



Establishing a Dose-response Toxicity for *Clarias gariepinus* Fingerlings Exposed to Ethanolic Extract of *Lantana camara*

U. U. George ^{a*}, O. O. Ajayi ^b, I. E. George ^c
and U. E. Vincent ^a

^a Department of Fisheries and Aquaculture, Faculty of Agriculture, Akwa Ibom State University, Ikot Akpaden, Akwa Ibom State, Nigeria.

^b Department of Pathology, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria.

^c Department of Zoology, Akwa Ibom State University, Ikot Akpaden, Mkpata Enin, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author UUG performed the conception, design and development of the topic, data collection and analysis, initial drafting and reviewing the manuscript and final approval of the prepared manuscript. Author OOA did the conception, design and development of the protocol, supervision of the experiments, data analysis and reviewing the manuscript. Author IEG was student who actually played the supporting role in the experiments and reviewing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The toxicity of *lantana camara* on the survival and histopathology of gills of *Clarias gariepinus* fish from Fisheries unit, Akwa Ibom State University Obio Akpa, farm was studied in two batches (A, and B) using the ethanolic extract (EE) of *Lantana camara* for 96 hours under laboratory

*Corresponding author: Email: ubonggeorge@aksu.edu.ng;

conditions. Five concentrations ranging from 0, 2, 3, 4, and 5 mg/L were prepared from the (EE) of *L. camara* for the toxicity test. The experimental animals showed different percentage mortalities with toxicant concentrations. The 96 hours LC₅₀ for *C. gariepinus* for both batches (A, and B) was given at 2.983 mg/l representing a log transformed concentration of 0.475 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment. The different batches of *C. gariepinus* (P>0.05) had no significant difference in mortality indicating that the ethanolic extract had same toxic effects on the test organisms. Toxicant exposure induced behavioral changes such as abnormal and uncoordinated swimming movement, restlessness, respiratory difficulties and attempt at jumping out was observed during the study. The results of the present study suggest that the ethanolic extract of *L. camara* had severe impacts on the test organism resulting in mortality. The effects of the plant extract on the gills of *C. gariepinus* fingerlings had severe impacts on the test organisms. Samples were taken from each of the concentrations to examine the effects of the extract on the gills of *C. gariepinus*. There was no observable change in the gills of the test organism in the control group. In the 2 mg/l concentration, the gill showed diffused epithelial degeneration of the primary lamella., in the 3 mg/l and 4mg/l concentration it was observed that the gills showed complete diffused epithelial degeneration of the lamella. Although cartilaginous filament of the epithelium was preserved between the two adjacent filament in skeletal muscle. Finally, in the 5 mg/l concentration the gill section showed diffused epithelial degeneration of the primary lamella and few retained processes of the secondary lamella. From the findings, it is observed that extract obtained from *L. camara* is toxic to aquatic life. Therefore, effective management strategies should be put in place to ensure safety compliance.

Keywords: Dose-response; toxicity; ethanolic extract; *Latana camara*; *Clarias gariepinus*; histopathology; gills.

1. INTRODUCTION

Lantana sp. (Verbenaceae) is a highly invasive tropical weed that attacks more than 60% of forests worldwide (Sharma, et al., 2005). The genus contains 150 species and is native to South America, Asia, and Africa's tropical and subtropical regions. *L. camara* L. is the most dominant species (Negi, et. al., 2019). Despite the fact that Lantana sp. is widely used as an ornamental plant, its negative effects, particularly when consumed by animals and leading to cholestasis, hepatotoxicity, and phototoxicity, have been linked to the plant's pentacyclic triterpenoids, including lantadenes A and B in their leaves and seeds (Negi, et al., 2019). The world has recently placed more emphasis on plant research, and a substantial body of evidence has amassed to demonstrate the enormous potential of medicinal plants employed in diverse traditional systems (Mouokeu, et al., 2011, Njateng, et al., 2010, Kuete, 2010). According to the World Health Organization (WHO), traditional medicine serves as the primary source of healthcare for 70 to 80 percent of people in developing countries. Many people, however, underestimate the toxicity of natural chemicals and are unaware that these substances might be as hazardous as or more toxic than synthetic drugs. Many plants have been documented to be hazardous to humans as

