

Research Article

Lethal Toxicity of Aqueous Extract of Sodom Apple (*Calotropis procera*) on African Catfish (*Clarias gariepinus*) (Burchell, 1822) Juveniles

Cynthia Ahemen Orpin¹, Armayau Hamisu Bichi¹, Akeem Babatunde Dauda¹ and James Bemshima Orpin^{2*}

¹Department of Fisheries and Aquaculture, Federal University Dutsin-Ma, Nigeria

²Department of Biological Sciences, Federal University Dutsin-Ma, Nigeria

*Corresponding Author: jorpin@fudutsinma.edu.ng

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ABSTRACT

The experiment was performed to check the lethal toxicity of aqueous extract of *Calotropis procera* on *Clarias gariepinus* juveniles. A range finding test was done and the concentrations for the definitive tests were determined. A total of 150 African catfish juveniles of average weight 9.37 ± 0.08 g were randomly selected and distributed into four treatments in triplicates of 20mg/L, 40mg/L, 60mg/L, 80mg/L concentrations and a control (0.00) using a static bioassay test over a period of 96 hours. Behavioural responses observed during exposure of the fish to lethal concentrations included erratic swimming, air gulping, haemorrhage, loss of equilibrium and sudden death. All data obtained were summarized using descriptive statistics for the mortality, water quality parameters, growth performance and nutrient utilization. While one-way analysis of variance (ANOVA) was used to assess differences in means among the concentrations and Duncan multiple range tests was used to separate the means, where there was a difference. Physicochemical water parameters and haematological parameters showed various significant difference among the treatments ($P < 0.05$). *C. procera* at higher concentrations of 40mg/L and above was highly toxic to fish, which led to erratic behaviour and mortality.

Keywords: *Clarias gariepinus*, *Calotropis procera*, Toxicity, Haematology, Mortality

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INTRODUCTION

Fish is one of the cheapest protein sources in Sub-Saharan Africa and the importance of fish in developing countries increased greatly after the Sahelian drought of 1971 to 1974, which decimated the cattle population and hence, shot up the prices of beef (Ayoola *et al.*, 2014). There is a huge market value for fish in Nigerian markets due to its taste hence, the proliferation of fish farming business in Nigeria using different fish production methods and other factors for proliferation of fish farming business apart from taste e.g. economic, health implications etc.

Clarias gariepinus (Burchell, 1822) belongs to the family Clariidae, and is widely distributed in Nigerian waters. The fish is known for its high tolerance and high survival ability and resilience in harsh habitat conditions.

In order to increase catch per unit effort (CPU) in traditional fishing, different toxic plants that can stupefy fish have been exploited (Ayoola *et al.*, 2014; Sogbesan and Ngadina, 2015). Fish live in contact with their environment and are therefore very susceptible to many physical and chemical changes, which may be detected in their physiology (Ayoola *et al.*, 2014). Human destructive influence on the

aquatic environment is in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Amoatey and Baawain, 2019). Stress response is characterized by physiological changes and the effect of pollutants on fish is assessed by acute and chronic toxicity tests (Chinnadurai *et al.*, 2022). Normal physiological processes are affected long before death of an organism hence the need for physiological and biochemical indicators of health and sub-lethal toxicant effects (Mensah *et al.*, 2014).

Phytochemical analyses of piscicidal plants showed that they contain diverse metals such as rotenones, saponins, flavonoids, alkaloids, glycosides, tannins, oxalic acids, solanine, selenium, nicotine, pyrethrum, ryania, coumerin, and resin (Al Ashaal *et al.*, 2010). Most of the substances are toxic to fish and other aquatic organisms at high concentrations and after some time fade out (Sehar *et al.*, 2014).

Calotropis procera belongs to the family Asclepiadaceae and it is a soft woody evergreen perennial shrub. This plant has few branches and relatively few leaves concentrated near the growing tips. A copious white sap referred to as the latex flows whenever the stems or leaves are cut. The plant is commonly found in Africa (North, Northeast, East tropical, West Central and West tropical), particularly the semi-arid regions of North West and North eastern Nigeria (Al Sulaibi *et al.*, 2020).

