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

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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 antioxident analysis. All auhtors contributed in the manuscript write up and approved the manuscript.

Key words
***C. idella*, Diafenthiuron, LC_{50}, Complete blood count, Antioxidant**

ABSTRACT

Diafenthiuron 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 LC_{50} value of diafenthiuron 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 LC_{50} value of diafenthiuron 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 diafenthiuron exposed treatments than contol group. Significant increase in serum triglycerides level was observed in *C. idella* treatments exposed to diafenthiuron. Concentrations of catalase, superoxide dismutase and malondialdehyde also varied significantly in liver and kidney when compared between diafenthiuron treated and untreated *C. idella.* In conclusion, we are reporting that diafenthiuron 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.

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). Diafenthiuron [1-tert-butyl-3-(2,

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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, coccinelid 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 leathal 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 diafenthiuron/L (T1). However, fish exposed to higher concentrations of diafenthiuron 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.

LC_{50} values of diafenthiuron for Ctenopharyngodon idella

We are reporting 5.67 mg/L to be the 96 h LC_{50} value of diafenthiuron for *C. idella*. No mortality observed for *C. idella* exposed to T1 till T5 but fish exposed to 6.67 mg (T6) diafenthiuron/L for 96 h suffered 60% morality. No mortality was recorded in control group.

