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Physiological effects of paraquat in juvenile African catfish *Clarias gariepinus* (Burchel 1822)

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PEER REVIEW

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Comments

This is an interesting research work in which authors have investigated the physiological effects of paraquat in juvenile African catfish *C. gariepinus*. The activity was assessed based on haematological and biochemical parameter in the blood and plasma while that of the morphology was based on the body weight and liver of the African catfish.
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ABSTRACT

Objective: To investigate the physiological effects of paraquat in African freshwater catfish *Clarias gariepinus*.

Methods: Two sublethal test concentrations of paraquat (1.37 and 2.75 mg/L) were chosen based on the 96 h LC_{50} value (27.46 mg/L). Some experimental fish were exposed to these concentrations and control group for 15 d. Peripheral blood samplings were taken at intervals for assessment of haematological and biochemical parameters.

Results: Exposure to paraquat affected behaviour and morphology of *Clarias gariepinus*. There were significant decreases (P<0.05) in the mean values of hemoglobin, red blood cells, packed cell volume, cellular hemoglobin, and cellular hemoglobin concentration. The levels of white blood cells, glucose, aspartate aminotransferase, and alanine aminotransferase significantly increased (P<0.05) while protein levels declined. However, no definite pattern of changes was observed in the number and type of leucocytes.

Conclusions: The results of the present study indicate that paraquat is toxic and has the potential to impair on the physiological activities in African catfish *Clarias gariepinus*. The use of paraquat should be strongly controlled and carefully monitored to avoid the possible damage done to the environment.

KEYWORDS

Paraquat, Toxicity, Morphological indices, Hematological, Biochemical parameters, *Clarias gariepinus*

1. Introduction

The growth of human population and increasing activities associated with agriculture, urbanization and industrialization have resulted in a staggering release of anthropogenic sources, including pesticides. Paraquat (1,1′-dimethyl-4-4′-bipyridinium dichloride) is the second widely used herbicide in the world for controlling weeds[1]. It is also used as a defoliant and desiccant to aid in the harvesting of cotton, beans, soybeans, potatoes, sunflowers, and sugarcane[2]. Paraquat is registered and sold under different trade names, such as Gramoxone, Crisquat, Dextrone X and Esgram in approximately 100 developed and developing countries. However, it

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has been banned in the European Union countries since 2007[3]. Due to the high solubility and repeated use of paraquat in agricultural and non-agricultural areas, such as horticulture, large quantities of paraquat could penetrate surface water through runoff with damaging effects to the biota[4]. Even at low concentrations, it still could elicit deleterious effects, such as cytogenetic damage, physiological effects, and even death, to exposed non-target species[3]. In plants, paraquat interferes with intracellular electron transfer systems with reactive oxygen species (ROS) including the superoxide anion and hydroxyl radicals, which interact with the unsaturated membrane lipids and results in the destruction of organelles and ultimately lead to cell death[5].

The mechanism of paraquat toxicity may also be attributed to its redox potential, which involves cyclic reduction-oxidation reactions that produce ROS and depletion of nicotinamide adenine dinucleotide phosphate hydrogen[6]. Photodegradation of paraquat results in the formation of N-methylisonicotinic acid, which further decomposes to yield methylamine-hydrochloride and carbon dioxide. In humans, paraquat poisoning has been reported to cause respiratory failure, severe central nervous system injury, and Pakinston's disease[7]. In fish, paraquat has been reported to alter the activity of several enzymes. Thus, paraquat can affect the cardiac contraction, opercula ventilation, and embryonic development[8].

Toxic substances in aquatic environment can affect fish growth indirectly by reducing food availability, or directly by changing their metabolism. Thus, toxins cause an increase in energy requirements for maintaining homeostasis[9]. Two morphological parameters, like condition factor (CF) and hepatosomatic index (HSI), have been proposed as indices of environmental contamination. CF is a measure of fish fitness for a given length. For instance a heavier fish is in a better condition[10]. HSI refers to the relative liver size and relates to the hepatic enzyme activity for chemical detoxification[11].

Haematological parameters have been used as biomarkers for physiological and pathological alterations in fishery management and disease investigation[12]. These parameters show the number of red blood cells (RBC), haemoglobin (Hb), white blood cells (WBC) and packed cell volume (PCV)[12]. In addition, blood indices have been used, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)[12]. To evaluate toxic stress of environmental contaminants, biochemical parameters have also been used widely, these parameters include the levels of plasma proteins, glucose, and other enzymes, like alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[13,14].

