JOBIMB, 2022, Vol 10, No 2, 42-45
https://doi.org/10.54987/jobimb.v10i2.756

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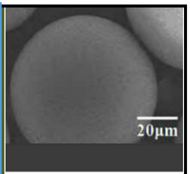

JOBIMB VOL 10 NO SP2 2022
ZnO nanoparticle

Acute Toxicity of Atrazine and its Morphological and Biochemical Effects on *Clarias gariepinus*

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HISTORY

Received: 27 July 2022
Received in revised form: 21 Aug 2022
Accepted: 10 Nov 2022

KEYWORDS

Biochemical Parameters
Clarias gariepinus
Histological Assay
Acute Toxicity
Atrazine

ABSTRACT

Atrazine is the second most widely used herbicide in Nigeria, an excellent polluter of surface and ground sources of water; it finds its way into the water bodies through percolation and surface run-off during rainy and dry season farming. The chemical has some effects on the ecosystem especially in aquatic habitats. different concentrations of atrazine have been prepared (0, 1, 2, 3, and 4 mg/l) and each treatment was replicated four times. a total number of twenty-five basins were used. Five test organisms (*Clarias garipienus*) were used in each basin. During 96-hour exposure, physicochemical parameters, morphological changes and abnormal behavior in the fish were observed. The research also considers biochemical changes in the kidney, liver and gills where the activities of cretinine, Urea, AST, ALT and Total bilirubin was assayed using colorimetric method. Based on the results obtained in this study, atrazine is found to be toxic to *Clarias gariepinus.* The result of the biochemical parameters and histological assay showed that *Clarias gariepinus* was seriously affected by atrazine.

INTRODUCTION

The changes in water quality adversely affect organisms and even lead to their mortalities in acute concentrations and severe exposure [1]. This may be due to herbicides used for agricultural and industrial purposes. In an attempt to increase farm output, man employs the use of heavy chemicals to prevent crops from insects and rodents, right from the period of pre-planting operation to the time of weed control in the farm up to the period of cultivation and storage of harvested products. All these chemicals are toxic to humans, plants, and aquatic organisms such as fish [2,3]. Any change in fish behaviour give information and knowledge regarding behavioral alterations which can be related to physiological biomarkers in aquatic species [4]. Behavioral bioassays have been widely used in toxicity assessment for the fact that is faster, more sensitive and ecologically more relevant [5].

At a higher concentration in the water bodies, pollutants can cause high mortalities in the aquatic habitat. Whereas when the concentration is low it leads to bio-accumulation and bio-magnification of the pollutants and eventually goes through the food web to human beings [5,6]. The issue of water pollution requires serious attention and should be handled with utmost attention in order to ensure that we consume healthy fish on a priority basis [7,8]. The resultant benefits derive from the use of chemical in farms are enormous, but environmental pollution and degradation are major problems that cannot be overlooked, and this problem is linked to their application [9].

Agrochemical pollution is a serious threat to environmental safety and exposure to such chemicals had deleterious health effects such as nervous system damage and cancer [10]. The presence of agricultural chemicals in the ecosystems has become a serious cause for concern that leads to considerable social and scientific anxiety globally, as their usage posed a serious threat to the ecosystem and all living organisms and easily contaminates water bodies, hence extensive damage to non-target species, including fish [11]. Contamination of the environment by agrochemicals has become a serious problem in terms of world conservation of [animals and human health [12]. Knowingly or unknowingly water is contaminated through direct application into the aquatic system, spray drifts, atmospheric fallout as rain and dust, sewage, industrial effluent and seldom by spillage.

https://doi.org/10.54987/jobimb.v10i2.756

Most cities of developing countries generate on the average 30–70 mm^3 of wastewater per person per year. Owing to lack of or improper wastewater treatment facilities, wastewater and its effluents are often discharged into surface water sources, which are receptacles for domestic and industrial wastes, resulting in pollution. The poor quality of wastewater effluents is responsible for the degradation of the receiving surface water body [13]. The EPA's review on atrazine has been criticized by some scholars and the safety of Atrazine remains controversial, hence the need for further research on the study of toxicity and maximum concentration level, following strict laboratory procedure for acute toxicity testing has become necessary. The purpose of this study is to assess the level of toxicity caused by atrazine on *Clarias garipienus*.