Hematolgical 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 diafenthiuron 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 diafenthiuron/L (T6) with untreated control group after acute toxicity test (96 h exposure time). Data is expressed as Mean ± 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 (x 10^3 μL^{-1})	65.2 ± 10	87.6 ± 10	106.79±24.06**	53.54 ± 10.04	101.9 ± 10.2*	76.32 ± 34.12	75.8 ± 11.0
Lymphocytes (x$10^3\mu L^{-1}$)	60.4 ± 9.2	83.3 ± 3.9 *	87.56 ± 15.96 *	47.81± 8.56	84.5 ± 7.4	65.83 ± 27.84	68.4 ± 10.0
Monocytes (x$10^3\mu L^{-1}$)	0.99 ± 0.3	2.47 ± 0.4 **	3.32 ± 1.36 ***	1.17± 0.30	3.1 ± 0.5 **	1.99 ± 1.15 *	1.56 ± 0.36*
Granulocytes (x$10^3\mu L^{-1}$)	3.83 ± 0.9	9.82 ± 2 *	15.91 ± 7.62***	4.56± 1.31	14.3 ± 3.1**	8.49 ± 5.53 *	5.83 ± 1.3
Lymphocytes (%)	93 ± 1.2	88.26 ± 1.8 *	83.29 ± 6.7 **	91.86 ± 1.65	84.9 ± 2.8 *	88.08 ± 4.91*	87 ± 4.0
Monocytes (%)	1.27 ± 0.3	2.4 ± 0.3 **	2.92 ± 0.96 ***	1.71± 0.31	2.7 ± 0.4 **	2.35 ± 0.77**	2.0 ± 0.2
Granulocytes (%)	5.73 ± 1.1	9.34 ± 1.5	13.79 ± 5.76 **	6.46 ± 1.35	12.4 ± 2.4 *	9.57 ± 4.15 *	11 ± 4.0
RBC (x10^6 μL^{-1})	1.2 ± 0.2	1.89 ± 0.1**	2.14 ± 0.58 **	1.917± 0.24*	2.2 ± 0.2 **	1.79 ± 0.25 *	1.331 ± 0.19*
Haemoglobin (gdl^{-1})	6.19 ± 0.8	7.92 ± 0.5	8.84 ± 1.94 *	8.55±0.73 *	9.5 ± 0.8 **	9.36 ± 1.01**	7.33 ± 0.66
Hematocrit (x10^3 μL^{-1})	17.11 ± 2.3	23.08 ± 1.7	25.94 ± 8.85 *	25.61± 2.8 *	27.8 ± 2.7 **	22.5 ± 3.37	18.33 ± 3.3
MCV (μm^3)	147.8 ± 8.2	122 ± 3. *7	119.78 ± 10.44*	135.63± 4.79	129.6 ± 5.4	125.8±11.99*	135.6 ± 10.0
MCH (pg)	53.73 ± 2.9	42.1 ± 0.5 **	41.99 ± 3.82 **	50.63± 5.21	45.7 ± 3.0	52.53 ± 3.18	76.1 ± 23.0
MCHC (gdl^{-1})	36.47 ± 1.3	34.73 ± 0.9	35.45 ± 5.06	37.09± 3.90	35.8 ± 3.01	42.0±3.87**	67.6 ± 30.0
RDW (%)	10.29 ± 1	8.65 ± 0.2	9.45 ± 1.87	7.09± 0.56 *	10.3 ± 1.6	7.73 ± 1.49 *	8.81 ± 0.88
RDW-SD(μm^3)	129 ± 39	49.86 ± 2.9	53.24 ± 24.08	86.67± 21.45	94.1 ± 25.6	55.2 ± 30.69	136 ± 36.0
Platelets (x10^3 μL^{-1})	101.3 ± 25	81.1 ± 26	147.9 ± 199.0	365.5 ±132.92	297.9 ± 153.3	110.2 ± 146.6	195 ± 47.0
MPV (μm^3)	6.01±0.09	6.45±0.095**	5.72 ± 2.05	5.62±0.07**	6.4 ± 0.1 **	5.62 ± 0.38 *	5.975 ± 0.17
PCT (%)	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.13	0.20±0.07	0.2 ± 0.1	0.06 ± 0.08	0.11 ± 0.03
PDW (%)	9.13 ± 0.48	7.43 ± 0.35 *	7.72 ± 2.93	8.68± 0.43	8.9 ± 0.3	8.42 ± 1.06	9.54 ± 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron treatments and their untreated control group. When the studied liver antioxidants metabolites were compared between Diafenthiuron 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 diafenthiuron/L (T6) and their untreated control group. Data is expressed as Mean ± 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±20	137.7±20	171.6±15***	169.4±14***	108.0±14	333±71 *	160.5±4.9
Cholesterol (mg/dl)	255 ± 59	285.8±16	288.5±13	297±19	257±12	429±68	375±48
Creatinine (mg/dl)	0.76±0.3	1.26±0.23	0.63±0.21	1.72±1.3	0.38±0.075	0.79±0.2	0.31±0.09
High density lipoprotein (mg/dl)	96.1±0.3	78.5±8.5	54.7±8.3	98.9±14	75.6±14	52.8±15	56.8±10
Low density lipoprotein (mg/dl)	101.8±33	169.9±16	182.4±17	182.8±27	161.7±19	316±86	277±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 diafenthiuron/L (T6) and their untreated control group. Data is expressed as Mean ± 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±0.09	0.27 ± 0.19	0.28±0.06	0.12 ± 0.11	0.37 ± 0.02	0.2±0.008*	0.202±0.08
	CAT (mg/dl)	1.71±0.005	1.62 ± 0.45	2.17±0.6	1.05±0.03*	1.53 ± 0.1	1.99±0.09	1.86±1.8
	MDA (nmol/gram)	0.17±0.03	0.23± 0.001	0.18±0.02	0.2 ± 0.007	0.22±0.01	0.2±0.05	0.16±0.02
Liver	SOD (unit/gram)	0.18±0.059	0.30 ± 0.16	0.4±0.09*	0.6 ± 0.23	0.4 ± 0.11	0.11±0.03	0.75±0.09
	CAT (mg/dl)	0.77 ± 0.1	0.84±1.9**	1.69±0.06**	1.74±0.38	1.96±0.12*	0.83±0.15	1.08±0.2
	MDA (nmol/gram)	0.14 ± 0.0003	0.13 ± 0.09	0.14 ± 0.02	0.11±0.00 **	0.13±0.02	0.17±0.01	0.17±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). Diafenthiuron and its metabolites are toxic for live forms as it has been documented that diafenthiuron 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). Diafenthiuron 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 diafenthiuron 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 diafenthiuron to be LC_{50} 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 diafenthiuron 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 diafenthiuron in all treatments (Table I). Haematocrit, haemoglobin and MCHC were also increased significantly in diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron 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 diafenthiuron doses (T1 and T2) while a significant decrease was observed in lymphocytes percentage in most diafenthiuron 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 diafenthiuron 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 P_{450} and monoxygenases 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 diafenthiuron (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 diafenthiuron 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 (H_2O_2), superoxide (O_2^-) 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 O_2 and H_2O_2 (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 diafenthiuron 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 O_2^- (Kohen and Nyska, 2002). It reacts with H_2O^2 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 Diafenthiuron 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.

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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.

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