Previous studies have investigated the toxicological effects of paraquat in fish[15-17]. However, the effects of paraquat on tropical fish species have not been completely shown. Due to its inexpensive cost, African catfish like *Clarias gariepinus* (*C. gariepinus*) is the main source of animal protein in most commercial food fish[18]. This species is commonly cultured in ponds and can be obtained freely in most natural freshwater bodies. This fish is in high demand in developing countries and can narrow the gap between demand and supply of animal protein in these areas. It can easily acclimatize to laboratory condition. Thus, it is an excellent model for ecotoxicological studies. The aim of the present study is to evaluate the effects of sublethal concentration of paraquat on morphology, hematology and biochemical parameters in *C. gariepinus*.

2. Materials and methods

2.1. Experimental fish and chemical

The live juvenile African catfish, *C. gariepinus* (n=250, average weight and length of (21.48±3.32) g and (11.37±1.23) cm, respectively) was collected from the Paseli Kuje fish farm (Abuja, Nigeria) and transported to our Fisheries Wet Laboratory where they were acclimatized for 20 d in a plastic tank (capacity of 300 L). Water was changed daily to help *C. gariepinus* to acclimatize to the new environment. The fish were fed daily with a diet (35% crude protein) that was 3% of their body weight at 8 h intervals. Feeding was terminated 24 h before the commencement of the experiment as recommended by Ward and Parrish[19], and Reish and Oshida[20]. The fish were treated in accordance with ethical rules for the experimental animal care set by the University Animal Care Committee. For the present study, a commercial formulation of paraquat (276 g/L), trade-name Gramoxone (CAS number 4685-14-7, manufactured by Hubei Xialong Chemical Industry Co. Ltd., China), was used as stock solution.

2.2. Acute toxicity testing

To determine the 96 h LC_{50} value of African catfish for paraquat, acute toxicity bioassays were conducted in 40 L glass aquaria (60×30×30 cm size) in a semi-static laboratory system with the test solution changed every second day to maintain a constant paraquat concentration. The study was conducted according to the Organization for Economic Cooperation and Development[21], guideline No. 203. A set of 10 fish specimen were randomly exposed to each test concentrations (0, 27, 32, 37, 42, 47 and 52 mg/L), obtained by serial dilution of the stock solution. The experiment was set in triplicate to obtain the 96 h LC_{50} value of African catfish for paraquat exposure. During the acute toxicity test, the mortality and survival rates of the juvenile African catfish were recorded after 24, 48, 72 and 96 h under each test concentrations. Deceased specimens were removed to avoid pollution of the water. During the treatment, fish behaviour was observed daily for hyperactivity, equilibrium status, swimming rate, fin movement, and jerky movements. The lethal concentration values of paraquat were determined using the probit analysis method described by Finney[22]. The physicochemical characteristics of the test water were analysed by standard methods[23], and were as follows: temperature 24.5-26.6 ˚C, pH 7.01-7.06, conductivity 252-284 μS/cm and dissolved oxygen 6.65-7.06 mg/L. The total hardness and alkalinity ranged from 170-185 mg/L and 250-270 mg/L as $CaCO_3$, respectively.

2.3. Sublethal concentrations and in vivo exposure experiment

The 96 h LC_{50} value of African catfish *C. gariepinus* for paraquat was determined by probit analysis. Based on the 27.46 mg/L (96 h, LC_{50}) value obtained, two test concentrations of 1.37 and 2.75 mg/L corresponding to 1/20th and 1/10th of LC_{50}, respectively, were estimated and used for the *in vivo* experiment. A total of 90 acclimatized fish were used for the *in vivo* experiment. A set of 30 randomly selected specimens in triplicate of 10 were exposed to each of the two sublethal test concentrations in a semi-static system with the test solution changed every second day to maintain a constant paraquat concentration. Control specimens were maintained in dechlorinating tap water without paraquat. Exposure lasted for 15 d and blood was collected from both treatment and control groups, on 1, 5, 10, and 15 d post-exposure at a rate of three specimens per interval. Prior to blood collection, each fish was anesthetized with tricaine methanesulfonate MS 222 to minimise stress. Blood was collected by puncturing the caudal vein with a heparinized syringe needle and stored in small ethylene diamine tetraacetic acid treated vials. The whole blood samples were used for the estimation of haematological parameters (Hb, RBC, and WBC counts). The remainder of blood sample was centrifuged at 10000 r/min in a cooling centrifuge for 5 min to obtain plasma, which was used for estimating glucose, protein, AST, and ALT. At the end of the duration of each exposure, body weight, liver weight and standard length of each fish were recorded, while CF and HSI were calculated following the method of White and Fietcher[24], as:

$$CF = \text{Body weight (g)} / \text{Standard length (cm)}^3 \times 100$$

$$HSI = \text{Liver weight (g)} / \text{Body weight (g)} \times 100$$

2.4. Haematological analysis

The RBC and WBC counts were determined using an improved Neubauer haemocytometer as described by Rusia and Sood[25], while counting, the number of various types of leucocytes was also observed. The haemoglobin content was estimated at a wave-length of 540 nm using the cyanmethemoglobin method[26]. The PCV was analysed according to Nelson and Morris[27], with the following modifications. Microcapillary tubes were filled with blood and centrifuged at 11000 r/min for 6 min using a Hawksley haematocrit centrifuge. The mean values were measured with a microcapillary reader. Erythrocyte indices, such as MCHC, MCH, and MCV, were calculated using standard formula[28]:

$$\text{MCHC (per cent)} = \frac{\text{Hb (g/100 mL)}}{\text{PCV/100 mL}} \times 100$$

$$\text{MCH (pg/cell)} = \frac{\text{Hb (g/dL)} \times 10}{\text{RBC count in millions/mm}^3}$$

$$\text{MCV (fL/cell)} = \frac{\text{PCV} \times 10}{\text{RBC count in millions/mm}^3}$$

2.5. Biochemical estimation

Plasma glucose levels were estimated using the o-toluidine method[29], while total plasma protein levels were determined following the methods of Lowry *et al*[30]. Plasma AST and ALT levels were determined following the methods of Reitman and Frankel[31].

2.6. Statistical analysis

The data were subjected to Two-way analysis of the anova variance followed by Duncan's multiple range tests, in statistic package for social science 17.0 (SPSS Inc. Chicago, Illinois, USA) to determine the significance of differences at a 5% probability level. The results were expressed as mean±SD error.

3. Results

3.1. Behavioural changes and mortalities of fish

The results showed that paraquat affected the behavioural characteristics of *C. gariepinus*. The control specimens were not hyperactive and showed normal swimming patterns and fin movements throughout the exposure period. However, with increasing paraquat concentrations and exposure duration time, hyperactivity and jerky movements increased. In contrast, the swimming rate, fin movement, and equilibrium status decreased. Fish mortalities increased as the concentration of paraquat increased (Table 1). For example, after 96 h exposure, the control group and the group exposed to 27 mg/L of paraquat had mortalities of 0% and 60% mortalities, respectively, whereas the group exposed to 47 mg/L paraquat had 100% mortality.

Table 1
Cumulative mortality of *C. gariepinus* exposed to various concentrations of paraquat.

Test concentration (mg/L)	Number of fish exposed	Number of mortality				Mortality (%)	Survival (%)
		24 h	48 h	72 h	96 h		
Control	30	0	0	0	0	0	100
22	30	0	3	6	9	30	70
27	30	0	3	12	12	60	40
32	30	0	6	18	21	70	30
37	30	6	12	24	24	80	20
42	30	15	21	24	27	90	10
47	30	24	27	30	30	100	0

3.2. Morphological parameters

There was a significant reduction ($P<0.05$) in the CF in the fish exposed to 1.37 mg/L by Day 15 and to 2.75 mg/L by Day 5 compared with the control (Figure 1A). In contrast, there was no significant difference ($P>0.05$) in HSI in the control and the exposed fish on Days 1 and 5 but HSI showed a significant decline from Day 10 at 2.75 mg/L (Figure 1B).

3.3. Haematological parameters

The changes in the various hematological parameters (PVC, Hb, RBC, and WBC) are presented in Table 2. The RBC, PCV, and Hb values in the exposed fish significantly decreased ($P<0.05$) compared to the control group. There was an increase in the WBC count in the exposed fish throughout the duration of the experiment. Variations in the hematological indices indicated that MCV, MCH, and MCHC were significantly lower ($P<0.05$) during the experiment in the treated groups when compared to the control. The lymphocytes

