and 6.67 (T6) mg/ L of diafenthiuron. An untreated control group was maintained in the parallel.

Fish mortality was observed after every 24 h following Qadir *et al.* (2014).

Blood and serum collection

At the end of 96 h diafenthiuron exposure, on average, one ml of blood was sampled through cardiac puncture from each fish and divided into two parts. One part of the blood was directly used to study hematological parameters like white blood cells (WBC), (lymphocytes, monocytes), granulocytes, lymphocytes percentage, monocytes percentage, granulocytes percentage, red blood cells (RBC), hematocrit, mean corpuscular volume (MCV), hemoglobin, hemoglobin concentration, mean corpuscular red blood cells distribution width, mean corpuscular hemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) platelets, platelet hematocrit, mean platelet volume, and platelet distribution width] were determined by using hematological analyzer (CBC Analyzer, Sysmex 21, Japan). While second part of blood was centrifuged at 13000 RPM for ten minutes for the separation of serum for the determination of serum biochemical parameters (triglycerides, cholesterol, creatinine, high density lipoproteins and low-density lipoproteins) by using O.R.I Reinbeker 4000 (Hamburg, Germany) and by using diagnostic kits following the instructions of manufacturer [Egychem (Egypt)].

Determination of antioxidant parameters in liver and kidney

Following sacrifice, kidney and liver were surgically isolated from each fish and stored at -20°C til further biochemical analysis was carried out. Antioxidants superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT) concentrations were reported in kidney and liver by using O.R.I, Reinbeker-75 (Hamburg, Germany).

Method of Salim *et al.* (2022) was used for estimation of SOD in kidney and liver of *C. idella*. Protocol of Noureen *et al.* (2021) was followed for estimation of lipid peroxidation in vital organs of fish. Hussain *et al.* (2020) protocol was used for CAT estimation.

Statistical analysis

All data was presented as mean standard error of mean. Mini Tab (version 16, Pennsylvania) was used to calculate one way analysis of variance (ANOVA) to compare all studied parameters between seven experimental conditions. Two sample t-test was also calculated to compare each parameter between control and a specific diafenthiuron treatment group.

RESULTS

General observations and behavioural response

No apparent behavioral changes were observed in *C. idella* exposed to 0.0038 mg diafenthion/L (T1). However, fish exposed to higher concentrations of diafenthion made fast movement at the start of experiment to avoid the toxic water. Increased mucous production from fish body was observed and pigmentation in fish body was reduced by the end of experiment in pesticide exposed groups. With the progression of experiment, fish lost scales and became sluggish in their movements. None of these signs were observed in control group.

LC₅₀ values of diafenthion for *Ctenopharyngodon idella*

We are reporting 5.67 mg/L to be the 96 h LC₅₀ value of diafenthion for *C. idella*. No mortality observed for *C. idella* exposed to T1 till T5 but fish exposed to 6.67 mg (T6) diafenthion/L for 96 h suffered 60% mortality. No mortality was recorded in control group.

Hematological profile

One-way analysis result revealed that red blood cell count ($P = 0.006$), hemoglobin ($P = 0.006$), hematocrit ($P = 0.003$) and red cell distribution width ($P = 0.05$) varied significantly when compared between all diafenthion treatments and their untreated control group. Two sample t test based analysis of hematological data revealed that RBC count increased significantly in all treatments (T1 ($P < 0.01$), T2 ($P < 0.01$), T3 ($P < 0.05$), T4 ($P < 0.01$), T5 ($P < 0.05$) and T6 ($P < 0.05$)), haematocrit was significantly higher in T2 ($P < 0.05$), T3 ($P < 0.05$) and T4 ($P < 0.01$) while red blood cells distribution width significantly decreased in T3 ($P < 0.05$) and T5 ($P < 0.05$) (Table I). It was observed that haemoglobin was increased significantly in T2 ($P < 0.05$), T3 ($P < 0.05$), T4 ($P < 0.05$) and T5 ($P < 0.01$) while was significantly higher in T5 ($P < 0.01$) than control group. A significant decrease in MCV was observed in T1 ($P < 0.05$), T2 ($P < 0.05$) and T5 ($P < 0.05$) and decreased MCH in T1 ($P < 0.01$) and T2 ($P < 0.01$) was also observed upon their comparison with control group (Table I).

Table I. Comparison of various hematological parameters between juvenile grass carp (*Ctenopharyngodon idella*) exposed to 0.0038 mg (T1), 0.05 mg (T2), 0.5 mg (T3), 1 mg (T4), 5 mg (T5) and 6.67 mg diafenthion/L (T6) with untreated control group after acute toxicity test (96 h exposure time). Data is expressed as Mean $\pm$ SEM. P-value indicates the result of 2 sample t –test calculated for each comparison.