MATERIALS AND METHODS

Materials

Experimental Design

Twenty-five (25) farms raised *Clarias garipienus* were used for the study. The fish were divided into five (5) groups. Five (5) fish were used in each group.

Preparation of Stock

Atrazine (philozine) 80 % WP was obtained at a concentration of 800 g/L in a one-liter container. From the 800 g/L, a stock solution was prepared by adding 1 ml of the herbicide to 999 mL of water [10]. The stock solution was then used to prepare different nominal concentrations of the toxicant by diluting measured volumes of the toxicant with borehole water. The control solutions had only the borehole water without the toxicant.
Different concentrations of atrazine were used to contaminate the bowls during the study. The concentrations ranged from 0 mg/L, to 1 mg/L, 2 mg/L, 3 mg/L and 4 mg/L, respectively.

Water Quality Assessment

Water properties (Electrical conductivity, Dissolved oxygen, Temperature, pH, Total hardness and Alkalinity) of the test solution and the control were monitored every 24 hours using the appropriate apparatus, these parameters were measured, and records were taken to ensure they are within a suitable range for the growth of the fish [14], the Fish were not fed during the toxicity test. Behavioural changes were observed at 12, 24, 48, 72 and 96 hours after the exposure to the atrazine herbicide as described by [15].

Blood Sampling for Biochemical Analysis

After 96-hour toxicity test was conducted, blood samples were collected via caudal vein puncture as described by [16-18]. Fish were held for collection of the blood in a slanting and/or vertical position with the ventral portion facing the person. The samples of the blood were drawn with a sterile 5 mL syringe and 21g needle. The needle was pierced on the ventral midline connecting the anal opening and the beginning of the anal fin to assess the dorsal blood vessel lying below the vertebral column, In order to prevent the blood from clothing the sample was transferred immediately into the EDTA bottle. Blood samples were collected in order to get enough blood for biochemical analysis. The biochemical parameters analysed were urea, cretinine, aspatate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin.

Determination of Urea

Urea was determined using the most popular method for the determination of urea (urease method), which is based on Berthelot principle. The urea in presence of urease produces ammonia and CO_2, the ammonia reacts with salicylate in the presence of nitroprusside and hypochlorite to produce a compound non as 2,2-dycaboxylindophenol which is a green colored compound read calorimetrically at 580 nm [19].

Determination of Creatinine

Creatinine has been determined based on Jaffe principle. This method is based on the production of the red coloured complex by the reaction of creatinine in an alkaline solution with picrate to form a red-coloured complex, the optical density of which is calorimetrically determined at 520 nm.

Determination of Alanine aminotransferase (ALT)

Principle: Alanine aminotransferase has been determined using colourimetric analysis following the procedure of Reitman and Frankel.

Determination of Aspartate Aminotransferase (AST). Principle

Aspartate aminotransferase has been determined using colourimetric analysis following the method of Reitman and Frankel [10].

Determination of Total Bilirubin using the Calorimetric Method

The Gendragsik and Grof bilirubin react with diazotized sulphanilic acid which produces azobillirubin which is a pink or purple color, the reaction was read calorimetrically at 540 nm. The assay procedure was carried out based on vandev bengh reaction as outlined below

Billuribin + Diazotized sulphanilic acid =Azobilluribin pink or purple color

The reaction was read colourimetrically at 540 nm.

Data Analyses

One-way Analysis of Variance (ANOVA) was employed for data analysis. mean difference was determined using Benferoni multiple comparison tests.

RESULTS AND DISCUSSION

The result of the mortality rate of the sampled specimens showed 40% of the specimens were dead after 96-hours with the highest mortality at concentration of 4mg/L and no mortality in 0 mg/L (control) as stated in **Table 1**. The rate of mortality in **Table** 1 is directly proportional to the concentration of atrazine (mg/L) in the test groups (B, C, D and E). There was no mortality recorded in the first 48 hours, but death was recorded on group C, D and E after 72 hours and also death at 96-hours in group B, C, and D while two deaths at same hours were recorded in group E. There was no death in group A (control) throughout the experiment period.