well as animals (Mouokeu et al., 2011, Aguinaga et al., 2014). It should therefore be underlined that the historic usage of any plant for medical reasons in no way guarantees its safety. Plants used in folk medicine should therefore be examined for safety and toxicity, and appropriate recommendations on their use provided. Natural products have always been important in the advancement of organic chemistry. The varied structural types of natural products not only contribute to new discoveries or face challenging synthetic difficulties, but also inspire optimism that they may serve as the foundation for new physiologically active molecules of commercial value. Biological testing has been crucial in plant extract toxicity investigations. Plant extracts have been the subject of several bioassay experiments. The first such study using several local plants was published in 1965 (Nakanishi et al., 1965). The purpose of toxicity testing is to detect potential detrimental effects of environmental agent exposure, to build a dose-response relationship that can elucidate the severity of effects associated with known exposures, and, eventually, to forecast the consequences of exposures on human populations. Toxicologists conduct carefully designed investigations and experiments in order to explore and access the impacts of substances. These studies aid in determining the precise amount of chemical that may cause harm, as

well as the possible hazards of being near or using products containing certain chemicals. Therefore, the aim of this study was to assess the toxic effect of *L. camara* on *C. gariepinus* fingerlings, in order to provide critical information on the toxicity of the plants.

2. MATERIALS AND METHODS

2.1 Collection of Test Organism

Fingerling of *Clarias gariepinus* were collected from Akwa Ibom State University fish farm, Obio Akpa Akwa Ibom State, Nigeria located within 4^o 57'52" N and 7^o45'29" E. The climate of the area is tropical and is characterized by distinct wet and dry seasons. The vegetation of the study area is generally rainforest close to the mangrove belt. Human activities in the area include farming, hunting, boat building and sand mining. A total of two hundred (200) fingerling were collected and used for the study.

2.2 Acclimatization of Specimen's

The fingerlings were acclimatized in a recirculatory glass aquaria measuring 96 x 50 x 29 cm containing habitat water for 24 hours in the fisheries and aquaculture laboratory of Akwa Ibom State fish farm. This enhanced the stability of the fingerlings from stress of collection and transportation [1].

2.3 Collection of Plant Sample

Fresh leaves of (*L. camara*) was collected for the study. The collection site of the plant was at Oron road in Uyo Local Government Area, Akwa Ibom State. The date of Collection was 20th January, 2023. The plants material was taken for identification and authentication by a plant systematics at the Department of Botany Herbarium, Akwa Ibom State University, Ikot Akpaden, Mkpata Enin Local Government Area.

2.4 Preparation of Plant Material

After the identification, the leaves were washed and sun dried. The leaves were shredded and spread on cellophane and allowed to dry for 72 hours under room temperature. The dried leaves were pulverized (grinded) into fine powder using wooden pestle and mortar.

2.5 Preparation of Ethanolic Extract (Maceration and Extraction)

Cold extraction method (Maceration) was used in this research according to Hidayat and Wulandari

(2021), in the extraction procedure, 1000 ml of 99% Concentrated Ethanol was used to Macerate 240 g of the plant materials in an airtight container and kept in the laboratory under room temperature for 72 hours (3 days). In the due date of filtration, the mixture was filtered with Muslim cloth to acquire the filtrate. The extract was stored in 250 ml conical flasks. The conical flask was well labelled, the mouth of the conical flask was covered with foil paper and masking tape rapped around the mouth to ensure that it is tightly covered.

