© by PSP Volume 25 – No. 12a/2016, pages 5935-5943 Fresenius Environmental Bulletin

STUDY ON ACUTE TOXICITY, HAEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS INDUCED BY THE EXPOSURE OF DDT TO CATFISH

Zubair Ahmad*, Khalid A Al-Ghanim, Hmoud F Alkahem Al-Balawi, F Al-Misned, Shahid Mahboob

Zoology Department, College of Science, P. O. Box 2455, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia.

ABSTRACT

Healthy and active *Clarias gariepinus* were exposed to acute and sub-lethal concentrations of DDT for a period of 96 hours and 30 days, respectively. The 96 h LC_{50} was registered as 1.55 mg/l. It has been observed that protein concentration was decreased, and the glucose was elevated in the plasma of treated fish. Sub-lethal DDT exposure has decreased significantly ($P<0.05$) the level of glycogen in muscle and liver. The DDT has caused a decrease in the counts of erythrocyte (RBC) and leucocyte (WBC), haematocrit (Hct) values and concentration of haemoglobin (Hb). Various hematological indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also changed. The concentration of magnesium (Mg) was unchanged but calcium (Ca) was reduced in the blood of exposed fish. The glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activity was increased in the DDT treated fish. It has been concluded that these effects were more pronounced in higher concentration and in the last period of exposure.

KEYWORDS:
Clarias gariepinus, DDT, Acute concentration, Biochemical parameters, Blood profile

INTRODUCTION

It is an undeniable fact that use of insecticides in agriculture and to control the vector of different diseases contributes to pollution of aquatic ecosystem. The DDT most widely applied chlorinated hydrocarbon poses a more serious problem due to its stability in the natural environment and biological magnification. It is quite obvious that by way of surface runoff considerable quantities of DDT enter the aquatic environment. Hence, it is imperative that effect of this toxicant on fish should be thoroughly investigated to motivate the environmental concerns for applying remedial measures before the situation gets worst. Recent years have witnessed the surge of interest in investigating the physiological, haematological and pathological disorders caused by DDT [1-4].

Fishes are sensitive to environmental quality. Behavior is the first sign of their reaction to altered medium. Besides, there are chemical constituents of the body which respond to deterioration of water quality. Some of the chemical changes are directly caused by the toxicants but other form part of fishes' physiological adaptive syndrome in situations of serious stress. Alterations in somatic and organ conditions are a manifestation of cumulative effect of the interaction between the external and internal environment of fish. Earlier works [1, 4, 5-7] amply emphasized the need to evaluate biochemical composition of tissues and haematological parameters in assessing the health and general condition of fish. Many investigaters have studied the effect of several organochlorine insecticides on the fish and possible consequences on human health [8-12]. Because these are good source of food for human, it is possible that when DDT or its metabolites are deposited in different organs of fish, they will reach to mankind. The high ability of fish to tolerate the effects of these insecticides and long persistance build the interest regarding this condition because both the fish and pollutants remain in the environment for long time, and humans are, therefore, accessable to pesticide exposure. In the present investigation an attempt was made to evaluate the variations in the blood profile and chemical composition in the cat fish exposed to DDT. Previous studies have shown that when the water quality is affected by toxicants, any physiological change will be reflected in the values of one or more of the haematological parameters [13]. Undoubtedly, blood cell responses are important indicators of changes in the internal and /or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Presently, for environmental monitoring and research in toxicology, the different hematological parameters are investigated to evaluate the pathological and physiological changes and disease demonstration of fishery management and

aquaculture [14]. Moreover,, the studies related to the detection of aberration in the fish (chemical composition and haematological profile) could provide symtoms of exposure to toxicants before any massive evidence become apparent and therefore are used as reliable indices of fish health. It is also worth to mention that changes in chemical composition and haematological profile are considered to provide symptoms about the type of toxicants and degree of pollution in the environment [15]. In the present investigation the parameters selected include RBC and WBC counts; levels of haemoglobin and haematocrit; MCV, MCH and MCHC indices; plasma glucose, protein, GOT and GPT enzymes, Ca and Mg, and glycogen and protein contents in liver and muscle.

MATERIALS AND METHODS

Healthy and active individuals of catfish (length 22-26 cm and weight 55-78 g) were collected from an artificial pond at Mozahmia, Riyadh. The fish were kept in large aquaria to acclimatize to physical and chemical conditions of lab for two weeks. The fish were fed a commercial fish food twice daily to satiety during this period. When the time of acclimation overs, ten fishes were transferred in each aquarium with different concentrations (1.0, 1.25, 1.5, 1.75, 2.0 and 2.25 mg/l) of dichlorodiphenyltrichloroethane (DDT). A known quantity of DDT was dissolved in known volume of acetone and this solution was added to aquarium water to obtain the required concentration. Same volume of acetone as used in experimental tanks was also added to control tanks. The experiment was run for 96 hours in triplicates. To maintain the concentration of DDT constant the medium of aquarium renewed on daily basis. The dead fishes from each concentration were removed and their number registered. The LC_{50} was calculated from the graph prepared between probit of kill (percent mortality is transformed into probit) and $\log_{10}$ concentration of DDT (Concentration of DDT is transformed into $\log_{10}$) using the method of Finney [16].