Increase demand for fish and fishery products and reduction in fish population in the wild has led to increase in the use of obnoxious chemicals and plant poisons by fishermen. In other to sustain fish biodiversity for conservation and continuous catch there is a strong need for this type of research because fisherfolks have reported using some of these plants poison to increase catches (Sogbesan and Ngadina, 2015) hence the need to determine the lethal concentrations of *Calotropis procera* which fish is exposed to is very vital.

MATERIALS AND METHODS

Study Area

This study was carried out in the Department of Fisheries and aquaculture Federal University Dutsin-ma Mini Research Centre, Located in Dutsin-ma town, Dutsin-ma Katsina State.

Experimental Design

The design of the experiment was a completely randomized design (CRD) with four treatments and a control all in triplicates for 96 hours acute toxicity test

Source of Experimental Fish management and acclimation

Four hundred (400) apparently healthy African catfish (*Clarias gariepinus*) juvenile with average weight 9.37 ± 0.08 g were procured from a reputable fish farm in Kaduna State. The *Clarias gariepinus* juveniles were distributed randomly into batches of 10 each and placed in 40-litre capacity container with 20 litres of borehole water. The juveniles were acclimated to laboratory conditions for a week and were fed with 40% crude protein pelleted feed during this period. Twelve hours before the commencement of the lethal experiment, the fish were not fed, the toxicants were then introduced into the test containers for further evaluation (APHA, 2012).

Collection and Identification of Plant Sample

Calotropis procera leaves were collected from the biological garden of the take off campus Federal University Dutsin-Ma Katsina State and sample was taken to the herbarium unit of the Department of Botany, Ahmadu Bello University Zaria for identification.

Aqueous Extraction of Phytochemicals from Leaves of *Calotropis procera*

The fresh leaves of the plant *C. procera* were collected between 08.00am and 09.00am from the Botanical garden of the Department of Biological Sciences, Federal University Dutsin-Ma, leaves were washed under running tap water, weighed using the weighing balance and blended in 200 ml of distilled water using Kenwood® electric blender and allowed to soak for 24 hours (Odulate *et al.*, 2016). The mixture was sieved through a No. 1 Whatman filter paper as described by (Odulate *et al.*, 2016) and the filtrate (stock solution) diluted (v/v) serially to the required level of concentration.

Experimental Procedure

The experiment was in two phases, the first phase was the range finding test, and the lethal experiment which lasted for 96hours.

Range Finding Test

The range finding test was conducted to determine the concentration of toxicant that was used for the definitive test. This was done by placing four concentrations and a control (10, 20, 40, 80 and 0.00 mg/L) of the toxicant in separate tanks and 5 fishes were stocked in each container, mortality of the stocked fish was observed at 0, 12, 24, 48, 72, and 96 hours. The concentrations were graded using lower ranges until 80-90% mortality was recorded in the highest concentration and 20-30% for the lowest concentration as suggested by Salim *et al.* (2021).

Lethal Experiment

One hundred and fifty (150) of the juveniles of *Clarias gariepinus* were procured and used for the lethal experiment. The desired *Calotropis procera* concentrations of 20mg/L, 40mg/L, 60mg/L, 80mg/L and a control (0.00) was measured and introduced into the four plastic containers in triplicate with a control carrying 10 fishes respectively. The fishes were observed for appearances, behaviour, breathing and mortality after 12, 24, 48, 72 and 96 hours respectively APHA (2012). The containers were examined for fish mortality on daily basis. Fish were confirmed dead when they showed no response to touch by glass rod. Dead fish were removed immediately and recorded (Odulate *et al.*, 2016).

Behavioural Response of Fish

The behaviour of the fish in the experimental set up and response was observed during lethal experiment, observations of the behaviour was carried out at intervals of 12, 24, 48, 72 and 96 hours respectively. The behaviour of the fish toward air gulping, loss of equilibrium, erratic swimming and haemorrhage was observed and recorded. While the appearance of the skin was also observed and recorded.