2.6 Preparation of Experimental Aquaria

Ten (10) rectangular plastic aquaria measuring 25 x 10 x 15 cm were thoroughly washed with tap water and properly rinsed with fresh water of similar salinity and allowed to drain dry for 24 hours on the laboratory bench based on Dede and Kagbo [2].

2.7 Stocking of Specimen

Each of the Ten (10) plastic aquaria was filled with two liters of water and 10 *C. gariepinus* was stocked in each aquarium [3]. The EE of *L. camara* with varying concentrations was added to each stocked aquaria and allowed to stand for 96 hours for mortality examination. A preliminary test was conducted to give the actual variations in concentration to be used for the bioassay. Each of the aquarium had a replicate to ensure accuracy [3].

2.8 Monitoring of Water Quality

Water Quality Parameters was monitored prior to commencement of the experiment and also periodically according to Standard Method [4,5] Parameters that were monitored include dissolve Oxygen (DO), pH, And Temperature (^oC). Temperature and pH was measured using portable pH /Ec/ TDs/ Temperature HANNA, H1 991301 Model instrument while oxygen was measured using digital portable analyser JPB - 607A from "Search Tech Instrument".

2.9 Monitoring of Specimen for Mortality

The effects of the various concentration of the EE of (*L. camara*) on the fingerlings was monitored on a 24 hours' basis for 96 hours as recommended by Udo et al., [1] and Ekanem and Ekpo [6].

2.9.1 Determination of Mortality and Survival rates of Fingerlings

The percentage mortality and survival rates of the fingerlings in the different concentrations of the ethanolic extract of *L. camara* during the period of study was determine using the formula;

$$\% \text{ mortality} = n/N \times 100 \text{ [7].}$$

Where;

n = number of dead fish per aquarium per concentration
N = Total Individual Stocked

The difference between dead fish and survivors will give the percentage survival of the fingerlings at the end of the experiment (96 hours) [1].

2.9.2 Determination of mortality lethal median concentration (96 hours LC₅₀)

The effects of the various concentrations of the ethanolic extract of plant (*Lantana camara*) on the fingerlings was determined by graphical method (Probit Level Determination as recommended by Omoregie [8], Omoregie and Ufodike [9], Ekanem and Ekpo [6] and Udo et al. [1]. At Lethal Median Concentration LC₅₀, after 96 hours of test, the number of fingerlings that are expected to die was determined from the graph. Similarly, the concentration that will kill 5% of the stocked fingerlings at the end of the test (96 hours) was determined at the probit level Omoregie, [8] Omoregie and Ufodike [9], Udo et al., [1]; Ekanem and Ekpo [6].

2.10 Collection of Sample for Histopathology Extractions

The gill's tissues were isolated from the test animal and fixed in formalin -saline for 48 hours. The fixed tissue was processed manually through graded ethanol, cleared in xylene impregnated and embedded in paraffin wax, sections of the tissue sample were cut with a rotary microtome, stained by hematoxylin and eosin technique, prepared tissues were finally observed using a microscope for pathological changes at x100 and x400 magnification.

2.11 Data Analysis

The results of the respective concentration effects of the ethanolic extract of *L. camara* was presented in tables. Two-way analysis of variance (ANOVA) was used to test for significant (P<0.05) difference considering the extract concentration and mortality time. p-value will allow to affirm if the observed differences in the variation of the studied parameters is significant. Also, the LC₅₀ was determined using Probit analysis. All statistics were carried out using SPSS version 20.0.

3. RESULTS

3.1 Initial Water Quality Parameters

The initial water quality parameters prior to stocking are shown in Table 1. Dissolved oxygen had a value of 5.2 mg/l, with a value of 29.8°C for Temperature and 6.77 for pH.

3.2 Summary of the Percentage Mortality and survivors of *C. gariepinus* Exposed to the EE of *L. camara* at the end of the Experiment (96 hours)

The percentage mortality and survivors of *C. gariepinus* at the end of the test period in each of the concentrations are shown in Table 2 for the two batches of the experiment.