After determining the LC_{50}, three sub-lethal doses (0.155, 0.233 and 0.310 mg/l; these concentrations correspond to 10, 15 and 20 percent of LC_{50}) of DDT were selected to expose the fish for 30 days. Parallel, a control set was run with water without DDT. These experiments were run in triplicates. The medium of aquarium was renewed on daily basis. The physical and chemical conditions of aquarium water like temperature (24.8±0.7 ºC), pH (7.6±0.5) and dissolved oxygen (7.3±0.6 mg/l) were determined weekly.

Three fish from each exposed and unexposed groups were collected from aquaria, and recorded for their total length and body weight. Collection of blood samples were made by cutting the caudal region to allow the blood from dorsal aorta to flow out. This method is easy and increasingly used for smaller and medium sized fishes. Blood samples were collected in heparinized vials. The method of Blaxhall and Daisley [17] was used to determine the hemoglobin. A micro-hematocrit centrifuge was used to determine the hematocrit values. The blood was diluted with Dace's solution and Turk's solution for the counting of erythrocytes and leucocytes, respectively, using Neubar haemocytometer. The MCV, MCH and MCHC were estimated using the methods outlined by Ghai [18]. The blood was centrifuged for 10 minutes at 6000 rpm at 4˚C and the plasma was collected and stored at -20 ˚C for biochemical analysis. Different kits obtained from BIOMERIEUX (France) were used for the estimation of glucose, total protein, Mg, Ca, GOT and GPT.

The liver was dissected out, placed on absorbent paper to absorb excess liquid and weighed. Samples of white muscle were collected from the site of origin of dorsal fin and above the lateral line. The method of Ashman and Seed [19] was followed for the extraction of glycogen and the method described by Montgomery [20] for its determination. Protein was estimated in dry samples. The technique of Webb and Levy [21] was used for the preparation of dry fat-free samples. The method of Lowry et al. [22] was followed for the determination of protein in dry fat-free samples.

A statistical analysis was done to demonstrate the significance of the differences between the values of different groups. For this test one way analysis of variance (ANOVA) was applied. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The percent mortality of fish caused by different concentrations of DDT is given in table 1. The 96 hours LC_{50} value of DDT for the fish enumerated from the graph (Fig. 1) was 1.55 mg/l.

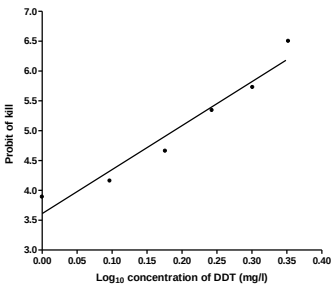

FIGURE 1
Relationship between probit of kill and Log_{10} concentration of DDT

TABLE 1
Number of dead fish, *Clarias gariepinus,* and their percentage mortality (in parentheses) following the treatment of DDT

Concentrations of DDT (mg/l)	Exposure Time (hour)			
	24h	48h	72h	96h
Control (0.0)	-	-	-	-
1.0	-	-	2 (6.66)	4 (13.33)
1.25	-	-	4 (13.33)	6 (20.00)
1.5	-	3 (10.00)	7 (23.33)	11 (36.66)
1.75	2 (6.66)	5 (16.66)	10 (33.33)	19(63.33)
2.0	3 (10.00)	6 (20.00)	14 (46.66)	23 (76.66)
2.25	6 (20.00)	14 (46.66)	22 (73.26)	28 (93.33)

TABLE 2
Changes in different hematological parameters of *Clarias gariepinus* induced by DDT. Values are mean ± standard error.