Determination of Physicochemical Parameters of Test Water

All physicochemical parameters of the water for the lethal experiment were determined as described by APHA, 2012.

Fish Mortality

During the observation for the behaviour and appearance of the test fish samples, the dead ones were removed, counted and recorded (Dauda *et al.*, 2018).

Haematology

Two fishes were randomly taken from each experimental trial with different concentrations of *Calotropis procera* for the lethal experiment, the blood samples were collected and analysed according to Dauda *et al.* (2018).

Procedure for Red Blood Cell Count

The standard RBC diluting pipette and a 1:200 dilutions was used for the red blood cell count (Dauda *et al.*, 2018).

Procedure for White Blood Cell Count

White blood cell was counted using Neubauhaemocytometer (Dauda *et al.*, 2018).

Data Analysis

The data obtained was summarized using descriptive statistics for the mortality, water quality, haematological parameters, growth performance and nutrient utilization. The difference among the concentrations was assessed using one-way analysis of variance, where there were significant difference Duncan multiple range tests was used to separate means (Dauda *et al.*, 2018).

RESULTS AND DISCUSSION

Behavioural Responses of *Clarias gariepinus* Juveniles Exposed to Different Concentrations of *C. procera* for 96hours

Clarias gariepinus juveniles showed different behavioural responses on exposure to *C. procera*. Immediately the fish were introduced into the bowls containing *C. procera* they became restless and agitated. Fishes came to the surface of water more frequently, they occasionally tried to jump out of the water. The fishes progressively became sluggish with increased mucous secretion and lethargic. The fish stood vertically with their head above the water surface as the time of exposure increased. Abnormal swimming movement such as air loss of equilibrium, loss of buoyancy and spasms before the death of fish was observed.

Physicochemical Parameters of Water during the Exposure of *Clarias gariepinus* Juveniles to different Concentrations of *Calotropis procera* for 96hours

The physicochemical parameters of water such as temperature, dissolved oxygen, hardness, alkalinity, ammonium nitrate, pH and TDS were presented in

Table 1. There was significant difference ($p < 0.05$) in all the selected water quality parameters after 96hours. Table 1: Lethal effect of *Calotropis procera* on some selected water quality parameters within 96hours.

Mortality rate of *Clarias gariepinus* Juveniles exposed to different concentrations of *Calotropis procera* extract.

Mortality occurred in all concentrations except for control and concentration of 20mg/L as shown in Fig

2. Mortality increased with increase in concentration, 20mg/L, 40mg/L, 60mg/L and 80mg/L.

The Haematological Indices of *Clarias gariepinus* Juveniles exposed to different Concentrations of Extracts for 96hrs

The result of haematological indices including PCV, RBC, WBC, Hb, Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils is presented in Table 2.