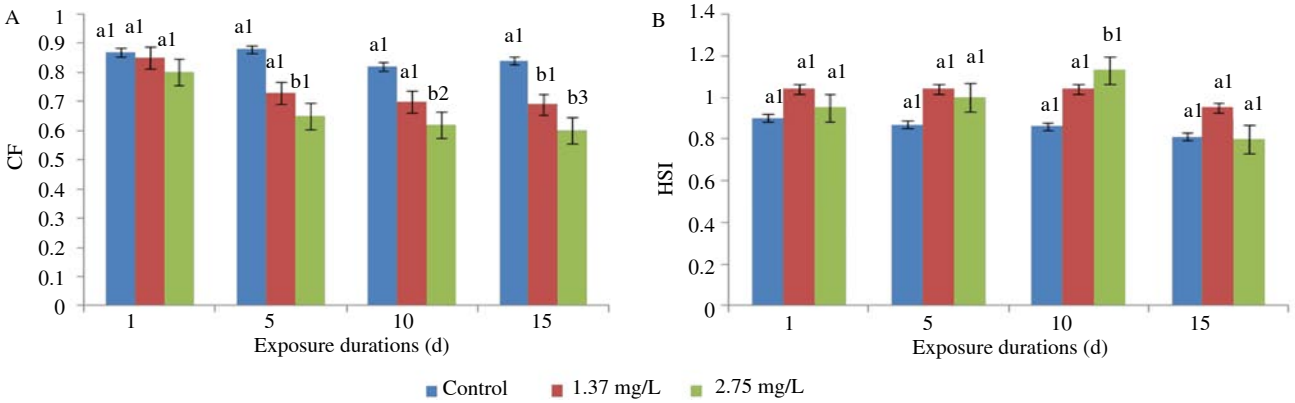

Figure 1. The values of CF and HSI of *C. gariepinus* exposed for 15 d in control and paraquat (1.37 and 2.75 mg/L) groups.

A: Values in CF of *C. gariepinus* exposed for 15 d in control and paraquat (1.37 and 2.75 mg/L) groups; B: Values in HSI of *C. gariepinus* exposed for 15 d in control and paraquat (1.37 and 2.75 mg/L) groups. Data represent mean±SE (n=5). Letters indicated significant difference (P<0.5) in mean values among pesticide concentrations, and numerals indicated significant difference (P<0.05) in mean values among durations of exposure.

Table 2

Effects of paraquat on some haematological parameters in *C. gariepinus*.

Parameter	Concentration (mg/L)	Exposure duration (d)			
		1	5	10	15
RBC ($\times10^6$)	Control	9.12±0.52^{a1}	10.11±0.13^{a1}	8.93±0.35^{a1}	8.51±0.28^{a1}
	1.37	6.37±0.33^{b1}	5.90±0.12^{b1}	6.41±0.45^{a1}	6.17±0.42^{a1}
	2.75	5.57±0.42^{b1}	4.68±0.42^{b1}	6.33±0.33^{a1}	5.87±0.74^{b1}
PVC (%)	Control	31.50±0.42^{b1}	32.00±1.03^{a1}	33.50±1.02^{a1}	29.00±0.89^{a1}
	1.37	17.50±0.38^{b1}	18.00±0.45^{b1}	20.00±0.51^{b1}	16.00±0.13^{b1}
	2.75	17.00±0.44^{b1}	16.00±0.31^{b1}	18.00±0.21^{b1}	15.50±0.18^{b1}
WBC ($\times10^3$)	Control	8 175.00±4.45^{a1}	8 450.00±5.38^{a1}	8 475.00±6.12^{a1}	8 200.00±4.11^{a1}
	1.37	8 675.00±5.32^{b1}	9 150.00±6.42^{b2}	8 900.00±5.11^{b3}	8 325.00±4.83^{b4}
	2.75	13 825.00±7.09^{c3}	8 475.00±8.11^{c2}	9 175.00±6.12^{c3}	8 475.00±5.13^{b4}
HB (g/dL)	Control	13.70±0.88^{a1}	14.10±0.54^{a1}	14.50±0.44^{a1}	13.40±0.45^{a1}
	1.37	6.50±0.18^{b1}	6.30±0.23^{b1}	5.40±0.16^{b1}	13.40±0.45^{a1}
	2.75	5.60±0.38^{b1}	7.30±0.33^{b1}	5.50±0.42^{b1}	6.50±0.61^{b1}
MCV (fL/cell)	Control	34.54±1.13^{a1}	35.65±1.46^{a1}	37.51±1.73^{a1}	34.08±1.18^{a1}
	1.37	27.47±0.64^{b1}	30.50±0.55^{a1}	31.20±0.53^{b1}	25.93±0.61^{b1}
	2.75	30.52±0.66^{b1}	30.19±0.33^{a1}	28.44±0.67^{c1}	26.41±0.71^{b2}
MCH (pg/cell)	Control	15.02±0.23^{a1}	13.95±0.31^{a1}	16.24±0.23^{a1}	15.57±0.23^{a1}
	1.37	10.21±0.41^{b1}	10.68±0.26^{b1}	8.42±0.63^{b1}	11.07±0.55^{b1}
	2.75	10.05±0.33^{b1}	10.60±0.27^{b1}	8.69±0.84^{b1}	11.07±0.55^{b1}
MCHC (g/dL)	Control	44.48±1.54^{a1}	44.06±0.93^{a1}	43.28±0.96^{a1}	46.21±1.22^{a1}
	1.37	37.14±0.77^{b1}	35.00±0.66^{b1}	27.00±0.73^{b2}	38.75±0.54^{b1}
	2.75	32.94±0.83^{c1}	40.63±1.12^{c2}	30.56±0.83^{c1}	41.94±1.08^{c3}