In the 0 mg/l concentration of the extract, no mortality was recorded throughout the test period in both batches A and B. in the 2 mg/l concentration of the extract, 70 % mortality was recorded leaving behind 30 % survivors in both bathes.

At the end of the 96-hour bioassay 100 % mortality was observed in the 3, 4 and 5 mg/l concentration of the extract leaving behind no test organisms in the test media for both batches (Table 2). Statistical Analysis using two way Anova (SPSS 20.0) showed that there was no significant difference (p<0.05) in mortality between the two batches in the different concentration and time.

Table 1. Initial Physico-chemical parameters of the test water prior to stocking of test organism

Fish Species	Initial physico-chemical parameters prior to stocking		
	DO (mg/l) Concentration	Temp (°C) value	pH value
<i>Clarias gariepinus</i>	5.2	29.8	6.77

Table 2. Summary of the percentage mortality and survivors of *C. gariepinus* exposed to the EE of *L. camara* at the end of the experiment (96 hours)

Conc. of extract (mg/l)	Batch A				Batch B			
	Mortality (M)	% M	Survivors (S)	% S	Mortality (M)	% M	Survivors (S)	% S
0	0	0	10	100	0	0	10	100
2	7	70	3	30	7	70	3	30
3	10	100	0	0	10	100	0	0
4	10	100	0	0	10	100	0	0
5	10	100	0	0	10	100	0	0

3.3 96 Hours LC₅₀ Determination

The 96 hours LC₅₀ for *C. gariepinus* exposed to the different concentrations of the ethanolic extract of *L. camara* is shown in Table 3 for both batches. The 96 hours LC₅₀ is given at 2.983 mg/l representing a log transformed concentration of 0.475 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment.

3.4 Histopathology of the gill of *C. gariepinus* Exposed to the Different Concentrations of the Ethanolic Extract of *L. camara*

The histo-morphological changes observed in the gills of fingerlings exposed to *L. camara* indicate that exposure to the plant can cause degenerative changes in the gill structure. The control (0 mg/l) group 1 (Ai & Aii) displayed normal highly cellular primary lamella epithelium, indicating that the gills were healthy and without any significant histological changes. Group 2 (2 mg/l) exhibited diffused epithelial degeneration of the primary lamella and supporting cartilage epithelium as shown in Fig. 1 (Bi & Bii). Groups 3 (3 mg/l) and 4 (4 mg/l) Fig. 1 (Ci & Cii) (Di & Dii) respectively displayed complete diffused epithelial degeneration of the filament, but preserved supporting cartilage epithelium. This indicates that the degenerative changes caused by *L. camara* were limited to the epithelium of the lamella and did not extend to the cartilage. The highest dose Group 5 (Ei & Eii) showed diffused epithelial degeneration of the primary lamella and few retained processes of the secondary lamella.

The results suggest that exposure to *L. camara* can cause histomorphological changes in the gills of fingerlings, affecting the primary lamella, supporting cartilage, and secondary lamella in varying degrees.

4. DISCUSSION

Three basic physico-chemical parameters were taken in line with standard practice in toxicological studies prior stocking of the experimental fish. Dissolved Oxygen value of 5.2 mg/l with a value of 29.8°C recorded for temperature and a value of 6.77 was recorded for pH. In aquaculture operations, there are recommended values for these parameters. For dissolved oxygen a range of between 4.0 – 6.0 mg/l is suitable, 6.7 – 8.6 for pH and 25.0 – 30.0°C for temperature are recommended values for standard operation of aquaculture [10,11, 3,12-16].

Before the experiment commenced, it was discovered that the physico-chemical parameter ranges of the experimental water fell within the acceptable limits, as previously reported by the authors under reference. The preservation of the standard values of the physico-chemical parameters by the experimental water prior to the commencement of the experiment might have been as a result of absence of impurities or the toxicant and the organisms themselves [17,18]. Impurities, pollutants and toxicants are known to play a role either to elevate or reduce the different physico-chemical parameters in aquatic environment [18,19,20].

