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Full-Length Research Paper

Combined Effects of Agrochemicals on the Serum Biochemistry of *Clarias Gariepinus* (Burchell, 1822) Juveniles

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ABSTRACT: Changes in serum biochemical parameters in response to combined-agrochemicals exposure were studied in an indigenous African fish *Clarias gariepinus*. Fish in 3 groups were exposed to sublethal concentrations of a mixture of paraquat, profenofos, cypermethrin, and NPK 15:15:15 fertilizer. Treatment groups 1, 2, and 3 received concentrations at 0.002mg/L, 0.007mg/L, 0.012mg/L obtained from 1/10, 1/30, and 1/50 of the 96h LC_{50} value which was recorded at 0.024mg/L. Results of ALT showed a high deviation from control values of 75.98 u/L to treatment values of 78.19, 86.77, and 88.28 u/L after 56 days. AST values at day 56 showed a similar increase from 116.35 u/L (control value) to 154.26, 170.98 and 178.38 u/L (treatment values). Similar elevations in values of biochemical parameters at $P<0.05$ were seen in ALP, CRT and Urea values. The elevated activities of serum ALT and AST indicate liver damage or enhanced transamination. It can therefore be hypothesized that the different toxicants used in this study induced stress in the fish, this is consequently related to the disruption of organ function. This study indicates that biochemical parameters in fish blood can be used as an indicator of toxicity in water bodies.

Keywords: Agrochemicals, Toxicity, *Clarias gariepinus*, Serum biochemistry

INTRODUCTION

Food from aquatic environments makes an important contribution to human nutrition and health and is also sought and enjoyed by people for cultural and gastronomic reasons. Maintaining the long-term production and supply of such food, both wild-capture fisheries and aquaculture, is a significant and ongoing challenge for society (FAO, 2014). Aquatic food plays a pivotal role in daily nutrition and provides various essential nutrients in an already healthy and ample diet. To meet the growing demand of food due to the ever-increasing human population, especially under seemingly unfavorable conditions, farmers have been known to use a variety, and in most cases, a combination of agrochemicals for crop improvement (Ito *et al.,* 2020). This is worrisome because following the application of these agrochemicals on the farms, there are undefined proportions that have been reported to enter into the aquatic environment through sources such as runoffs and spray drifts. Aquatic animals are reported to be variably sensitive to a wide variety of toxicants in water. The toxicities of agrochemicals to aquatic organisms have been reported to be determined by the toxicity of the agrochemical, the species of aquatic organism and the duration of exposure of the organism to the toxicants (Khaled *et al.,* 2015). Also, various species of fish show uptake and accumulation of many contaminants or toxicants. Some aquatic organisms are known to have the ability to bioaccumulate chemicals in considerable amounts and more often in concentrations far above the amounts in their environments, these high levels of toxicants have been reported to cause deleterious effects in growth parameters, hematology and serum biochemistry (Pandey *et al.,* 2018). The African Catfish (*Clarias gariepinus)* is a fish species indigenous to most

water bodies in Africa and the biochemical parameters of the resident fish serve as indicators of water quality which can be used to detect pollution in water. Prominent among these biomarkers are physiological variables, such as serum or plasma levels of metabolites and ions, levels of hormones like cortisol (Martins *et al. 2008*) and biochemical variables such as detoxifying enzymes activities. Other fish species, such as Nile tilapia, *Oreochromis niloticus* have been assessed for an effect of transported profenofos, (Khan, 2019; Sharafeldin *et al*., 2015), the results included a deviation for the normal range of blood parameters values. The aim of this study was to show the effects of chronic toxicity of a mixture of paraquat, cypermethrin, profenofos and NPK 15:15:15 fertilizer on the biochemical analysis of the serum of African Catfish, *Clarias gariepinus*.

MATERIALS AND METHODS

Fish sampling

384 juvenile African Catfish of similar sizes (40g – 60g) were held in aerated plastic receptacles and allowed to acclimatize under laboratory conditions for 14 days under natural photo period (OECD, 2019).

Test compounds

Paraquat, Sharpshooter (Profenofos 40% + Cypermethrin 4%) and NPK (15:15:15) fertilizer were used in this study.