Parameters	Time (Days)	Control (0.0)	DDT concentrations (mg/l)			
			0.155	0.233	0.310	P<0.05
Erythrocytes	10	1.89 ±0.25	1.65±0.08	1.64 ±0.09	1.65 ±0.06	
(Cellx10^6/mm^3)	20	1.78 ±0.08	1.63±0.05	1.68 ±0.08	1.43 ±0.08	*
	30	1.78 ±0.09	1.64±0.06	1.63 ±0.07	1.42 ±0.07	*
Leucocytes	10	39.55 ±0.62	36.54±0.68	35.65 ±0.75	36.38 ±1.95	
(Cellx10^3/mm^3)	20	38.98 ±0.84	36.87±0.98	34.79 ±0.58	32.12 ±1.50	*
	30	39.45 ±0.85	35.42±0.86	33.01 ±0.52	32.12±1.78	*
Hematocrit	10	35.85 ±0.94	35.23±1.12	34.68 ±0.59	34.14 ±1.15	
(%)	20	36.25 ±0.83	35.25±1.06	34.13 ±1.13	32.46 ±1.21	*
	30	36.49 ±0.91	34.21±1.12	33.45 ±1.06*	30.42 ±1.20	*
Hemoglobin	10	6.75 ±0.19	5.12±0.12	5.62 ±0.13	5.25 ±0.15	*
(g/dl)	20	7.34 ±0.15	5.68±0.15	4.88 ±0.11	4.86 ±0.17	
	30	6.97 ±0.15	5.42±0.14	4.69 ±0.18	4.42 ±0.14	*
MCV	10	189.69±3.85	213.52±4.12	211.06±4.85	206.90±4.58	
(fl/cell)	20	203.65±4.45	216.25±3.45	203.74±3.96	226.99±5.41	*
	30	205.00±4.63	208.60±3.54	205.17±4.55	214.22±5.65	
MCH	10	35.84±1.85	31.03±1.32	34.26±2.65	31.85±2.18	
(Pg/cell)	20	41.23±2.33	34.84±1.25	29.06±2.15	33.87±2.25	*
	30	39.15±2.35	33.05±1.56	28.77±2.45	31.12±2.25	*
MCHC	10	18.82±1.45	14.53±1.24	16.29±1.66	15.79±1.35	
(%)	20	20.24±1.25	16.11±1.12	14.34±1.26	14.47±1.85	*
	30	19.10±1.35	16.60±1.36	14.07±1.46	14.29±1.45	*

The LC_{50} value of DDT for *Moina macrocopa* was documented by Liu et al. [23] as 324 microg/L. In a report of UNEP [9] the LC_{50} of DDT for shrimp was as 0.4 µg/L and 42 µg/L for rainbow trout. Little higher value (2.95 ppm) of 96 h LC_{50} is presented by Mustafa and Murad [5] for cat fish, *Heteropneustes fossilis*. A survey of literature [24-25] revealed that the LC_{50} values of DDT for other teleost were less than the value registered in the present study. This may be ascribed to the lesser susceptibility of air breathing fish to DDT when compared to fishes which are exclusively aquatic breathing. The LC_{50} value of DDT for *Carassius auratus* was reported as 0.648 mg/kg by Yong et al. [4]. A higher LC_{50} value (2.94 mg/l) of fenvalerate dissolved in acetone was reported for *C. gariepinus* [26]. In another report, Pimentel [8] has summarized the LC_{50} values of DDT for different fish species varying from 0.002-0.08 ppm. These differences in the toxic level of DDT may be attributed to the differences in tolerance and susceptibility among the fish and on its accumulation, bio-transformation and excretion. The different fish species may have dissimilarity in metabolic pathways which develop varied patterns of bio-transformation, leading to the formation of varied toxic metabolites [27]. The level of toxic effects of pesticides may also be attributed to the length and weight, corporal surface/body weight ratio and rate of breathing [28]. Oh et al. [29] have reported three causative factors such as varied inhibition of acetylcholinesterase, detoxification and absorption for the selective toxicity of pesticides for various fish species. In general the toxicity varied with respect to the duration of exposure, species and size of fish [29-30].

The present investigation indicated that sub-

lethal exposure of DDT has altered the level of different blood parameters of *Clarias gariepinus*. Reduction in the red blood cell count, haematocrit values and haemoglobin levels was evident in the fish exposed to different concentrations of DDT (Table 2).

"Fall in the leucocyte count was also occurred in the insecticide (DDT) exposed fish. The reduction was more pronouced in the fishes exposed to higher dose. Change in the values of hematological indices (MCV, MCH and MCHC) was evident in DDT treated fish" [7]. Generally, insecticide exposure to fishes adversely affects and results alterations in different blood parameters. "Complying to the view presented above a fall in the RBC count, hemoglobin level and haematocrit values in fish treated with insecticides was reported by Banaee et al. [31-32] and Ahmad [33-34] and the reduction in these parameters was ascribed to loss of cells and/or reduction in size of cells due to detrimental effects induced by pesticide" [7]. Concordant views have been suggested by Zaki et al. [35]. Decreased level of haemoglobin, RBC count and haematorit values in metal (lead nitrate) exposed *H. fossilis* was reported by Adeyemo [36]. It is believed that toxicants would exert detrimental effects on the haematopoietic system of the fish, thus suppressing the production or consequential fast eradication of different constituents of blood. The results registered in this investigation are similar to those mentioned above and can be related to afore-said factors.

The results obtained in this study showed a decreased leukocyte count (Table 2) which would be attributed to impairments of the haematopoietic system resulted by DDT exposure. The reduction in the leucocyte count may be related to lower supply of these cells to the blood circulation due to less formation or alternatively an increased rate of elimination from circulation and subsequent faster destruction of cells. Same reason was presented by Al-Kahem et al. [37] for the reduction in the leucocyte count. Significant reduction in the number of thrombocytes and lymphocytes may be the main factor for the reduction of leucocyte count in the fish treated with chromium [38]. Reduction in the leucocyte count was demonstrated by the fish exposed to diazinon- based pesticide [39].