Table 3. LC₅₀ determination for *C. gariepinus* at the end of the 96-hours bioassay

Plant	Species	Probit	S.E	LC ₅₀	Log Con.
<i>L. camara</i>	<i>Clarias gariepinus</i>	P= 3.403 -- 7.170X	2.741	2.983	0.475

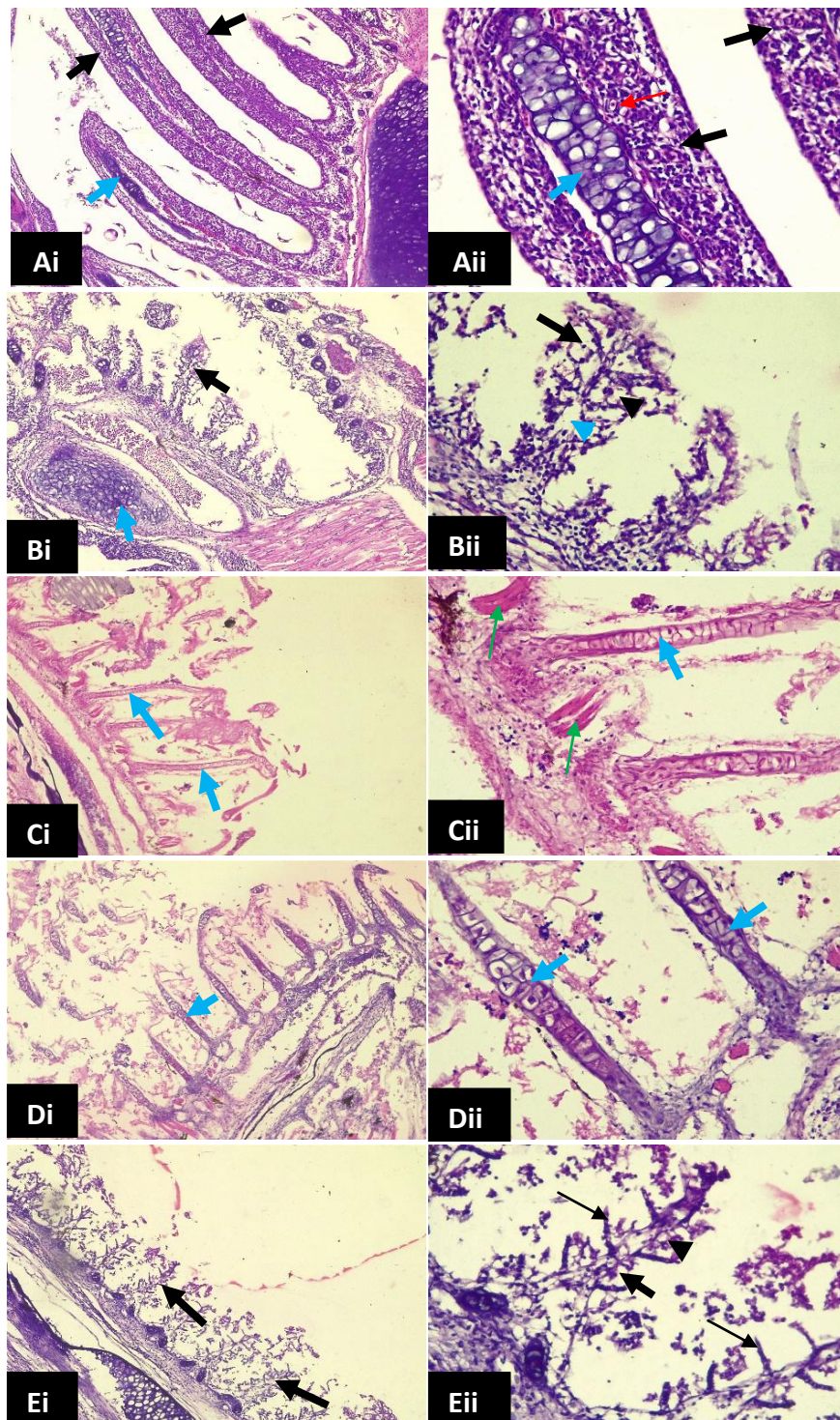


Fig. 1. Photomicrograph of the gill arch tissue section across the 5 groups. Haematoxylin and Eosin (H&E) stain, X100 and X400 Magnification

Control Group (Ai&Aii) showed gills section with normal filament epithelium (thick black arrow), the supporting cartilage epithelium (thick blue arrow), muscle fiber (arrowhead) and the capillaries (thin red arrow). The 2 mg/l (Bi&Bii) showed moderate epithelium degeneration of the filament and the supporting cartilage, while 3 mg/l (Ci&Cii) & 4 mg/l (Di&Dii) showed retained cartilage epithelium but diffused degeneration of filamentous epithelium and 5 mg/l (Ei&Eii) depicted diffused epithelium degeneration of both primary (thick black arrow) and secondary (thin black arrow)

The percentage mortality of *C. gariepinus* in the ethanolic extract of *L. camara* ranged from 0 – 100% in both batches A and B at the end of the 96-hours bioassay. No mortality was recorded in the 0 mg/l concentration of the toxicant. However, 70% mortality was recorded in the 2 mg/l concentration in each of the batches while 100% mortality was recorded in each of the 3 mg/l, 4 mg/l and 5 mg/l concentration of the toxicant. The results of the present findings which shows that mortality was concentration-dependent are in consonance with the earlier reports by George et al., [3] when reporting on the laboratory bioassay of the potential effect of rubber extract (*H. brasiliensis*) on the Survival of fingerlings of *O. niloticus*; George et al., [12] during their studies on the effect of lethal concentrations of rubber extract (*H. brasiliensis*) on the survival on fingerlings of *C. gariepinus* under laboratory condition; George et al., [15,16] when working on the toxic effect of crude oil on hatchery reared *O. niloticus* fingerlings and earlier assertion made by George et al., [13,14] when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *C. gariepinus* juveniles.