Toxicity tests

Range finding and acute toxicity test

The concentration of agrochemicals used for the acute toxicity test was determined by a preliminary (Range finding) test (Solbe, 1995). Results obtained from the range finding test served as a guide for the definitive (acute toxicity) test. Concentrations used for the definitive (acute toxicity) test were 0.000, 0.010, 0.020, 0.030, 0.040 and 0.050mg/l for the control group and Group 1-5.Two replicates per test concentration was used to avoid test repetition due to system failure and to provide a stronger statistical baseline. Each test chamber contained an equal volume of test solution (10 liters) and equal number of fish (n=8). The 96 h LC_{50} values were obtained by Probit Analysis.

Chronic toxicity test

1/10, 1/30, 1/50 (0.002mg/L, 0.007mg/L, 0.012mg/L)of the 96h LC_{50} value recorded at 0.024mg/L were used for the sub-lethal experiment for 56 days. A total of 240 acclimated fish were divided into 1 control group and 3 experimental groups (n=20/ group) which ran in triplicates.

Sample extraction and analysis

The test animals were randomly picked for serum biochemical studies at an interval of 0, 7, 14, 28 and 56 days using a hand-held scoop net. Biochemical analysis was carried out on the blood of the fish which was obtained by bleeding the fish via caudal veni-puncture using a 23G needle fitted on a 5 ml syringe. Blood in plain bottles were allowed to cloth and then spurn with a bench centrifuge at 3000 RPM for 5minutes. Serum was separated from the plasma using an automated pipette and dispensed into sample tubes which were put into the biochemistry analyzer (Merck microlab 300) loading tray. Parameters such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Urea (U), Creatinine (CRT) were investigated.

Statistical analysis

The data were subjected to two-way analysis of variance (ANOVA) and results giving p values ≤ 0.05 were considered significantly different and further analyzed by Duncan's multiple range tests, in statistic package for social science 17.0 (SPSS Inc. Chicago, Illinois, USA). Differences between groups were considered statistically different for p values ≤ 0.05. The results were expressed as mean±SD error.

RESULTS

The variations in ALT, AST, ALP, UN and CRT are indicated in (Table 1). Values for ALT of treated fishes with 0.000, 0.002, 0.007 and 0.012 mg/L of a mixture of Paraquat, Profenofos (40%) + Cypermethrin (4%) and NPK 15:15:15 fertilizer for 56 days ranged from 64.06 to 75.98 u/L in the control group; and 71.18 to 78.19; 68.75 to 86.77 and 72.27to 88.28 u/Lin the experimental group 1, 2 and 3 respectively. Values for AST ranged from 115.30 to 118.17 u/L in the control group; and 116.18 to 172.54; 112.95 to 170.98and 115.14 to 178.38 u/Lin the experimental group 1, 2 and 3 respectively.

ALP values of fish after exposure to the combined toxicants for 56 days ranged from 13.14 to 14.20 u/L in the control group; and 14.03 to 18.02; 13.66 to 18.35; and 13.94 to 18.13 u/L in the experimental group 1, 2 and 3 respectively. An increase in CRT values was also recorded and ranged from 0.65 to 1.44 mg/dL in the control group; and 1.05 to 2.49; 0.94 to 2.69 and 1.31 to 2.89 mg/dL in the experimental group 1, 2 and 3 respectively. Similar elevation was also seen in Urea values of fish which ranged from 2.98 to 3.66 mg/dL in the control group; 3.96 to 5.96; 4.14to 5.88 and 4.82 to 6.08 mg/dL in the experimental group 1, 2 and 3 respectively.