Values with different alphabetic (lowercase) superscripts differ significantly (P<0.05) between concentrations within exposure duration. Values with different numeric superscripts differ significantly (P<0.05) between exposure durations within concentrations.

were the most abundant type of leucocyte in the peripheral blood of *C. gariepinus* exposed to paraquat (Table 3). This was followed by the neutrophils, while the least abundant type of leucocyte was the eosinophils. Compared with the control, the exposed paraquat fish suffered acute neutropenia on Day 5. Monocytes, basophils and eosinophil activity in the exposed fish were not significantly different from the control group (P>0.05) throughout the experiment.

3.4. Biochemical parameters and enzyme assays

The plasma protein level significantly decreased (P<0.05) in the treated group when compared to the control group throughout the experiment. In contrast, glucose level increased (Figure 2AB). Variations in enzyme parameters indicated that AST and ALT significantly increased in the exposed group as compared to control during the experiment (Figure 3AB).

Table 3

Effects of paraquat on differential white blood cell counts (in percent) in *C. gariepinus*.

Parameter	Concentration (mg/L)	Exposure duration (d)			
		1	5	10	15
Lymphocytes	Control	74.00±0.70^{a1}	72.50±0.71^{a1}	66.00±0.66^{a1}	81.00±0.67^{a1}
	1.37	84.00±1.23^{b1}	86.00±1.11^{b1}	79.00±0.81^{b2}	76.30±0.63^{b3}
	2.75	80.50±1.71^{c1}	82.00±0.93^{b1}	81.00±0.64^{b1}	73.00±0.81^{b2}
Neutrophils	Control	25.00±0.33^{a1}	22.50±0.33^{a1}	18.00±0.61^{a1}	19.00±0.28^{a1}
	1.37	15.50±0.22^{b1}	14.00±0.38^{b1}	20.00±0.23^{b2}	22.50±0.21^{b1}
	2.75	19.00±0.18^{c1}	27.00±0.41^{c2}	19.00±0.41^{b2}	27.00±0.18^{c3}
Monocytes	Control	1.00±0.01^{a1}	0.50±0.01^{a1}	0.50±0.01^{a1}	0.60±0.02^{a1}
	1.37	0.50±0.01^{a1}	0.51±0.02^{a1}	1.00±0.02^{a1}	0.50±0.01^{a1}
	2.75	0.50±0.02^{a1}	0.58±0.02^{a1}	0.80±0.03^{a1}	0.70±0.03^{a1}
Basophils	Control	0.04±0.01^{a1}	0.03±0.00^{a1}	0.02±0.00^{a1}	0.03±0.00^{a1}
	1.37	0.05±0.02^{a1}	0.01±0.00^{a1}	0.03±0.01^{a1}	0.03±0.01^{a1}
	2.75	0.05±0.01^{a1}	0.01±0.00^{a1}	0.04±0.01^{a1}	0.02±0.00^{a1}
Eosinophils	Control	0.05±0.00^{a1}	0.03±0.00^{a1}	0.04±0.00^{a1}	0.05±0.00^{a1}
	1.37	0.04±0.00^{a1}	0.04±0.00^{a1}	0.06±0.01^{a1}	0.03±0.00^{a1}
	2.75	0.04±0.00^{a1}	0.03±0.00^{a1}	0.06±0.00^{a1}	0.05±0.00^{a1}

Values with different alphabetic (lowercase) superscripts differ significantly (P<0.05) between concentrations within exposure duration. Values with different numeric superscripts differ significantly (P<0.05) between exposure durations within concentrations.