In the present study, percentage mortality was concentration dependent. The higher the concentration, the higher the percentage mortalities. Similar results have been reported by different authors; Ayuba and Ofojekwu [21], Adedeji et al., [22] and Ogundiran et al., [23] while investigating on the toxicity of different plant extracts on *C. gariepinus*. Calta and Ural, [24] also reported a dose-dependent toxic response of *C. carpio* to synthetic pesticides. Ayotunde et al., [25] noted that *C. gariepinus* generally tolerate and adapt to environments with rapid changes in water chemistry and increasing contaminants concentration. This explains the response of the species to different concentration. However, the differential mortality observed in the different concentration of the extract over the 96 hours' period, vindicates the law of tolerance according to (Shellford, 1941).

Mechanism for the selective toxicity of toxicants for various fish species depend on four factors which include, nature of the toxicant, varying concentration of the toxicant, Exposure time and fish species. The aforementioned parameters were most likely responsible for the various harmful reactions displayed by the fish at various concentrations and times during the experiment. The reactions are frequently more severe at greater concentrations due to increasing

acetylcholinesterase inhibition, which ultimately leads to the death of the test organisms [25,22].

The 96 hours LC₅₀ of any toxicant is the dose or concentration which killed 50 % of the stocked organisms at the end of the experimental period of 96 hours (4 days) [1,3,12-16].

The 96 hours LC₅₀ is known to vary from toxicant [4] and from concentration to concentration of the toxicant [26,27].