Parameters	Control	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
WBC ($\times 10^3 \mu\text{L}^{-1}$)	65.2 $\pm$ 10	87.6 $\pm$ 10	106.79 $\pm$ 24.06**	53.54 $\pm$ 10.04	101.9 $\pm$ 10.2*	76.32 $\pm$ 34.12	75.8 $\pm$ 11.0
Lymphocytes ($\times 10^3 \mu\text{L}^{-1}$)	60.4 $\pm$ 9.2	83.3 $\pm$ 3.9 *	87.56 $\pm$ 15.96 *	47.81 $\pm$ 8.56	84.5 $\pm$ 7.4	65.83 $\pm$ 27.84	68.4 $\pm$ 10.0
Monocytes ($\times 10^3 \mu\text{L}^{-1}$)	0.99 $\pm$ 0.3	2.47 $\pm$ 0.4 **	3.32 $\pm$ 1.36 ***	1.17 $\pm$ 0.30	3.1 $\pm$ 0.5 **	1.99 $\pm$ 1.15 *	1.56 $\pm$ 0.36*
Granulocytes ($\times 10^3 \mu\text{L}^{-1}$)	3.83 $\pm$ 0.9	9.82 $\pm$ 2 *	15.91 $\pm$ 7.62***	4.56 $\pm$ 1.31	14.3 $\pm$ 3.1**	8.49 $\pm$ 5.53 *	5.83 $\pm$ 1.3
Lymphocytes (%)	93 $\pm$ 1.2	88.26 $\pm$ 1.8 *	83.29 $\pm$ 6.7 **	91.86 $\pm$ 1.65	84.9 $\pm$ 2.8 *	88.08 $\pm$ 4.91*	87 $\pm$ 4.0
Monocytes (%)	1.27 $\pm$ 0.3	2.4 $\pm$ 0.3 **	2.92 $\pm$ 0.96 ***	1.71 $\pm$ 0.31	2.7 $\pm$ 0.4 **	2.35 $\pm$ 0.77**	2.0 $\pm$ 0.2
Granulocytes (%)	5.73 $\pm$ 1.1	9.34 $\pm$ 1.5	13.79 $\pm$ 5.76 **	6.46 $\pm$ 1.35	12.4 $\pm$ 2.4 *	9.57 $\pm$ 4.15 *	11 $\pm$ 4.0
RBC ($\times 10^6 \mu\text{L}^{-1}$)	1.2 $\pm$ 0.2	1.89 $\pm$ 0.1**	2.14 $\pm$ 0.58 **	1.917 $\pm$ 0.24*	2.2 $\pm$ 0.2 **	1.79 $\pm$ 0.25 *	1.331 $\pm$ 0.19*
Haemoglobin (gdl ⁻¹)	6.19 $\pm$ 0.8	7.92 $\pm$ 0.5	8.84 $\pm$ 1.94 *	8.55 $\pm$ 0.73 *	9.5 $\pm$ 0.8 **	9.36 $\pm$ 1.01**	7.33 $\pm$ 0.66
Hematocrit ($\times 10^3 \mu\text{L}^{-1}$)	17.11 $\pm$ 2.3	23.08 $\pm$ 1.7	25.94 $\pm$ 8.85 *	25.61 $\pm$ 2.8 *	27.8 $\pm$ 2.7 **	22.5 $\pm$ 3.37	18.33 $\pm$ 3.3
MCV (μm^3)	147.8 $\pm$ 8.2	122 $\pm$ 3. *	119.78 $\pm$ 10.44*	135.63 $\pm$ 4.79	129.6 $\pm$ 5.4	125.8 $\pm$ 11.99*	135.6 $\pm$ 10.0
MCH (pg)	53.73 $\pm$ 2.9	42.1 $\pm$ 0.5 **	41.99 $\pm$ 3.82 **	50.63 $\pm$ 5.21	45.7 $\pm$ 3.0	52.53 $\pm$ 3.18	76.1 $\pm$ 23.0
MCHC (gdl ⁻¹)	36.47 $\pm$ 1.3	34.73 $\pm$ 0.9	35.45 $\pm$ 5.06	37.09 $\pm$ 3.90	35.8 $\pm$ 3.01	42.0 $\pm$ 3.87**	67.6 $\pm$ 30.0
RDW (%)	10.29 $\pm$ 1	8.65 $\pm$ 0.2	9.45 $\pm$ 1.87	7.09 $\pm$ 0.56 *	10.3 $\pm$ 1.6	7.73 $\pm$ 1.49 *	8.81 $\pm$ 0.88
RDW-SD (μm^3)	129 $\pm$ 39	49.86 $\pm$ 2.9	53.24 $\pm$ 24.08	86.67 $\pm$ 21.45	94.1 $\pm$ 25.6	55.2 $\pm$ 30.69	136 $\pm$ 36.0
Platelets ($\times 10^3 \mu\text{L}^{-1}$)	101.3 $\pm$ 25	81.1 $\pm$ 26	147.9 $\pm$ 199.0	365.5 $\pm$ 132.92	297.9 $\pm$ 153.3	110.2 $\pm$ 146.6	195 $\pm$ 47.0
MPV (μm^3)	6.01 $\pm$ 0.09	6.45 $\pm$ 0.095**	5.72 $\pm$ 2.05	5.62 $\pm$ 0.07**	6.4 $\pm$ 0.1 **	5.62 $\pm$ 0.38 *	5.975 $\pm$ 0.17
PCT (%)	0.06 $\pm$ 0.01	0.05 $\pm$ 0.01	0.09 $\pm$ 0.13	0.20 $\pm$ 0.07	0.2 $\pm$ 0.1	0.06 $\pm$ 0.08	0.11 $\pm$ 0.03
PDW (%)	9.13 $\pm$ 0.48	7.43 $\pm$ 0.35 *	7.72 $\pm$ 2.93	8.68 $\pm$ 0.43	8.9 $\pm$ 0.3	8.42 $\pm$ 1.06	9.54 $\pm$ 0.36.0