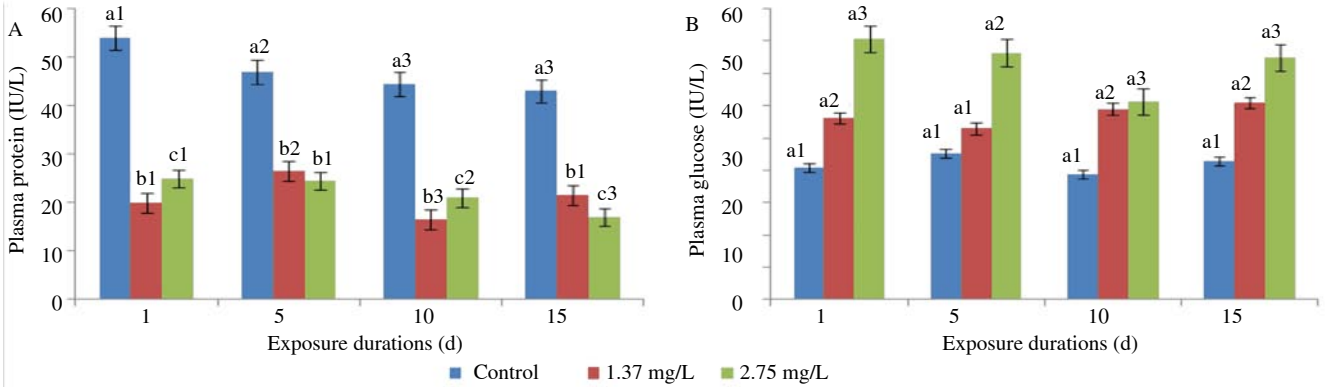

Figure 2. Changes in plasma protein and plasma glucose of *C. gariepinus* exposed for 15 d in control and paraquat (1.37 and 2.75 mg/L) groups.
A: Changes in plasma protein of *C. gariepinus* exposed for 15 d in control and paraquat (1.37 and 2.75 mg/L) groups; B: Changes in plasma glucose of *C. gariepinus* exposed for 15 d in control and paraquat (1.37 and 2.75 mg/L) groups. Letters indicated significant difference (P<0.5) in mean values among pesticide concentrations, and numerals indicate significant difference (P<0.05) in mean values among durations of exposure.

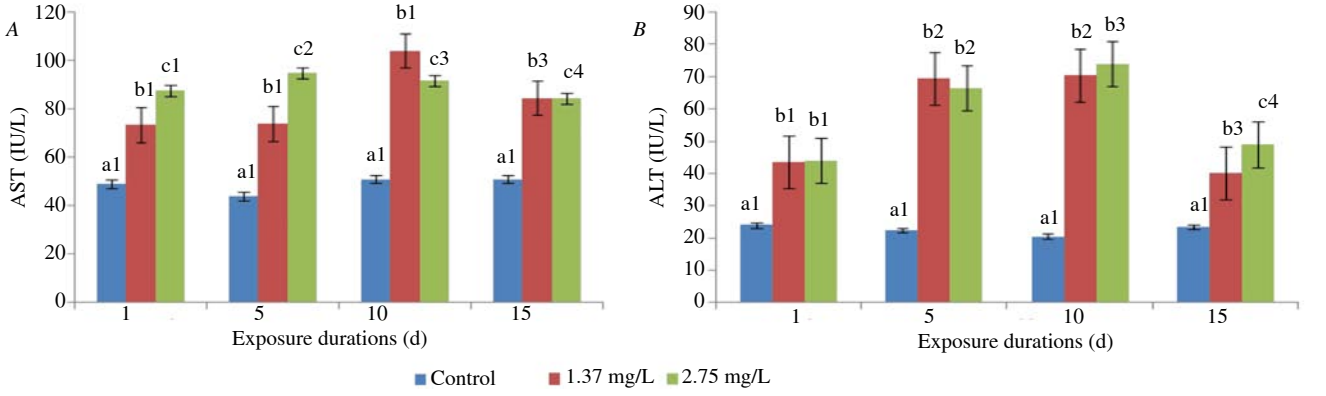

Figure 3. Changes in AST and ALT enzymes activities in *C. gariepinus* exposed for 15 d to control and paraquat (1.37 and 2.75 mg/L test concentrations).
A: Values in AST in *C. gariepinus* exposed for 15 d to control and paraquat (1.37 and 2.75 mg/L test concentrations); B: Values in ALT in *C. gariepinus* exposed for 15 d to control and paraquat (1.37 and 2.75 mg/L test concentrations). Letters indicated significant difference (P<0.5) in mean values among pesticide concentrations, and numerals indicated significant difference (P<0.05) in mean values among durations of exposure.