Different blood cell indices such as mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) seem to be sensitive changes in the fish treated with pesticides. "Fluctuations in the values of MCV, MCH and MCHC are related with the RBC count, hemoglobin concentration and haematocrit values" [7]. Rao [15] reported variations in these indices after the exposure of pesticide to common carp. Similar results were also presented by Ahmad [40] in the catfish, *H. fossilis* exposed to Uranyl acetate. Reduction in the concentration of Ca and magnesium ions were registered in the fish exposed to DDT especially in the last period of exposure. The pesticide certainly pose detrimental effect on the fish and transform the activity of essential organs like liver and kidneys, interrupting the homeostatic situation of the fish. Similar observations were reported by Al-Akel et al. [41] and Ahmad [33] in the different species of fish after the exposure of various toxicants.

The content of glucose in insecticide exposed fish was significantly ($P<0.05$) elevated (Table 4) which may be caused by the conversion of glycogen into glucose to cope with the augmented demand for energy. Stimuli of stress elicit rapid secretion of hormones like glucocorticoids and catechol amines from adrenal gland of the fish [42] and these are known to produce hyperglycemia in animals. Other reason for such elevation may also be the increased gluconeogenesis response of exposed fish in their attempt to meet their elevated energy demands [43]. The hyperglycemic condition registered in this investigation can also be ascribed to enhanced secretion of these hormones which may cause glycolysis in the DDT exposed fish. The result obtained in the present study agrees with the findings of Abalaka et al. [44]; Ahmad [33-34]; Alkahem-Al-Balawi et al. [37] and Al-Ghanim [45]. The insecticide might changes the activities of kidney and liver causing disruption in the homeostatic situation of the body which might change the levels of metals. Similar to present observations Al-Akel et al. [41] have reported alterations of metal concentrations in the carp, *Cyprinus carpio*, after feeding copper added feed and lend a considerable support to present study.

Hypoproteinaemia in the plasma of DDT exposed fish was registered, especially at higher concentration and in the last period of exposure. Results presented in table (3) clearly indicate the depletion in the concentration of protein in different tissues of the exposed fish. The DDT treatment to catfish influenced the metabolic performance and modified the tissue accumulation of protein and glycogen (Table-3).

"Probably, DDT interrupts in the protein biosynthesis pathway, especially by preventing the functions of polymerases, inhibiting the epigenetic origin of these macromolecules, or else it can increase the break down of preformed molecules of protein" [7]. The investigations made by Mazeaud et al. [46] and Strange et al. [47] have established that toxicants exert stresses on the animal's body, which enhance the secretion of adrenocorticotrophic hormone (ACTH) from pituitary gland. The adrenal gland is stimulated by this hormone to secrete increased amount of corticosteroids which results in higher level of glucose by amplifying the enzymatic transformation of glycogen, protein and fat. Significant hypoproteinaemia was reported by

TABLE 3
Concentrations of protein and glycogen in the liver and white muscle of *Clarias gariepinus* exposed to DDT. Values are mean ± standard error.

Parameters	Concentrations (mg/l)	Exposure Time (Days)					
		Liver			Muscle		
		10	20	30	10	20	30
Protein (mg/100 mg)	Control (0.0)	69.23 ±3.81	67.69 ±2.79	68.37 ±2.88	58.37 ±2.15	56.91 ±2.12	56.51 ±1.99
	0.155	62.31 ±2.11	55.45 ±2.01	54.39 ±2.36	54.33 ±2.03	44.93 ±1.99	43.15 ±3.05
	0.233	61.56 ±3.59	56.34 ±3.75	55.12 ±2.04	56.16 ±3.11	43.86 ±1.99	44.12 ±2.13
	0.310	59.23 ±3.23	54.62 ±2.99	51.98 ±3.11	55.55 ±3.23	42.21 ±2.21	42.23 ±2.14
	P<0.05		*	*		*	*
Glycogen (mg/g)	Control	10.52 ±0.16	10.32 ±0.25	10.60 ±0.27	3.92 ±0.09	3.85 ±0.07	3.68 ±0.08
	0.155	8.25 ±0.16	7.25 ±0.19	7.32 ±0.17	2.95 ±0.06	2.18 ±0.07	2.38 ±0.06
	0.233	8.16 ±0.15	6.25 ±0.16	6.35 ±0.15	2.75 ±0.05	2.15 ±0.04	2.35 ±0.08
	0.310	7.85 ±0.65	5.21 ±0.68	4.88 ±0.45	2.52 ±0.11	2.11 0.09	1.95 ±0.11
	P<0.05		*	*		*	*

TABLE 4
Changes in plasma biochemical composition of *Clarias gariepinus* exposed to sub-lethal concentrations of DDT. Values are mean ± standard error.