P > 0.05 = Non significant; P < 0.05 = Least Significant (*); P < 0.01 = Significant (**); P < 0.001 = Highly significant (***). WBC, White blood cells, RBC, Red blood cells, MCV, Mean corpuscular volume, MCH, Mean corpuscular hemoglobin, MCHC, Mean corpuscular hemoglobin concentration, RDW, Red blood cells distribution width; MPV, Mean platelet volume, PCT, Platelet hematocrit, PDW, Platelet distribution width.

One-way analysis result revealed that white blood cell count ($P = 0.04$), lymphocyte ($P = 0.02$), monocyte ($P = 0.003$), monocyte % ($P = 0.02$) and granulocytes ($P = 0.004$) varied significantly when compared between all diafenthuron treatments and their untreated control group. When the data regarding differential leukocyte count was analyzed by two sample student t test, it was observed that lymphocyte count increased significantly in T1 ($P < 0.05$) and T2 ($P < 0.05$) while lymphocytes percentage significantly decreased in T1 ($P < 0.05$), T2 ($P < 0.01$), T4 ($P < 0.05$) and T5 ($P < 0.05$). When compared between control and treated groups, it was observed that monocyte count increased significantly in all diafenthuron exposed treatments except T3 in which it varied non-significantly ($P > 0.05$). Monocyte percentage was significantly increased in T1 ($P < 0.01$), T2 ($P < 0.001$), T4 ($P < 0.05$) and T5 ($P < 0.01$) as compared to control group. Significant increase was observed in granulocyte count in T1 ($P < 0.05$), T2 ($P < 0.001$), T4 ($P < 0.01$) and T5 ($P < 0.05$) and granulocytes percentage was higher in T2 ($P < 0.01$), T4 ($P < 0.05$) and T5 ($P < 0.05$) than control. Total WBC count was significantly higher in T2 ($P < 0.01$) and T4 ($P < 0.05$) than control group (Table I).

One-way analysis result revealed that mean platelet volume ($P < 0.001$) and platelet distribution width ($P = 0.007$) varied significantly when compared between all diafenthuron treatments and their untreated control group. Two sample t test analysis indicated that mean platelet volume increased significantly in T1 ($P < 0.01$) and T4 ($P < 0.01$) while it was significantly decreased in T3 ($P < 0.01$) and T5 ($P < 0.05$) and platelet distribution width was found significantly decreased in T1 ($P < 0.05$) than control group. All other analyzed parameters, except mentioned above, varied non-significantly ($P > 0.05$) when compared between a specific diafenthuron treatment and the untreated control group (Table I).

One-way analysis result revealed that serum

triglyceride concentration ($P < 0.001$) varied significantly when compared between all diafenthuron treatments and their untreated control group. Two sample t test based analysis of serum biochemical profile revealed that triglyceride concentrations were the only significantly increased parameter in T2 ($P < 0.001$), T3 ($P < 0.001$) and T5 ($P < 0.01$) than control group. The concentrations of other studied parameters did not differ significantly when they were compared between a specific diafenthuron treatment and control group (Table II).

Antioxidants parameters of fish kidney

One-way analysis result revealed that all studied antioxidant metabolites in kidney varied nonsignificantly when compared between all diafenthuron treatments and their untreated control group. Two sample t test based data analysis of antioxidants metabolites of kidney revealed that superoxide dismutase decreased significantly in T5 ($P < 0.05$) while catalase concentrations significantly decreased in T3 ($P < 0.05$) as compared to control group. All other studied parameter varied non-significantly ($P > 0.05$) when compared between a specific diafenthuron treatment and control group (Table III).

Antioxidant parameters of fish liver

One-way analysis result revealed that catalase concentrations in liver ($P < 0.001$) varied significantly when compared between all diafenthuron treatments and their untreated control group. When the studied liver antioxidants metabolites were compared between Diafenthuron treated and untreated groups by two sample t test analysis, it was observed that SOD significantly increased in T2 ($P < 0.05$) and T6 ($P < 0.05$), CAT increased significantly in T1 ($P < 0.001$), T2 ($P < 0.01$) and T4 ($P < 0.05$) while MDA significantly decreased in T3 ($P < 0.01$) as compared to control group (Table III).