In the present study the 96 hours LC₅₀ was 2.983 mg/l representing a log concentration of 0.475 for both batches (A and B). The 96 hours LC₅₀ of toxicants are known to vary as previously reported by the authors earlier cited above. For instance, Ogundiran et al., [23] reported 96 hours LC₅₀ of 0.0166 mg/l and 0.0038 mg/l for batch A and B *C. gariepinus* fingerlings under the toxicity effects of detergent effluents, 96 hours LC₅₀ of 0.1 mg/l and 0.03 mg/l was reported by Adewoye et al., [28] when working on the effects of soap and detergent effluents on *C. gariepinus* fingerlings. Again, Ayotunde et al., [25] reported the 96 hours LC₅₀ of 0,033 – 0.33 mg/l on *C. gariepinus* adults using Carica papaya extract. The varied 96 hours LC₅₀ values usually obtained from different toxicants and test organisms is again reported by Ekanem et al., [29], when they reported a 96 hours LC₅₀ of 5.0 ± 1.76 and 4.0 ± 1.76 mg/l for *Macrobrachium macrobrachion* and *Macrobrachium vollenhovenii*. In this study the 96 hours LC₅₀ of 0.475 mg/l obtained for both batch A and B might have depended on the ranges of the toxicant finally used for the bioassay after conducting a preliminary test.

The effects of the ethanolic extract of *Lantana camara* showed pathological effects on the gill lamellae of *Clarias gariepinus* fingerlings. However, the gill lamellae in the control (0 mg/l) were not affected. Pathological effects were pronounced at 2 mg/l, 3 mg/l, 4 mg/l and 5 mg/l concentration of the extract which shows evidence of diffused epithelial degeneration of the lamella.

Gill lamellae cell disintegration has been reported by several authors, Diana et al., [30] when investigating on the biochemical and histological effects of deltamethrin on *Carassius auratus gibelio* with different effects such lamellae cells hypertrophy and nuclear pycnosis in the basal cells, Gabriel et al., [31] reported histopathological changes in the gills of *C.*

garipepinus exposed to refined petroleum oil and kerosene under laboratory conditions.

The histological changes observed in the present study were concentration dependent with severe alteration been pronounced at higher concentration. The results of this findings are similar to earlier assertion reported by George et al., [15,16] when reporting on the acute toxic effects of *H. brasiliensis* on the gills of hatchery reared *O. niloticus* fingerlings and observed histological changes in the gills of the exposed organisms which were concentration dependent, George et al., [13] when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *C. garipepinus* juveniles; Idowu et al., [32] when studying the effect of *Euphorbia hirta* leaf extract on histopathology of juveniles *Clarias garipepinus* and George et al., [14] when reporting on the histopathological alterations in gills of fingerlings of *C. garipepinus* following sub-lethal acute exposure to *Hevea brasiliensis* [33-36].

5. CONCLUSION

Establishing a dose-response toxicity for *C. garipepinus* fingerlings exposed to ethanolic extract of *Lantana camara* were investigated using static bioassay under laboratory condition. Prior to the toxicity test of the extract on the fish species physico-chemical parameters were taken before stocking of the experimental fish. The percentage mortality recorded in this study was concentration dependent with higher mortality recorder at higher concentrations in both batches. This assertion is true based on the law of tolerance as proposed by Shelford, (1941). From the mortalities ratio the 96 hour LC₅₀ was 0.475 mg/l for both batches. The results of histopathology showed similar observation of concentration dependent, the higher the concentration, the pronounced the effects of the extract on gill lamellae showing degeneration with no pathological changes seen on the gills of the control group in both batches. Based on the results of the study which showed high percentage mortalities when exposed to the ethanolic extract of *Lantana camara*, it is imperative that ecologically friendly methods should be put in place to checkmates invasive species within our environment. Further research is recommended on the toxicity of locally available plants within our environment. This recommendation stems from the fact that the toxicity level observed during the study was very high even at low concentrations. Based on the findings from the present studies, *L. camara*

cannot be used as s raw material in fish feed formulation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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