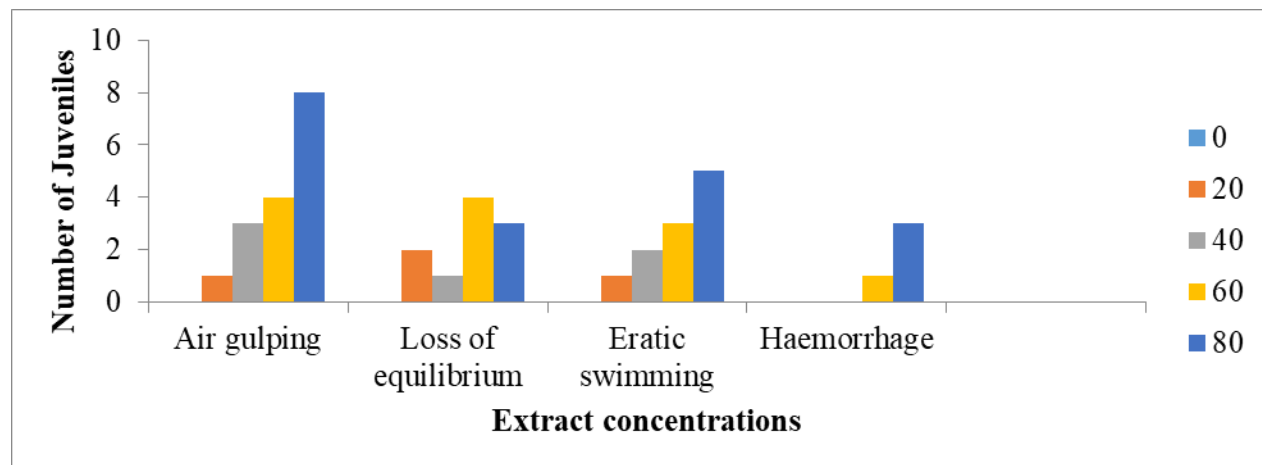


Fig. 1. Behavioural response of *Clarias gariepinus* within 96hours exposure to *Calotropis procera*

Table 1. Physicochemical Parameters of water after 94hours

Parameter	Control	20mg/L	40mg/L	60mg/L	80mg/L
Temperature °C	25.67±0.33 ^{ab}	26.00±0.67 ^{ab}	25.00±0.00 ^a	26.67±0.33 ^b	26.67±0.33 ^b
DO(mg/L)	4.20±0.00 ^c	4.20±0.00 ^c	2.20±0.67 ^b	2.20±0.00 ^b	1.00±0.00 ^a
Hardness	80.00±0.67 ^a	87.67±0.88 ^b	91.00±2.52 ^b	98.00±0.00 ^c	101.33±1.45 ^c
Alkalinity	30.00±0.00 ^a	30.00±0.00 ^a	30.00±0.00 ^a	36.67±3.33 ^a	40.00±5.77 ^a
Ammonium(mg/L)	1.96±0.42 ^a	2.1±0.58 ^a	2.48±0.00 ^b	2.79±0.77 ^c	2.94±0.00 ^c
pH(mg/L)	6.33±0.17 ^a	7.60±0.00 ^b	7.70±0.06 ^b	7.90±0.06 ^{bc}	8.27±0.09 ^c
TDS(mg/L)	169.67±7.62 ^{ab}	218.00±2.68 ^{ab}	251.33±6.23 ^a	253.67±9.84 ^b	350.33±6.44 ^{ba}

Different letters as superscript across the rows indicates significant difference ($P < 0.05$)