Table 1. The mortality rate of juvenile *Clarias garipienus* exposed to varied concentrations of atrazine.

No. of basins	No. of test fish	Conc. mg/L	Mortality					No. of mortality	Percentage mortality
			12 hours	24 hours	48 hours	72 hours	96 hours		
Control	5	0	0	0	0	0	0	0/5	0
Group B	5	1	0	0	0	0	1	1/5	20
Group C	5	2	0	0	0	1	1	2/5	40
Group D	5	3	0	0	0	1	1	3/5	60
Group E	5	4	0	0	0	1	2	4/5	80

Changes in physico-chemical parameters of the water
all the parameters measured are found to be within suitable range for the optimum survival and growth of *C. gariepinus*. Therefore, changes in behavior and death of the fish could not be as a result of poor quality of the test water. The range of pH for this study is (6.9–7.6) which is in agreement with desirable range (6.5–9) for fish production [20] . DO, dissolved oxygen ranges for this study (7.3–5.0 mg/L) which is also good for fish production. Likewise, the temperature range for this study (22.2–23.8) is within the normal range of tropical temperature to which fish are adapted (22–35 °C) as reported by Adeyemo et al. [21].

The result of the physiochemical parameters of *Clarias gariepinus* during a response to atrazine for day 1 as presented in **Table 2** indicated that there was a significant decrease (P<0.05) in dissolved oxygen in treated groups as compared with the control group. There was a significant increase (P<0.05) in conductivity in treated groups as compared with the control. There was no significant difference (P<0.05) in pH and temperature in the treated groups as compared with the normal control group. There was a significant decrease (P<0.05) in hardness in the treated group as compared with the normal control group. There was no significant difference (P<0.05) in alkalify in the group treated with 1 ppm as compared with the control but however there was a significant decrease (P<0.05) in the group treated with 2 ppm, 3 ppm and 4 ppm as compared with the normal control groups.

Table 2. Mean physiochemical parameters of test water at 12, 24, 48, 72 and 96-hours**.**

Groups/parameters	Dissolved oxygen	Conductivity	pH	Temperature	Hard-ness	Alkalinity
Group A	7.3 ± 0.14^a	343.1 ± 15.90^a	7.1 ± 0.05^a	22.4 ± 0.16^a	2.3 ± 0.01^a	0.5 ± 0.04^a
Group B	6.8 ± 0.07^b	415.2 ± 6.41^b	7.2 ± 0.06^a	22.8 ± 0.06^b	1.8 ± 0.04^b	0.5 ± 0.02^a
Group C	6.4 ± 0.08^c	456.8 ± 7.53^c	7.3 ± 0.01^b	23.0 ± 0.12^b	1.5 ± 0.03^c	0.5 ± 0.05^a
Group D	5.8 ± 0.12^d	490.0 ± 3.60^d	7.4 ± 0.01^c	23.2 ± 0.20^b	1.4 ± 0.02^d	0.7 ± 0.25^b
Group E	5.0±0.19e	540.2 ± 19.20^e	7.5 ± 0.03^d	23.5 ± 0.14^b	1.0 ± 0.05^e	0.4 ± 0.02^c

Values are expressed as Mean ± SEM Mean values with different superscript letters in a column are significantly different (P< 0.05).