Table II. Comparison of various studied serum biochemical parameters between juvenile *Ctenopharyngodon idella* exposed to 0.0038 mg (T1), 0.05 mg (T2), 0.5 mg (T3), 1 mg (T4), 5 mg (T5) and 6.67 mg diafenthuron/L (T6) and their untreated control group. Data is expressed as Mean $\pm$ SEM. P-value indicates the result of 2 sample t –test calculated for each studied parameter.

Parameters	Control	T1	T2	T3	T4	T5	T6
Triglycerides (mg/dl)	100.3 $\pm$ 20	137.7 $\pm$ 20	171.6 $\pm$ 15***	169.4 $\pm$ 14***	108.0 $\pm$ 14	333 $\pm$ 71 *	160.5 $\pm$ 4.9
Cholesterol (mg/dl)	255 $\pm$ 59	285.8 $\pm$ 16	288.5 $\pm$ 13	297 $\pm$ 19	257 $\pm$ 12	429 $\pm$ 68	375 $\pm$ 48
Creatinine (mg/dl)	0.76 $\pm$ 0.3	1.26 $\pm$ 0.23	0.63 $\pm$ 0.21	1.72 $\pm$ 1.3	0.38 $\pm$ 0.075	0.79 $\pm$ 0.2	0.31 $\pm$ 0.09
High density lipoprotein (mg/dl)	96.1 $\pm$ 0.3	78.5 $\pm$ 8.5	54.7 $\pm$ 8.3	98.9 $\pm$ 14	75.6 $\pm$ 14	52.8 $\pm$ 15	56.8 $\pm$ 10
Low density lipoprotein (mg/dl)	101.8 $\pm$ 33	169.9 $\pm$ 16	182.4 $\pm$ 17	182.8 $\pm$ 27	161.7 $\pm$ 19	316 $\pm$ 86	277 $\pm$ 89

P > 0.05 = Non significant; P < 0.05 = Least significant (*); P < 0.001 = Highly significant (***).

Table III. Comparison of different studied antioxidant parameters of kidney and liver of juvenile *Ctenopharyngodon idella* exposed to 0.0038 mg (T1), 0.05 mg (T2), 0.5 mg (T3), 1 mg (T4), 5 mg (T5) and 6.67 mg diafenthuron/L (T6) and their untreated control group. Data is expressed as Mean $\pm$ Standard Error of Mean. P-value indicates the result of 2 sample t –test calculated for each studied parameter.

Organ	Parameters	Control	T1	T2	T3	T4	T5	T6
Kidney	SOD (unit/gram)	0.57 $\pm$ 0.09	0.27 $\pm$ 0.19	0.28 $\pm$ 0.06	0.12 $\pm$ 0.11	0.37 $\pm$ 0.02	0.2 $\pm$ 0.008*	0.202 $\pm$ 0.08
	CAT (mg/dl)	1.71 $\pm$ 0.005	1.62 $\pm$ 0.45	2.17 $\pm$ 0.6	1.05 $\pm$ 0.03*	1.53 $\pm$ 0.1	1.99 $\pm$ 0.09	1.86 $\pm$ 1.8
	MDA (nmol/gram)	0.17 $\pm$ 0.03	0.23 $\pm$ 0.001	0.18 $\pm$ 0.02	0.2 $\pm$ 0.007	0.22 $\pm$ 0.01	0.2 $\pm$ 0.05	0.16 $\pm$ 0.02
Liver	SOD (unit/gram)	0.18 $\pm$ 0.059	0.30 $\pm$ 0.16	0.4 $\pm$ 0.09*	0.6 $\pm$ 0.23	0.4 $\pm$ 0.11	0.11 $\pm$ 0.03	0.75 $\pm$ 0.09
	CAT (mg/dl)	0.77 $\pm$ 0.1	0.84 $\pm$ 1.9**	1.69 $\pm$ 0.06**	1.74 $\pm$ 0.38	1.96 $\pm$ 0.12*	0.83 $\pm$ 0.15	1.08 $\pm$ 0.2
	MDA (nmol/gram)	0.14 $\pm$ 0.0003	0.13 $\pm$ 0.09	0.14 $\pm$ 0.02	0.11 $\pm$ 0.00 **	0.13 $\pm$ 0.02	0.17 $\pm$ 0.01	0.17 $\pm$ 0.01

P > 0.05 = Non significant; P < 0.05 = Least Significant (*); P < 0.01 = Significant (**).

CAT, Catalase; MDA, Malondialdehyde; SOD, Super oxide dismutase.