Key: DO=Dissolved Oxygen, TDS= Total Dissolved Solids, ND= Not Detected

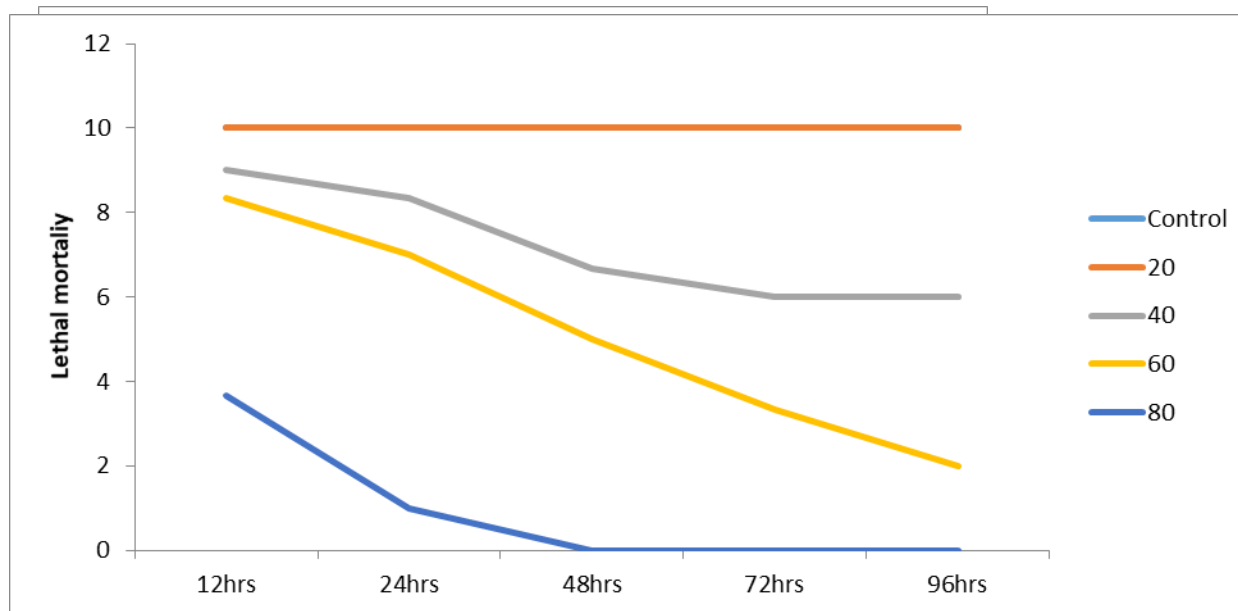


Figure 2. Mortality of *Clarias gariepinus* juveniles exposed to different Concentrations of extracts for 96hrs

Table 2. Mean haematological indices of *Clarias gariepinus* juveniles during the period of research

Blood parameters	Control	20 (mg/L)	40 (mg/L)	60 (mg/L)	80 (mg/L)
PCV (%)	30.33±2.03 ^b	28.33±0.88 ^b	27.66±0.33 ^b	29.33±1.20 ^b	21.66±0.88 ^a
RBC (×10 ¹² /ml)	2.04±0.11 ^b	1.88±0.05 ^{ab}	1.98±0.18 ^{ab}	1.99±0.75 ^{ab}	1.73±0.28 ^a
WBC (×10 ⁹ /ml)	12.27±0.26 ^c	8.90±0.56 ^{ab}	7.30±0.61 ^a	10.33±0.47 ^{bc}	8.73±0.43 ^{ab}
Hb (gm/100ml)	9.57±0.35 ^b	9.03±0.43 ^b	9.17±0.09 ^b	9.73±0.18 ^b	6.80±0.20 ^a
Neutrophils (%)	10.37±0.43 ^a	11.07±0.33 ^a	12.20±1.57 ^a	19.00±0.25 ^b	17.90±1.32 ^b
Lymphocytes (%)	89.63±2.18 ^b	87.80±0.98 ^b	85.47±1.74 ^{ab}	80.13±1.39 ^a	78.87±1.23 ^a
Monocytes (%)	ND	ND	ND	ND	ND
Eosinophils (%)	0.67±0.33 ^b	0.33±0.33 ^b	ND	0.33±0.33 ^b	ND
Basophils (%)	ND	ND	ND	ND	ND

Different letters as superscript across the rows indicates significant difference (P<0.05)

Key: PVC – Packed Cell Volume, RBC – Red Blood Cell, WBC – White Blood Cell, Hb – Haemoglobin, ND= Not Detected

DISCUSSION

Behavioural Responses Exhibited by *C. gariepinus* Juveniles

The behavioural changes exhibited by *Clarias gariepinus* juveniles exposed to *Calotropis procera* leave extract include, erratic swimming, haemorrhage, loss of equilibrium and air gulping. A similar response was documented by Odulate *et al.*, (2016). Fish exposed to toxins have been reported to have shown such behavioural responses (Sogbesan and Ngadina, 2015). The fish exhibited stressful behaviours, which were higher as the concentration of the extract increased. Gradual decrease in activity with time was observed until a state of calmness

followed by subsequent death. Olusola *et al.* (2023) and Idowu *et al.* (2017) reported similar behaviour on *C. gariepinus* and *C. punctatus* by Odulate *et al.* (2016). The loss of balance and direction in the tested fish, erratic swimming and haemorrhage can be attributed to a nervous reaction of the fish to the extract. The air gulping behaviour exhibited by the fish may be because of respiratory impairment of the gills resulting to the gills surface inability to actively carry out gaseous exchange and further death (FAO, 2014).