Parameters	Time (Days)	DDT concentrations (mg/l)				
		Control	0.155	0.233	0.310	(P<0.05)
Total Protein (g/dl)	10	30.55±2.52	28.65±1.98	27.55±1.87	26.69±2.02	*
	20	29.98±1.67	28.25±1.57	24.98±1.32	24.55±1.45	*
	30	30.85±18.88	28.21±1.23	24.82±1.65	224.12±1.86	*
Glucose (mg/dl)	10	55.65±6.21	65.25±6.25	71.35±7.76	77.24±7.96	*
	20	58.32±7.23	66.25±6.58	76.62±8.15	88.64±7.75	*
	30	57.52±7.84	64.55±5.89	78.25±7.64	98.23±9.25	*
Ca (mg/dl)	10	175.65±15.3	168.21±12.23	155.25±11.2	150.65±13.2	*
	20	185.21±13.2	170.12±10.23	156.45±11.1	148.35±11.6	*
	30	198.65±112.1	165.21±13.2	151.32±09.4	144.63±07.9	*
Mg (mg/dl)	10	38.22±3.15	37.65±2.35	37.03±2.45	36.82±4.15	
	20	37.24±5.01	36.85±2.56	36.75±5.41	35.45±4.23	
	30	36.83±4.51	35.78±2.35	35.52±4.45	34.95±3.44	
PGOT (IU/l)	10	85.26±12.2	94.25±10.23	99.82±10.5	110.26±11.4	*
	20	86.28±14.2	97.25±9.56	118.75±13.3	119.95±9.5	*
	30	88.35±11.2	99.65±12.45	125.25±14.2	128.31±12.2	*
PGPT (IU/l)	10	68.18±4.62	78.25±7.22	88.25±8.55	102.32±6.24	*
	20	70.21±12.3	81.26±9.65	88.28±10.53	99.23±12.25	*
	30	67.65±10.2	80.25±9.88	98.56±11.1	108.25±9.13	*

Omoniyi et al. [48] and Shalaby [49] in the fish treated with different pollutants.This fall in the protein level in the toxicant exposed fish was ascribed to the destruction of cell or necrosis, along with the disruption in protein synthesis pathways [50] or loss of proteins through kidney due to pathological impairments caused by toxicant [51]. In contrast to present observations, hyperprotenaemia was reported by Al-Attar [52]; Omitoyin [53]; Abalaka et al. [44] in animals exposed to toxicants.

The Insecticide exposure significantly (P<0.05) elevated the activity of GOT and GPT enzymes in the fish treated with DDT (Table 4). These enzymes (GOT and GPT) are used as distinguish diagnostic means to determine the toxic effects of different pollutants [54]. Metals exposures at various

concentrations enhance the activity of these enzymes [55]. Exposure of ammonia to fish has elevated the level of these (GOT, GPT) enzymes in the serum [56]. The perception of these authors was that GPT is highly responsive to any environmental alteration. Zaki et al. [35] have reported an enhanced level of ALT activity in malathion exposed fish whereas Abalaka et al. [44] documented that the fish treated with extract of *Porkia biglosa* pods exhibited elevated activity of ALT. These authors believe that the enhanced activity of this enzyme in the toxicant treated fish is due to impairment caused to liver and make their way into blood [57] and /or enhanced synthesis of enzyme in liver. Similar to present investigation, significantly higher activity level of glutamic–oxaloacetic acid transminase (GOT) were recorded [58] in the fish fed diet without docosahexaenoic acid but the activity of SGPT remain unchanged. They found that hepatic parenchyma develop into generalized massive steatosis, showing necrosis centers with diet free of docosahexaenoic acid. "An increased activity level of SGOT and SGPT in *C. punctatus* after the exposure of mercuric chloride and monocrotophos was reported" [59]. "Previous reports have suggested that liver is rich in GOT and GPT, and any damage caused by pollutants to it could result in liberation of large quantities of these enzymes into the blood" [60-61]. Therefore, any increase in the activity level of these enzymes (PGOT and PGPT) is a distinguish indicator of devastation at cellular level [33, 37, 61-62]. However, significant ($P<0.05$) enhancement in the activity of the enzymes registered in this study may be attributed to destruction of liver caused by DDT.

CONCLUSION

The present study revealed that DDT is highly toxic to fish *Clarias gariepinus*. It has high toxic potential to change the chemical composition and haematological profile of fish even at very low doses. Destruction of blood cells and alterations in chemical constituents of tissues and plasma was quite obvious in the fish treated with sub-acute level of pesticide (DDT). The erythrocyte and leucocyte counts and hemoglobin level uniformly showed decreasing trend with the increase in concentration and exposure period. It is hypothesized that the DDT either suppress the activity of haemopoitic organs and/or destroy the existing quantity of the blood constituents. It indicated that enough precaution and care must be taken to prevent discharge of effluent containing even low amount of DDT into water bodies.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Deanship of Scientific Research, King Saud University for funding this research through the Prolific Research Group No. PRG-1436-011.