DISCUSSION

Fish is an ideal model to study the effect of toxins in aquatic environment (Iqbal *et al.*, 2005) because their behaviors can be observed easily and can be quantified in controlled settings (Sindhe *et al.*, 2007). Accumulation of pesticides in vital organs of fish like kidney, liver and muscles results in dysfunction and mortality of fishes (Srivastava and Kaushik, 2001). Diafenthuron and its metabolites are toxic for live forms as it has been documented that diafenthuron is photo chemically converted into a carbodiimide derivative within a few h after its exposure to sunlight (Keum *et al.*, 2002) and these derivatives are much more powerful insecticide than its precursor (Ishaaya, 1993). Some of these chemicals are biologically degradable while rest becomes permanent part of aquatic environments as they are generally non-degradable and toxicological effects of these chemicals are extended to non- target organisms. Hence, there is a growing concern regarding the indiscriminate use of pesticides that is a serious threat to aquatic organisms (Weber *et al.*, 2010). Diafenthuron is among the very commonly used pesticides in Pakistan but we did not find any report in literature regarding its toxic effects on non target organisms. Although, there are reports regarding the detection of diafenthuron in vegetables (okra, bitter gourd, brinjal, tomato, onion, cauliflower, and chilies) collected from local market in Sindh Province (Sheikh *et al.*, 2013). During present study, we are reporting 5.67 mg/L of diafenthuron to be LC₅₀ value for *C. idella*. Our results are in contradiction with those documented by the manufacturers of this pesticide, syngenta, as they had reported 0.0038 mg/L of diafenthuron as median lethal concentration at 96 h for carp (Tavares-dias and Moraes, 2004) indicating that either the reported information is too much generalized or carps have developed resistance

against this pesticide with the passage of time.

Haematological parameters of fish assists in diagnosing adverse conditions and in understanding the relationship between blood characteristics, the health status of fish and their association with the (Heath, 1995). Data analysis indicated a significant increase in RBC count following 96 h exposure to diafenthuron in all treatments (Table I). Haematocrit, haemoglobin and MCHC were also increased significantly in diafenthuron treatments. It has been reported previously that an increase in RBC count and haemoglobin can occur in situations of acute stress as during such conditions adrenergic stimulations triggers spleen to contract and release large quantities of RBCs in circulating blood (Goede and Barton, 1990). These results are similar to Riaz-ul-Haq *et al.* (2018) as they have also reported increased RBC, hemoglobin, hematocrit, MCV and RBC distribution width in *Labeo rohita* after exposing it to sub lethal dose of 0.0075 mg L⁻¹ of diafenthuron for 4 days. These results are in agreement with those of Heath (1995) as he had reported a significant increase in RBCs count upon sub lethal fenvalerate administration to *C. idella*.

Analysis of haematological profile revealed a significant increase in WBC count and monocytes in diafenthuron exposed treatments (Table I). These findings are in agreement with those reported by Riaz-ul-Haq *et al.* (2018) who has reported a significant increase in monocyte and monocyte percentage in *Labeo rohita* following exposure to diafenthuron for 16 days. Increase in WBC and monocytes is a condition known as leukocytosis. Increased number of white blood cell count and monocytes may indicate a subclinical infection in fish as the immune system start producing antibodies as defense against infection (Lebelo *et al.*, 2001; Hassen, 2002; Pourgholam *et al.*, 2013). During toxic exposure period of diafenthuron during present study, the WBC

counts and monocytes were increasing indicating that fish tried to develop a defensive mechanism to overcome the toxic stress.

In our results, lymphocytes count was increased significantly upon exposure to lower diafenthion doses (T1 and T2) while a significant decrease was observed in lymphocytes percentage in most diafenthion exposed treatments. These results agree with those of Pourgholam (2013) as they had observed decreased lymphocyte count in *C. idella* after 12 h of exposure to sub-lethal concentration of diazinon. They have documented that this lymphopenia was probably due to decreased lymphocyte supply to the circulatory system due to compromised lymphocyte production or because of their sudden and rapid destruction (Alkahem, 1994).

Haematological profile analysis revealed a significant increase in granulocytes count and granulocytes percentage in diafenthion exposed treatments; a condition known as granulocytosis which is often associated with inflammation in fish and it is observed in combination with extreme physical or emotional stress, blood infection, failure of kidney function and due to drug exposure (Tonya *et al.*, 2008). In our result granulocytosis is probably due to exposure to the pesticide that might have resulted infection in *C. idella*. The susceptibility of aquatic organisms to most pesticides is due to several reasons. One of them is that they share the same neurological and respiratory mechanisms as insects but in addition to this factor, the lack a proper detoxification system to neutralize the effects of these imposed chemicals. This is because aquatic organisms are primitive in an evolutionary history and their isoenzymes of cytochrome P₄₅₀ and monooxygenases are somehow inefficient to degrade most toxic compounds that enter their bodies (Brown *et al.*, 2001).

Analysis of serum biochemical profile revealed a significant increase in triglycerides of *C. idella* exposed to diafenthion (Table II). Singh and Srivastava (1998) had observed increased serum triglycerides concentrations in *Heteropneustes fossilis* following pesticide exposure indicating hepatopathy that was probably generated due to oxidative stress due to free radicals generated following toxicant exposure. These results are in agreement with Prusty *et al.* (2011) when they exposed *Labeo rohita* fingerlings to fenvalerate. The fluctuations in the triglyceride concentrations in blood plasma in diafenthion exposed fish during present study are probably due to the disturbance created by the toxin that may have affected the normal liver, digestive system and related enzyme's functioning along with disturbing hormonal and metabolic imbalance (Lee and Gerking, 1983).