4. Discussion

In ecotoxicology, the 96 h LC_{50} is one of the most valuable parameters for assessing the toxic effects of pollutants. Herein, the 96 h LC_{50} value (*i.e.* 27.46 mg/L) was obtained from *C. gariepinus* exposed to paraquat, which suggests that this herbicide is highly toxic to fish. The observed fish mortality was both time and concentration dependent. The 96 h LC_{50} values of paraquat previously reported in *Mesopotamichthys sharpeyi*[17], *Trichogaster trichopterus*[32], and *Oreochromis niloticus* (*O. niloticus*)[16], were 1.48, 1.41 and 7.00 mg/L, respectively, which were lower than the value obtained from *C. gariepinus* in the present study. The toxicity of a pesticide for an organism is affected by the strains of species, size, age, sex, temperature, water quality and formulation of the test chemicals[33,34]. The sublethal concentrations of paraquat used in our study (1.37 and 2.75 mg/L) are environmentally realistic because environmental concentrations of 0.01 μg/L and 0.14-8.70 μg/L of paraquat have been reported in Elechi Creek, Nigeria[35], and in some water bodies in Thailand[36], respectively. Although our test concentrations exceeded the 0.1 μg/L safety limit established by the United State Environmental Protection Agency[37], considering the repeated application of the pesticide in agricultural fields and other anthropogenic sources, the actual concentration in aquatic environments may exceed this limit. The observed behavioural changes in *C. gariepinus* exposed to paraquat in the present study, which indicated internal effects on body physiology, may be attributed to a neurotoxic effect of paraquat. Our results are consistent with previous reports on fish exposed to paraquat[16,32,37], and in other pesticides, such as carbosulfan[38], malathion[39], dichlorvos[40], and chlorpyrifos[41].

CF and HSI are indicators of overall health in fish and have been used in toxicological studies as indicators of stress[42]. In the present study, there was a significant reduction in CF on Day 15, following exposure to 1.37 mg/L, and exposure at highest test concentration (*i.e.* 2.75 mg/L). A similar decrease in CF was reported by Khan[43], and Roussel *et al.*[44], in fish exposed to environmental pollutants. There was no significant difference in HSI between the exposed and the control fish on Days 1 and 5 post exposure. However, HSI significantly reduced at the highest test concentration (2.75 mg/L), at the beginning of Day 10 post exposure. Normal HSI have been reported for: *Cyprinus carpio*, exposed to alachlor and deltamethrin[9,45]. *Carassius auratus* exposed to alachlor[46], and *Oncorhynchus mykiss* (*O. mykiss*) exposed to carbamazepine[10]. Lower HSI have also been reported in *O. mykiss*, exposed to

pharmaceutical drug verapamil[47], and in juvenile *O. niloticus*, exposed to paraquat[16]. Normal CF and HSI may indicate that paraquat does not affect the liver at the beginning of the exposure. However, with the progression of the experiment, the liver was affected and this was indicated in a decline in CF and HSI. Therefore, the reduction in CF and HSI may indicate a decrease in the overall condition of the fish, which may be due to the effect of paraquat.

Some studies have demonstrated that pesticides cause a significant reduction in the haematological parameters of fish[16]. Li *et al*.[47], reported that verapamil reduced the erythrocyte count, Hb and PCV in *O. mykiss*. Reduction in the values of these parameters was also reported in *Prochilodus lineatus* exposed to clomazone[48], and in *Labeo rohita* exposed to fenvalerate[49]. Reduction in the erythrocyte count was reported in *Cirrhinus mrigala* exposed to ibuprofen[50], and *Clarias albopunctatus* exposed to acetellic[51]. Our observations are also consistent with the trend in *Heteropneustes fossilis*, exposed to adrin and fenvalerate[52], and in *Salmo gardneri* and *Mystus vittatus* exposed to pesticides[53].