REFERENCES

[1] Satyanarayan, S., Bejankiwar R.S., Chaudhari P.R., Kotangale J.P., Satyanarayan A. (2004) Impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntiu ticto*. J Environ Sci 16: 631-634.

[2] Okayi, R.G., Annune P.A., Tachia M.U., Oshoke O.J. (2010) Acute toxicity of glyphosate on *Clarias gariepinus* fingerlings. J Res Forest Wildlife Environ 2:150-155.

[3] Mnif, W., Hassine A.I.Z., Bouaziz A., Bartegi A., Thomas O., Roig B. (2011) Effect of endocrine disruptor pesticides: a review. Int J Res Public Health 8: 2265-2303.

[4] Yong, J.I., Guang-Hua L.U., Chao W., Jie Z. (2012) Biochemical responses of freshwater fish *Carassius auratus* to polycyclic aromatic hydrocarbons and pesticides. Water Sci Eng 5: 145-154.

[5] Mustafa, S., Murad A. (1984) Survival, behavioural responses and haematological profile of catfish *Heteropneustes fossilis* exposed to DDT. Japanese J Ichthyol 31: 55-65.

[6] Mustafa, S., Zofair S.M. (1984) Chemical analysis of internal environmental response of carp, *Puntius stigma* to DDT. Proc. III International Congress on Analytical, Technical and Environmental Chemistry, Spain.

[7] Al-Ghanim, K.A., Ahmad Z., Al-Kahem Al-Balawi H.F., Al-Misned F., Mahboob S., Suliman E.M. (2015) Effects of a low-radiotoxicity uranium salt (uranyl acetate) on biochemical and hematological parameters of the catfish, *Clarias gariepinus*. Chinese J Oceanol Limnol http://dx.doi.org/10.1007/s00343-015-4392-3

[8] Pimentel, D. (1971) Ecological effects of pesticides on non-target species. Published by Executive office of the president, Office of the Science and technology. Washington D.C., Pp-226.

[9] (UNEP) United Nations Environmental Programme (1995) Persistent organic pollutants: Informations on POPs, their alternatives and alternative approaches. Source: http://www.popstoolkit.com/about/chemical/ddt.aspx

[10] Turusov, V., Rakitsky V., Tomatis L. (2002) Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence and risks. Environ Health