The results of the liver function parameters of *Clarias gariepinus* during the response to atrazine are presented in **Table 3**. The table indicates that there was a significant decrease (p<0.05) in AST among treated groups as compared with the normal control group. There was a dose-dependent significant increase (p<0.05) in ALT and TBN among the treated groups as compared with a normal control group. Physiological stress biomarkers can be used as a criterion when defining the effect of atrazine on aquatic organism, as it serves as an indicator for toxicity effect of pollutant on fish. In the present study, blood biomarkers were analysed for ALT, TBN and AST. The result inferred that atrazine administration led to increasing levels of blood biomarkers (ALT and TBN). The values recorded for ALT and TBN increased across the treatment with significant (p<0.05) differences across the treatment. The significantly higher ALT activities in the fish exposed to increasing atrazine across the treatment when compared with the control group were as a result of leakage of alanine aminotransferase (ALT) enzymes from injured liver cells. These results are similar to that of Konstantinova and Russanov [22] who studied paraquat-induced oxidative stress in rat liver.

Table 3. Liver function parameters of *Clarias gariepinus* during response to atrazine.

Groups/parameters	AST	ALT	TBN
Group A	0.24 ± 0.01^a	0.25 ± 0.00^a	0.40 ± 0.10^a
Group B	0.03 ± 0.00^b	0.31 ± 0.00^b	1.60 ± 0.17^b
Group C	0.03 ± 0.00^b	0.37 ± 0.00^c	2.80 ± 0.22^c
Group D	0.04 ± 0.00^c	0.47 ± 0.01^d	3.60 ± 0.26^d
Group E	0.08 ± 0.00^d	0.50 ± 0.02^e	8.22 ± 1.91^e

Values are expressed as mean ± SEM of five (5) replicates. Mean values with different superscript letters in a column are significantly different at P< 0.05 using one way analysis of variance (ANOVA). AST: Aspartate amino transferase, ALT: Alanine amino transferase, TBN: Total bilirubin.

The result of kidney function parameters of *Clarias gariepinus* during the response to atrazine is presented in **Table 4**. There was a dose-dependent significant increase (P<0.05) in creatinine among treated groups as compared with the normal control group. There was no significant difference (P<0.05) in the urea level of the group treated with 1mg/L as compared with the normal control group. however, there was significant increase (p<0.05) in urea in group treated with 2 mg/L, 3 mg/L and 4 mg/L as compared with the normal control group.

Serum urea and creatinine are useful bio-indicators for evaluating renal function in both in vitro and in vivo studies. In this present study, urea and creatinine levels were increased significantly as compared with the normal control group. The observed increase in these renal function parameters is due to kidney damage as a result of atrazine toxicity. This result is in agreement with previous works [23, 24]. The increase in urea and creatinine serum levels results amongst other reasons from impaired excretion and/or decreased urinary clearance by the kidneys. Normally, urea and creatinine are excreted primarily by the kidneys. Creatinine is freely filtered by glomeruli because it is a small molecule with a molecular weight of 113 Daltons [25]. Thus, serum increases in these parameters particularly creatinine, would reflect overproduction and/or renal impairment most probably at the level of glomeruli [26].

Table 4. Kidney function parameters of *Clarias gariepinus* during response to atrazine.

Groups/parameters	Creatinine	Urea
Group A	12.2 ± 0.80^a	1.1±0.05a
Group B	$15.6 \pm 0.50_b$	1.1 ± 0.07^a
Group C	18.8 ± 0.66^c	1.3 ± 0.09^b
Group D	22.2 ± 0.37^d	1.7 ± 0.10^c
Group E	28.4 ± 3.25^e	4.1 ± 0.37^d

Values are expressed as mean ± SEM of five (5) replicates. Mean values with different superscript letters in a column are significantly different at p< 0.05 using one way analysis of variance (ANOVA).

CONCLUSION

The present investigation shows that mortality increases with an increase in the concentration of atrazine herbicide which has manifested in the alteration of biochemical parameters of the liver, kidney, gills. behavioural changes were also observed due to an increase in the concentration of atrazine. Hence, the higher the concentration the higher the mortality rate and changes in behavior. Therefore, atrazine can induce mortality in *Clarias gariepinus* juveniles which in turn can cause problems when such water and fish are consumed by humans. The use of atrazine in close proximity to aquatic environment should strictly be monitored to prevent its harmful effects on aquatic organisms.

ACKNOWLEDGEMENT

The effort of the Federal College of Education (T) Gombe is acknowledged for providing master's sponsorship to the first author.

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