Studies have shown that pesticides have cytotoxic effects in fish through the generation of ROS, which impose severe oxidative stress on the fish[48,54-56]. The anaemia reported in this study may be attributed to erythrocyte membrane lipid peroxidation that predisposed it to rapid sequestration or haemolysis brought on by paraquat exposure. Samthakumar *et al*.[57], noted that monocrotophos exposure resulted in the destruction of interstitial cells in fish and inhibited erythropoeitic processes through the inhibition of erythropoietin, which may result in anaemia. We believe that the reduction in RBC, Hb, and PCV in *C. gariepinus* exposed to paraquat could be the product of impaired erythropoiesis and rapid haemolysis of the RBC. Further, the reduction in MCV in the present study reveals erythrocytic shrinkage leading to microcytic anaemia. This reduction in MCV in the paraquat exposed fish could also arise due to osmoregulatory imbalances. The reduction in the Hb concentration, MCH, and MCHC suggested that paraquat could have inhibited the Hb biosynthetic pathway by interfering or inhibiting the utilization of delta-aminolevulinic acid[58,59]. This may have an adverse impact on the oxygen carrying capacity of the blood. Generally, leucocytes modulate immunological functions in animals, including fish. The observed leucocytosis in the present study indicated a normal immune protective response to paraquat intoxication. It also suggested that paraquat stimulated the immune system with a concomitant release of lymphocytes from the lymphomyeloid tissue as a defence response. It resulted in the leucocytosis and/or lymphocytosis, which altered body physiology. Leucocytosis was also reported in *Cyprinus carpio* exposed to lindane[60], and in *O. niloticus* exposed to deltamethrin[13].

The results of this study indicated that paraquat provoked AST and ALT to enhance activities of *C. gariepinus*. Similar increases in AST and ALT activities were reported in *Mystus vittatus* exposed to metasystox and sevin[53], in *O. mykiss* exposed to verapamil[47]. In contrast, Ogueji and Auta[61], reported a dose-dependent inhibition of AST and ALT and alkaline phosphatase activities in *C. gariepinus* exposed to lambda cyhalothrin. The elevated activities of serum AST and ALT indicate liver damage or enhanced transamination. Increased transamination during pesticide challenge has been attributed to the need to meet higher energy demanded by fish[62,63]. The glucose levels observed also increased, which may be a physiological response to meet the high metabolic demands caused by continued exposure to paraquat. Conversely, the reduction in the protein level may be associated with liver and kidney damage caused by toxicant stress and the consequent utilization of available protein for metabolic activities.

The results of the present study indicate that the commercial formulation of paraquat (gramoxone) is toxic and has the potential to impair the behaviour, morphology, hematology and biochemical activities in the African catfish. The use of paraquat at riverside and coastal areas should be strongly controlled and carefully monitored to avoid exposure to aquatic environments. Further studies on the chronic effects of paraquat, and the parameters examined herein, are still needed for a greater insight into the mechanism of paraquat toxicity.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Comments

Background

Owing to the growth of human population and the need to improve crop production to meet up with the demand for food, paraquat is used in agriculture as herbicide. It enters into the aquatic environment and cause havoc to aquatic life. Therefore, there is the need to investigate the toxicological consequences of paraquat on physiology of juvenile African catfish *C. gariepinus*.

Research frontiers

In the present study, the authors investigated the acute and sublethal effects of paraquat on the physiology of juvenile African catfish *C. gariepinus* in a semi-static laboratory system. All the parameters studied on expsure of the fish to paraquat suggested that they could be good biomarkers to assess the toxic effects of paraquat on aquatic organisms.

Related reports

In humans, paraquat poisoning has been reported to cause respiratory failure, severe central nervous system injury and

Parkinston's disease. The results of the present study indicate that paraquat is toxic and has the potential to impair the physiological activities of the African catfish *C. gariepinus.*

Innovations and breakthroughs

There is no published work on physiological effects of paraquat in juvenile African catfish *C. gariepinus*. The present investigation provides first hand information on toxic effects of paraquat on behavioural, morphological, haematological and biochemical responses of the African catfish. All biomarkers studied can be used to monitor and assess the residues of other herbicides in aquatic ecosystem.

Applications

Paraquat is the second widely used herbicide in the world for controlling weeds, also as a defoliant and a desiccant that aid in the harvesting of some agricultural crops. Furthermore, it can also be used in toxicological studies especially in fish to monitor and assess the possible effects of herbicides especially paraquat in aquatic biota.

Peer review

This is an interesting research work in which authors have investigated the physiological effects of paraquat in juvenile African catfish *C. gariepinus*. The activity was assessed based on haematological and biochemical parameters in the blood and plasma while that of the morphology was based on the body weight and liver of the African catfish. The study reports that paraquat is highly toxic, possess neurotoxic properties and can impair the physiological functions of African catfish *C. gariepinus.*

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