Perspect 110: 125-135.
[11] Pathak, R., Ahmed R.S., Tripathi A.K., Guleria K., Sharma C.S., Makhijani S.D., Banerjee B.D. (2009) Maternal and cord blood levels of organochlorine pesticides: Association with preterm labor. Clinical Biochem 42: 746-749.
[12] Azmi, M.A., Naqvi S.N.H. (2011) Pesticide pollution, resistance and health hazards: In Pesticides-The impact of pesticides exposure, Prof. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-531-0, In Tech publisher, Croatia and Shanghai. 446p.
[13] Van Vuren, J.H.J. (1986) The effects of toxicants on the haematology of *Labeo umbratus* (Teleosti: Cyprinidae). Comp Biochem Physiol 93: 155-159.
[14] Mulcahy, M.F. (1975) Fish blood changes associated with diseases. A haematological study of Pike lymphoma and Salmon ulcerative dermal necrosis. In: The pathology of fishes. W.E. Ribelin, C. Migaki Madison (eds.), University of Wisconsin, pp925-944.
[15] Rao, D.S. (2010) Carbaryl induced changes in the haematological, serum biochemical and immunological responses of common carp, *Cyprinus carpio,* (L.) with special emphasis on herbal extracts as immunomodulators. Ph. D. Thesis, Andhra University, India. Pp-235.
[16] Finney, D.J. (1971) Probit analysis. 3rd edition, Cambridge Press, New York. NY.
[17] Blaxhall, P.C., Daisley K.W. (1973) Routine haematological methods for use with fish blood. J Fish Biol 5: 771-781.
[18] Ghai, C.R. (1986) A text book of practical physiology. Medical Publishers, New Delhi.
[19] Ashman, P.U., Seed J.R. (1973) Biochemical studies in vole, *Micritous montamus.* I. The daily variation of hepatic-6-phosphatase and liver glycogen. Comp Biochem Physiol 45: 365-378.
[20] Montgomery, R. (1957) The determination of glycogen. Arch Biochem Biophys 67: 378-386.
[21] Webb, J.N., Levy H.B. (1955) A sensitive method for the determination of deoxyribonucleic acid in tissues and micro-organisms. J Biol Chem 213: 107-117.
[22] Lowry, O.H., Rosebrough J.N., Farr A.L., Randall R.J. (1951) Protein measurement with Folin phenol reagent. J Biol Chem 103: 265-275.
[23] Liu, X.B., Xi Y.L., Wang J.L., Hu K. (2008) Acute toxicity of DDT and its effects on life table demography of *Moina macrocopa*. J Appl Ecol 19: 1343-1348.
[24] Johnson, D.W. (1968) Pesticides and fishes: A review of selected literature. Trans Am Fish Soc 97: 398-424.
[25] Spehar, R.L., Christensen G.M., Curtis C., Lemke A.E., Norberg T.J., Pickering Q.H. (1982) Effects of pollution on freshwater fish. J Water Pollut Control Fed 54: 877-922.
[26] Bhattacharya, B., Kaviraj A. (2009) Toxicity of pyrethroid pesticide fenvalerate to freshwater catfish *Clarias gariepuinus:* Lethality, biochemical effects and role of dietary ascorbic acid. J Environ Sci Health Part B, 44: 578-583.
[27] Johnsson, C.M., Toledo M.C.F. (1993) Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. Archiv Environ Conta Toxicol 24:151-155.
[28] Murty, A.S. (1986) Toxicity of pesticides to fish. CRC Press Inc Boca Raton, FL 143p.
[29] Oh, H.S., Lee S.K., Kim Y.H., Roh J.K. (1991) Mechanism of selective toxicity of diazinon to killifish (*Oryzias latipes*) and loach (*Misgurnus anguillicaudatus*). *A*quatic Toxicol Risk Assessm 14: 343-353.
[30] Dutta, H.M., Munshi J.S.D., Dutta G.R., Singh N.K., Adhikari S., Richmonds C.R. (1995) Age related differences in the inhibition of brain acetylcholinesterase activity of *Heteropneustes fossilis* (Bloch) by malathion. Comp Biochem Physiol 111A: 331-334.
[31] Banaee, M., Mirvaghefi A.R., Rafei G.R., Majazi Amiri B. (2008) Effects of sub-lethal diazinon concentrations on blood plasma biochemistry of common carp. Int J Environ Res 2: 189-198.
[32] Banaee, M., Sureda A., Mirvaghefi A.R., Ahmadi K. (2011) Effects of diazinon on biochemical parameters of blood in rainbow trout (*Onchorhynchus mykiss)*. Pest Biochem Physiol 99: 1-6.
[33] Ahmad, Z. (2011) Toxicity bioassay and haematological changes induced by diazinon in common carp, *Cyprinus carpio.* Afr J Biotech 10: 13852 -13859.
[34] Ahmad, Z. (2012) Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus*. Afr J Biotech 11: 8578-8585.
[35] Zaki, M.S., Mostafa S.O., Nasr S., Noor El-Deen A.I., Ata N.S., Awad I.M. (2009) Biochemical, clinicopathological and microbial changes in *Clarias gariepinus* exposed to pesticide malathion and climate changes. Rep and Opn 6-11.
[36] Adeyemo, O.K. (2007) Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. Turk J Fish Aquat Sci 7: 163-169.
[37] Alkahem Al-Balawi, H.F., Ahmad Z., Al-Akel A.S., Al-Misned F., Suliman E.M., Al-Ghanim K.A. (2011) Toxicity bioassay of lead acetate and effects of its sub-lethal exposure on growth, haematological parameters and reproduction in *Clarias gariepinus*. Afr J Biotech 10: 11039-11047.
[38] Alkahem, H.F. (2005) Behavioral responses

and changes in some haematological parameters of the cichlid fish, *Oreochromis niloticus*, exposed to trivalent chromium. J King Abdul Aziz Univ Sci 7: 5-13.

[39] Jaffer Ali, H.A., Rani V.J. (2009) Effect of phosalone on haematological indices in the tilapia, *Oreochromis mossambicus*. Turk J Vet Anim Sci 33: 407-411

[40] Ahmad Z. (2014) Biochemical and haematological changes induced by low-radiotoxicity uranium salt (uranyl acetate) in *Heteropneustes fossilis.* J Pure Appl Microbiol 8(Spl. Edn. 2): 593-603.

[41] Al-Akel, A.S., Alkahem-Al-Balawi H.F., Al-Misned F., Mahboob S., Ahmad Z., Suliman E.M. (2010) Effects of dietary copper exposure on accumulation, growth and hematological parameters in *Cyprinus carpio.* Toxicol Environ Chem 92: 1865-1878.

[42] Pickering, A.D. (1981) Stress and compensation in teleostean fishes: Response to social and physical factors. In: Pickering, A.D., (eds) Stress and fish. Academic Press, New York, USA. Pp295-322.

[43] Winkaler, E.U., Santosh T.R.M., Machdo-Neto J.G., Martinez C.B.R. (2007) Acute lethal and sub-lethal effects of neem leaf extracts on neotrapical freshwater fish, *Prochilodus lineatus*. Comp Biochem Physiol Part C 145: 236-244.