Selected Physicochemical Water Quality Parameters for Lethal Concentrations

The water physicochemical parameters for lethal analysis were negatively affected as seen from the experiment. The water quality parameters in the extract concentrations varied significantly from what was obtained in the control. Similar observation was reported by Agbebi *et al.* (2012) Akinwande *et al.* (2007) and Dan-Ologe and Sogbesan (2007) when fish were exposed to different concentrations of different extracts. The reduction in dissolved oxygen recorded in this experiment coupled with increase in the alkalinity as the concentrations of the extract increased could be attributed to the effect of the extracts and could have contributed to the mortality recorded in the treatments with the different concentrations of *C. procera* extracts. The pH values observed increased with increasing levels of plant extract concentrations, this may be an indication that *C. procera* extract is not acidic but alkaline in nature. This finding agrees with that of Odulate *et al.* (2016) and Agbebi *et al.* (2012).

Mortality Rate of the Fish from the Lethal Experiment

Mortalities were observed in all the treatments except in the control. Increase in fish mortality with increase in concentration of the extract in the medium showed a dose-response relation, which has been reported by Omoniyi *et al.* (2013). The LC₅₀ value of 50.12mg/L obtained in this study indicates that aqueous extract from *C. procera* was moderately toxic to juveniles of *C. gariepinus* and is in agreement with previous research (Ashraf *et al.*, 2010; Ayotunde *et al.*, 2010; Keremah *et al.*, 2010; Agbebi *et al.*, 2012).

Haematological response of *Clarias gariepinus* to lethal concentrations of *C. procera*

The haematological data obtained in this study for lethal analysis of *Clarias gariepinus* is similar to finding reported by Ochang *et al.* (2007); Pharm *et al.* (2007) and Adewolu and Adamson (2012). The significant decreases in PCV, RBC and Hb levels were in line with observation of Fafioye (2014) and Ajani, (2008). They also noted significant decreases in these blood cell parameters between the control and experimental specimens of both *C. gariepinus* and *O. niloticus* on exposure to sub-lethal concentrations. The significant reduction in these blood parameters is an indication of severe anaemia that might have been caused by the destruction of red blood cells (Kori-Siakpere *et al.*, 2009), and

haemodilution resulting from impaired osmoregulation across the gill epithelium (Aderolu *et al.*, 2010; and Salim, *et al.*, 2021). The decreased values of haemoglobin concentration, packed cell volume and erythrocyte in the present study could also be attributed to the inability of the fish to deliver sufficient oxygen to hematopoietic tissues suggesting poor physical activity (Hussain *et al.*, 2014; Salim *et al.*, 2021).

The significant decrease observed in white blood cell level on exposure to *C. procera* may be due to the function of the white blood cells. They are the "soldiers" of the body. This is in consonance with the result of Ajani (2008) and Ojikutu *et al.* (2013) where leukocyte counts were decreased after been exposed to an organophosphate pesticide for 96 hr. Low leucocyte count in this study is attributed to the reduction in the number of lymphocytes. Sotolu and Faturoti (2011) also noted that stress levels affect the number of leukocytes in immune organs.

CONCLUSION

This study has demonstrated that exposure of *C. gariepinus* juveniles to the aqueous extract of *Calotropis procera*, resulted in erratic swimming, air gulping, haemorrhage and death as a result of damage done by such extract as well as increase in mortality with increase in concentration of the extract. Thus, it could be said that frequent exposures to lower concentrations may not cause any behavioural responses to the fish due to biodegradability of most botanicals.

The study also showed that some physicochemical parameters including dissolved oxygen, alkalinity, total dissolved solid, temperature and pH were adversely affected by the aqueous extract at various concentrations. The extract caused a decrease in the dissolved oxygen level and an increase in the pH level of the water with increase in extract concentrations. Policies that will regulate the use of *C. procera* plant on any water body should be enacted and enforced. Awareness should be created by government and stakeholders on the effect of *C. procera* in fishing operation so as not to eliminate other living biota in an ecosystem. Further studies on the histology of some organs is also recommended.

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