It is a general consideration that pesticides are not reaching the non-target organisms but if by some

how these chemicals will reach them, they can cause an enhanced ROS generation in aquatic organisms leading to compromised cell functioning resulting in oxidative stress (Akhtar *et al.*, 2018). Due to oxidative stress, reactive oxygen species (ROS), oxygen free radicals such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻) and hydroxyl radicals (OH) are produced in the cell (Soares *et al.*, 2008). Antioxidants are reported from many fish species and they are supposed to protect their lipids and other double bond containing compounds against the damages that are caused by ROS. SOD is the enzyme that catalyzes the dismutation of the superoxide anion to O₂ and H₂O₂ (Blaha *et al.*, 2004). SOD is among the first enzymes that try to neutralize the oxidative stress in animals (Ali *et al.*, 2004). In the present study, SOD decreased significantly in kidney of T5 but in liver of T2 and T6, its concentration was increased indicating a detoxifying mechanism against diafenthion exposure. Similar results were reported by Abhijith *et al.* (2016) as they had documented enhanced SOD concentration in kidney and liver of *Catla catla* following exposure to methyl parathion.

MDA is the ultimate product of lipid peroxidation and its concentration provides a direct evidence of toxicity that is caused by free radicals (Sieja and Talerczyk, 2004). In the present study, MDA varied non-significantly in kidney but attained significant decrease in liver in T3 as compared to the control group indicating decreased lipid peroxidation in of *C. idella*. Our results are in contrast with those of Abhijith *et al.* (2016) as they had reported significant increase in MDA in liver and kidney of *Catla catla* following an exposure to methyl parathion pesticide.

CAT is a component of primary antioxidant defence system that is used as biomarker of pollutant induced oxidative stress in aquatic organisms (Borkovi *et al.*, 2005). CAT is one of the sensitive enzymes and its activity is modulated by various factors including overproduction of O₂⁻ (Kohen and Nyska, 2002). It reacts with H₂O₂ to form water and oxygen molecule (Sayeed *et al.*, 2003; Blaha *et al.*, 2004). In the present study, the CAT activity decreased significantly in T3 in liver but in kidney it was increased significantly in T1, T2 and T4 as compared to control group indicating that applied pesticide has disturbed the superoxides in the analyzed organs.

CONCLUSION

In conclusion, we are reporting that exposure to Diafenthion for 96 h can lead to alterations in the haematological, serum biochemical parameters and antioxidant metabolites of liver and kidney of *C. idella* and thus this pesticide is potent to adversely affect the physiology of non-target organisms. Strict actions must

be taken in order to prevent pesticidal entry in aquatic habitats. One of such measures is the construction of conservation buffer areas that are meant to trap chemicals and prevent their entry in surface water.

ACKNOWLEDGEMENT

Authors are grateful to Government Fish Hatchery Muzaffargarh for providing fish samples for these experiments.

Funding

No specific grant was available for this project.

Ethical statement

All the experimental protocols and animal handling procedures were approved by the ethical committee of Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan (Pakistan).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Abhijith, B.D., Ramesh, M. and Poopal, P.K., 2016. Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of *Catla catla* during methyl parathion exposure *J. Basic appl. Zool.*, **77**: 31-40. <https://doi.org/10.1016/j.jobaz.2015.11.002>
- Akhtar, S., Saba, S., Rehman, S., Hassan, A., Samad, N., Amir, M., Akram, M., Ijaz, M., Asif, K., Ashiq, M.N. and Iqbal, F., 2018. Microemulsion based synthesis of strontium hexaferrite cobalt iron oxide nanoparticles and their biocompatibility in albino mice. *J. exp. Nanosci.* **13**: 199-210. <https://doi.org/10.1080/17458080.2018.1475759>
- Ali, M., Parvez, S., Pandey, S., Atif, F., Kaur, M., Rehman, H. and Raisuddin, S., 2004. Fly ash leachate induces oxidative stress in freshwater fish *Channa punctata* (Bloch). *Environ. Int.* **30**: 933-938. <https://doi.org/10.1016/j.envint.2004.03.004>
- Alkahem, H.F., 1994. The toxicity of nickel and the effects of sub lethal levels on haematological parameters and behavior of the fish, *Oreochromis niloticus*. *J. Univ. Kuwait Sci.*, **21**: 243-252.
- Asifa, H., Aziz, M.A. and Aheer, G.M., 2010. Susceptibility of *Bemisia tabaci* Gen. (Homoptera: Aleyrodidae) to selected insecticides. *Pakistan J. Zool.*, **42**: 295-300.
- Blaha, L., Kopp, R. and Simkova, K., 2004. Oxidative stress biomarkers are modulated in silver carp (*Hypophthalmichthys molitrix* Val) exposed to microcystin-producing cyanobacterial water bloom. *Acta Vet. Brno*, **73**: 477-482. <https://doi.org/10.2754/avb200473040477>
- Borkovi, S.S., Borkovi, S.S., Pavlovic, S.Z., Kovacevic, T.B., Stajn, A.S., Petrovic, V.M. and Saicic, Z.S., 2005. Antioxidant defence enzyme activities in hepatopancreas, gills and muscle of spiny cheek crayfish *Orconectes limosus* from the river Danube. *Comp. Biochem. Physiol.*, **147**: 122-128. <https://doi.org/10.1016/j.cbpc.2007.08.006>
- Brown, C.D., Fryer, C.J. and Walker, A., 2001. Influence of topsoil tilth and soil moisture status on losses of pesticide to drains from a heavy clay soil. *Pest Manage. Sci.* **57**: 1127-1134. <https://doi.org/10.1002/ps.410>
- Crab, R., Deforidit, T., Bossier P. and Verstrete, W., 2012. Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*, **356**: 351-356. <https://doi.org/10.1016/j.aquaculture.2012.04.046>
- Goede, R.W. and Barton, B.A., 1990. Organismic indices and an autopsy based assessment as indicators of health and condition of fish. *Am. Fish. Soc. Symp. Bethesda*, **8**: 93-108.
- Hassen, F.E.Z.M., 2002. *Studies on diseases of fish caused by Henneguya infestation*. Ph.D thesis. Faculty Veterinary Medical Suez Canal University, Egypt.
- Heath, A.G., 1995. *Water pollution and fish physiology*. CRC Press, Florida, USA.
- Hussain, M.F., Ashiq, M.N., Gulsher, M., Akbar, S., Iqbal, F., 2020. Exposure to variable doses of nickel oxide nanoparticles disturbs serum biochemical parameters and oxidative stress biomarkers from vital organs of albino mice in a sex specific manner. *Biomarkers*, **25**: 719-724. <https://doi.org/10.1080/1354750X.2020.1841829>
- Iqbal, F., Qureshi, I.Z., and Ali, M., 2005. Histopathological changes in liver of farmed Cyprinid fish, *Cyprinus carpio*, following nitrate exposure. *Pakistan J. Zool.*, **37**: 297-300.
- Ishaaya, I., 1993. Insect detoxifying enzymes: Their importance in pesticide synergism and resistance. *Physiology*, **22**: 263-276. <https://doi.org/10.1002/arch.940220119>
- Keum, Y.S., Seo, J. and Kim, P.J., 2002. Dissipation of foliar residues of difenthiuron and its metabolites. *Bull. environ. Contam. Toxicol.*, **68**: 845-851. <https://doi.org/10.1007/s00128-002-0032-7>
- Kohen, R. and Nyska, A., 2002. Oxidation of

- biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.* **30**: 620–650. <https://doi.org/10.1080/01926230290166724>
- Kranthi, S., Kranthi, K., Siddhabhatti, M. and Dhepe, V.R., 2004. Baseline toxicity of Cry1Ac toxin against spotted bollworm, *Earias vittella* (Fab) using a diet-based bioassay. *Curr. Sci.*, **87**: 1593–1597.
- Lebelo, S.L., Saunders, D.K. and Crawford, T.G., 2001. Observations on blood viscosity in striped bass, *Morone saxatilis* (Walbaum) associated with fish hatchery conditions. *Kansa. Acad. Sci.*, **104**: 183–194. [https://doi.org/10.1660/0022-8443\(2001\)104\[0183:OOBVIS\]2.0.CO;2](https://doi.org/10.1660/0022-8443(2001)104[0183:OOBVIS]2.0.CO;2)
- Lee, R.N. and Gerking, S.D., 1983. Electrolyte balance and energy mobilization in acid stressed rainbow trout *Salmo gairdneri* and their relation to reproductive stress. *Environ. Biol. Fish.* **8**: 115–123. <https://doi.org/10.1007/BF00005178>
- Noureen, H., Salim, M., Nadeem, A., Shafiq, S., Yousuf, S., Samad, N., Ashiq, M.N. and Iqbal, F., 2021. Synthesis, characterization and biocompatibility of holmium zirconate/zinc sulphide nanocomposite in albino mice in a gender specific manner. *J. Chem. Soc. Pak.* **43**: 46–56. <https://doi.org/10.52568/000552/JCSP/43.01.2021>
- Pourgholam, R., Hassan, M.D., Kakoolaki, S., Rostami, H.A.K., Rostami, A.M. and Pourgholam, M.A., 2013. Some hematological and biochemical changes in blood serum of grass carp (*Ctenopharyngodon idella*) vaccinated with *Aeromonas hydrophila* following exposure to sublethal concentration of diazinon. *Iran. J. Fish. Sci.* **12**: 12–23.
- Prusty, A.K., Kohli, M.P.S., Sahu, N.U., Pal, A.K., Saharan, N., Mohaptra, S. and Gupta, S.K., 2011. Effects of short term exposure of fenvalerate on biochemical and haematological responses in *Lebeo rohita* (Hamilton) fingerlings. *Pest. Biochem. Physiol.*, **100**: 124–129. <https://doi.org/10.1016/j.pestbp.2011.02.010>
- Qadir, S., Latif, A., Ali, M., and Iqbal, F., 2014. Effects of imidacloprid on the hematological and serum biochemical profile of *Labeo rohita*. *Pakistan J. Zool.*, **46**: 1085–1090.
- Qadir, S., Xiwei, C., Wei, W., Feng, F., Sultan, S., Kareem, K., and Iqbal, F., 2017. Short and long-term exposure to imidacloprid disturbs the elemental composition and free amino acid profile in muscles of *Labeo rohita*. *Comp. Clin. Pathol.*, **26**: 1339–1346. <https://doi.org/10.1007/s00580-017-2538-8>
- Riaz-ul-Haq, M., Javeed, R., Irum, S., Rasheed, M.A., Amjad, M., and Iqbal, F., 2018. Effect of Diafenthuron exposure under short and long term experimental conditions on hematology, serum biochemical profile and elemental composition of a non-target organism, *Labeo rohita*. *Environ. Toxicol. Pharmacol.*, **62**: 40–45. <https://doi.org/10.1016/j.etap.2018.06.006>
- Ruder, E.J. and Kayser, H., 1993. The carbodiimide product of diafenthuron inhibits mitochondria *in vivo*. *Pest. Biochem. Physiol.*, **46**: 96–106. <https://doi.org/10.1006/pest.1993.1041>
- Salim, M., Shahzeen, M., Khan, M.N., Tariq, R., Muhammad, G., Shabbir, G., Nisar, L., Ashiq, M.N. and Iqbal, F., 2022. Induction of selenium nanoparticles disturbs behavior, blood and serum biomarkers and oxidative stress markers from vital organs of male and female albino mice. *J. Chem. Soc. Pak.*, **44**: 59–68. <https://doi.org/10.52568/000979/JCSP/44.01.2022>
- Sayed, I., Parvez, S. and Pandey, S., 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Environ. Safe.* **56**: 295–301. [https://doi.org/10.1016/S0147-6513\(03\)00009-5](https://doi.org/10.1016/S0147-6513(03)00009-5)
- Sheikh, S.A., Nizamani S.M., Panhwar A.A. and Mirani, B.N. 2013. Monitoring of pesticide residues in vegetables collected from markets of Sindh, Pakistan. *Fd. Sci. Technol. Lett.*, **4**: 41–45.
- Sieja, K. and Talerczyk, M., 2004. Selenium as an element in the treatment of ovarian cancer in women receiving chemotherapy. *Gynecol. Oncol.*, **93**: 320–327. <https://doi.org/10.1016/j.ygyno.2003.12.013>
- Sindhe, S.C.S., Pala, I. and Butchiram, M.S., 2007. Toxicity and behavioural changes in the freshwater fish *Labeo rohita* exposed to ziram. *J. Ecotoxicol. Environ. Monit.*, **17**: 537–542.
- Singh, N.N. and Srivastava, A.K., 1998. Formothion induced biochemical changes in blood and tissue of freshwater catfish *Heteropneustes fossilis*. *Malys. J. appl. Biol.*, **27**: 39–43.
- Soares, S.S., Martins, H., Gutierrez-Merino, C. and Aureliano, M., 2008. Vanadium and cadmium *in vivo* effects in teleost cardiac muscle: metal accumulation and oxidative stress markers. *Comp. Biochem. Physiol.*, **147**: 168–178. <https://doi.org/10.1016/j.cbpc.2007.09.003>
- Srivastava, N. and Kaushik, N., 2001. Use of fish as bioindicator of aquatic pollution. *Proc. Int. Congr. Chem. Environ. Indore, India.* pp. 227–229.
- Stanley, J., Chandrasekaran, S., Gnanadhas, P., Kuttalam, S. And Jasmine, R.S., 2016. Selective