[44] Abalaka, S.E., Esievo K.A.N., Shoyinka S.V.O. (2011) Evaluation of biochemical changes in *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods. Afr J Biotech 10: 234-240.

[45] Al-Ghanim, K.A. (2012) Acute toxicity and effects of sub-lethal malathion exposure on biochemical and haematological parameters of *Oreochromis niloticus.* Sci Res Essays 7: 1674-1680.

[46] Mazeaud, M.M., Mazeaud F., Donaldson F.M. (1977) Primary and secondary effects of stress in fish: Some new data with general review. Trans Am Fish Soc 106: 201-212.

[47] Strange, R.J., Schreck C.B., Golden J.T. (1977) Corticoid stress responses to handling and temperature in salmonids. Trans Am Fish Soc 106: 213-218.

[48] Omoniyi, I., Agbon A.O., Sodunke S.A. (2002) Effects of lethal and sub-lethal concentrations of Tobacco (*Nicotiana tobaccum)* leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell). J Appl Sci Environ Management 6: 37-4.

[49] Shalaby, A.M.E. (2009) The opposing effects of ascorbic acid (Vitamin C) on ochratoxin toxicity in Nile tilapia (*Oreochromis niloticus*). Link: http://www.ag.arizona.edu/ista/ista6web/pdf/209.pdf.Rterieved:05-04-9

[50] Bradbury, S.P., Symonic D.M., Coats J.R., Atchison G.J. (1987) Toxicology of fenvalerate and its constituent isomers to the fathead minnow (*Piephales promeos*) and blue gill *(Lepomis macrochirus*). Bull Environ Contm Toxicol 38: 727-735.

[51] Salah El-Deen, M.A., Sharada H.I., Abu-El-Ella S.M. (1996) Some metabolic alteration in grass carp (*Ctenopharyngodon idella*) induced by exposure to cadmium. J Egypt Ger Soc Zool 21: 441-457.

[52] Al-Attar, A.M. (2005) Biochemical effects of short-term cadmium exposure on the freshwater fish, *Oreochromis niloticus*. J Biol Sci 5: 260-265.

[53] Omitoyin, B.O. (2007) Plasma biochemistry changes in *Clarias gariepinus* (Buchell, 1822) fed poultry litter. Asian J Anim Sci 7: 45-52.

[54] Nelson, D.L., Cox M.M. (2000). Lehninger principles of biochemistry. Worth Publishers. New York.

[55] McKim, J.M., Christensen G.M., Hunt E.P. (1970) Changes in the blood of brook Trout (*Salvelinus fontinalis*) after short-term long-term exposure to copper. Can J Fish Aquat Sci 27: 883–889.

[56] Jeney, G., Nemcsok J., Jeney Z.S., Olah J. (1991) Acute effect of sublethal ammonia concentrations on common carp (*Cyprinus carpio* L.). II. Effect of ammonia on blood plasma transminases (GOT, GPT), GDH enzyme activity and ATP value. Aquaculture 104: 49-156.

[57] Mousa, M.M.A., El-Ashram A.M.M., Hamed M. (2008) Effects of Neem leaf extract on freshwater fishes and zooplankton community. 8th International symposium on tilapia in aquaculture. The Central Laboratory for Aquaculture Research, Cairo, Egypt. October 12-14.

[58] Lemaire, P., Drai P., Mathieu A., Carriere S., Giudicell J., Lafaurie M. (1991) Changes with different diets in plasma enzymes (GOT, GPT, LDH, ALP) and plasma lipids (Cholesterol, triglycerides) of sea- bass (*Dicentrarchus labrax).* Aquaculture 93: 63-75.

[59] Agrahari, S., Pandey K.C., Gopal K. (2007) Biochemical alteration induced by monocrophos in the blood plasma of fish, *Channa punctatus* (Bloch). Pest Biochem Physiol 88: 268-272.

[60] Vaglio, A., Landriscina C. (1999) Changes in liver enzyme activity in the teleost *Sparus aurata* in response to cadmium intoxication. Ecotoxicol Environ Saf 43: 111–6.

[61] Palanivelu, P., Vijayavel K., Balasubramanian S.E., Balasubramanian M.P. (2005) Influence of insecticidal derivatives (Cartap Hydrochloride) from the marine polychaete on certain enzymes of the freshwater fish

Oreochromis mossambicus. J Environ Biol 26: 191-196.

[62] Van-Der, R.O., Jonny B., Vermeulen N.P.E. (2003) Fish bioaccumulation and biomarkers in environmental risk assessment, a review. Environ Toxicol Pharmacol 13: 57-149.

Received: **01.03.2016**
Accepted: **20.09.2016**

CORRESPONDING AUTHOR

Zubair Ahmad
Zoology Department, College of Science, P. O. Box 2455, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia.

e-mail: zahmed@ksu.edu.sa