Table 1: Effects of a mixture of Paraquat, Profenofos (40%), Cypermethrin (4%) and NPK 15:15:15 fertilizer on some biochemicals parameters in *C. gariepinus*

PARAMETERS	CONC (mg/L)	EXPOSURE DURATION (Days)				
		0	7	14	28	56
ALT (u/L)	control	71.19±3.0	68.01±6.0	64.06±4.1	68.38±6.02	75.98±6.4
	0.002	71.18±2.3	75.49±3.7	71.23±0.8	81.60±1.5	78.19±9.6
	0.007	68.75±0.9	75.49±9.3	81.95±1.4	86.75±1.1	86.77±4.8
	0.012	72.27±1.7	79.55±2.7	82.44±4.9	81.56±1.6	88.28±2.7
AST (u/L)	control	118.17±2.4	116.06±9.5	120..7±2.1	115.30±4.0	116.35±8.0
	0.002	116.18±0.7	160.08±0.4	167.11±6.6	172.54±5.0	154.26±2.5
	0.007	112.95±7.3	163.24±6.6	168.54±6.6	170.54±4.2	170.98±9.4
	0.012	115.14±6.5	171.43±7.3	175.32±5.3	174.65±9.3	178.38±2.3
ALP (u/L)	control	13.85±1.9	14.04±0.4	13.42±0.8	14.20±0.7	13.14±0.7
	0.002	14.03±1.2	16.38±0.6	16.82±0.1	17.39±0.1	18.02±0.1
	0.007	13.66±0.5	17.52±0.4	17.58±0.2	17.86±0.1	18.35±0.1
	0.012	13.94±0.8	16.63±0.3	17.89±0.4	17.99±0.02	18.13±0.4
CRT (mg/dL)	control	0.65±0.18	1.26±0.1	1.44±0.23	1.12±0.2	0.78±0.2
	0.002	1.05±0.06	1.89±0.15	1.44±0.1	1.96±0.06	2.49±0.03
	0.007	0.94±0.1	1.99±0.1	1.71±0.1	2.07±0.05	2.69±0.04
	0.012	1.31±0.1	1.98±0.1	1.84±0.1	2.25±0.06	2.89±0.08
U (mg/dL)	control	2.98±1.37	3.35±0.01	3.66±1.01	3.53±0.7	3.27±0.8
	0.002	3.96±0.3	5.01±0.01	5.09±0.04	5.60±0.1	5.96±0.1
	0.007	4.14±0.8	5.07±0.04	5.21±0.06	5.68±0.06	5.88±0.2
	0.012	4.82±0.1	5.13±0.01	5.29±0.06	5.75±0.2	6.08±0.3

Key
ALT - Alanine aminotransferase
AST - Aspartate aminotransferase
ALP - Alkaline Phosphatase
U - Urea
CRT - Creatinine

DISCUSSION

In this study, variations in enzyme parameters indicated that AST, ALT, ALP, UN and CRT significantly increased in the exposed group as compared to control during the experiment. The use of serum AST and ALT enzyme activities in fish as bioindicators of toxicant of aquatic ecosystems have been recorded by Kumar, (2016) and Khaled *et al*. (2015). This study is in consonance with Das *et al*. (2004) who recorded an elevation in the activity level of AST, ALT and ATP of major Indian carps following exposure to nitrite toxicity. ALT and AST activities were also seen to increase in the study of John (2007) following exposure of *Mystusvittatus* to metasystox and sevin, similarly increase in ALT and AST activities were also recorded in *Oncorhynchus mykiss* exposed to verapamil (Li *et al*., 2011). The elevated activities of serum ALT and AST indicate liver damage or enhanced transamination. Increased transamination during pesticide challenge has been attributed to the need to meet higher energy demanded by fish (Saravanan *et al.,* 2011). ALP values similarly showed an increase at $P<0.05$. CRT and UN values in this study showed elevation in comparison to the control values. CRT and UN are the most abundant non-protein nitrogen constituents in the body and their determination are the most requested tests of the kidney's ability to excrete metabolic wastes (Tresseler, 1988). Increase in these values are used as indicators of renal failure, it can be hypothesized that the different toxicants used in this study induced stress in the fish which is consequently related to the impairment of the renal function. The presence of increasing Creatinine concentration in the blood suggests that the function of glomerular filtrate may also be impaired. Increase in enzyme parameters are synonymous with the findings of Nwani *et al*. (2015) which demonstrated that biochemical activities in the African catfish is impaired by toxicants. The high levels of serum biochemical values are a clear indication of fish stress. This study highlights the need for new studies to explore howcomplex mixtures of agrochemicals can affect biochemical parameters of fish species.

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