- toxicity of diafenthiuron to non-target organisms: Honey bees, coccinellids, chelonus, earthworms, silkworms and fish. *J. Pl. Protect. Res.*, **56**: 1-5. <https://doi.org/10.1515/jppr-2016-0001>
- Tavares-Dias, M. and Moraes, S., 2004. Biochemical characteristics of *Brycon cephalus* and *Brycon orbignyanus* teleost *Dulci aquicolas*. *Braz. Ecol. Imp.*, pp. 282-285.
- Tilak, K.S., Veeraiah, K., Thathaji, P.B. and Butchiram, M.S., 2007. Toxicity studies of butachlor to the freshwater fish *Channa punctata* (Bloch). *J. environ. Biol.*, **28**: 485-487.
- Tonya, M.C., Alistair, D.M. and Jill, E.A., 2008. Hematologic disorders of fish. *Vet. Clin. Exot. Anim.*, **11**: 445-462. <https://doi.org/10.1016/j.cvex.2008.03.007>
- Velisek, J., Piackova, V. and Zuskova, E. 2009. Effects of acute exposure to metribuzin on some hematological biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). *Bull. environ. Contam. Toxicol.*, **82**: 492-495. <https://doi.org/10.1007/s00128-009-9648-1>
- Weber, J., Halsal, C., Muir, D., Texieria, C.R., Small, J., Solomon, K.R., Hung, H. and Tery, B., 2010. Endosulfan, a global pesticide: A review of its fate in the environment and occurrence in the Arctic. *Sci. Total Environ.*, **408**: 2966-2984. <https://doi.org/10.1016/j.scitotenv.2009.10.077>