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RESEARCH ARTICLE

Investigating the Toxicity and Histopatho-Morphological Alterations Induced by Cana Indica Extract on *Clarias gariepinus* Fingerlings

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Abstract

This study aimed to assess the toxic potentials and histopatho-morphological changes in *Clarias gariepinus* fingerlings exposed to the ethanolic extract of Cana indica. Upon introduction of the extract to the test media, significant variations were observed in the water quality parameters, following a concentration-dependent pattern. Behavioural changes and modifications in the gill tissues were evident in the exposed groups, with severe alterations observed, excluding the control group. Mortalities were recorded in a concentration-dependent manner, with the LC_{50} value estimated at 20.5 mg/l representing a log transformed concentration of 1.31mg/l. These findings underscore the potential toxicological impact of *Cana indica* extract on aquatic organisms, highlighting the need for further investigation into its environmental implications and management strategies.

Keywords: *Cana indica, Clarias gariepinus,* Toxicity, Histopatho-morphology, Fingerlings.

1. Introduction

Plant's part has been reported to cause massive fish mortality, behavioural changes, haematological, biochemical responses and histopathological effects on Clarids (Gabriel and Okey, 2009; Okey *et al.*, 2013). Alteration in water parameters by the presence of contaminants makes it potentially harmful to life, instead of sustaining them (Agrawal *et al*., 2010; Michael *et al*., 2015; Ekpo *et al*., 2015; George and Atakpa, 2015 a, b). The buildup of toxicants in an aquatic environment can lead to reduced reproductive capacities; alteration in growth rates and reduced ability to with stand to variation in water quality parameters (Adamu *et. al*., 2008; George and Effiom, 2018, Jonah and George, 2019; Jonah *et al*., 2019; George *et. al*., 2020 a, b).

Plants are infinite sources of biological active substances and more than 65,000 species have been reportedly used all over the world for numerous purposes (Istvan, 2000; George, *et. al*., 2023 e). Ichthyotoxins plants contains different active ingredient such as nicotine, pyrethrum, rotenone, resins, tannins, alkaloids, flavenoids and saponins. Studies by several authors have demonstrated that herbal plant and their products have been used as natural alternatives for treatment and management of various diseases including dental decay (Abiola, 2008), pesticide (Maikai, *et. al*., 2008), molluscicide

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(Azare, *et. al*., 2007), Anaethetic (Okey, *et. al*., 2013) and Piscicide (Gabriel, *et. al*., 2008).

Piscicidal plants such as *Blighia sapida*, *Kegelia biglobosa*, *Lepidagathis alopercuriodes* and *Agave americana* are commonly used by fisher folks to harvest fishes (Audu, *et. al*., 2014). The use of Agave americana by fishermen in catching fish has been reported by Yadav, (2000) while the use of *L. alopercuroides* for the quick kill of hardy mudskipper fish was documented by Obumanu, *et. al* (2007).

Assessment of the effects of ichthyotoxic plants on fish has proven that exposed fish showed altered behavioral changes, disruption of the internal biochemical and physiological processes, histopathological alterations and eventually will result to death (Okey, *et. al*., 2013).

Cana indica commonly known as Indian shot, is a plant species known for its diverse medicinal properties. Extracts from *Cana indica* have been traditional used in various herbal remedies due to their purported therapeutic benefits. However, alongside its medicinal potential, there is a growing concern about the potential toxicity of *Cana indica* extract particularly in aquatic environments.

Clarias gariepinus or African catfish, is a commercially important freshwater fish species widely cultured in aquaculture systems. The fingerlings stage of *C. gariepinus* represents a critical developmental phase where susceptibility to environmental stressors, including toxic substances is heightened. Understanding the effects of *Cana indica* extract on the early life stages of *Clarias gariepinus* is crucial for assessing the impact on aquaculture practices and ecosystem health.

This study aims to investigate the acute toxicity of *C. indica* extract on fingerlings of *C. gariepinus*. By determining the lethal concentration (LC_{50}) or median lethal dose (LD50) of the extract, this research will provide valuable insights into the potential risks posed by *C. indica* extract to the survival and health of *C. gariepinus* fingerlings. Additionally, elucidating the mechanisms underlying the observed toxicity will contribute to a comprehensive understanding of the interactions between *C. indica* extract and aquatic organisms, aiding in the development of mitigation strategies to minimize adverse effects on fish populations and aquatic ecosystems. Toxicological data on *C. indica* extract can informed regulatory agencies responsible for setting guidelines and standards for the use of herbal remedies and environmental protection.

2. Materials and Methods

2.1 Collection of Plant Sample

Fresh leaves of *Cana indica* were collected for the study. The collection site of the plant was Obio Akpa in Oruk Anam Local Government Area, Akwa Ibom State. The date of Collection was 15th November, 2023. The plants material was transported to University of Uyo, Uyo, Akwa Ibom State for identification and authentication of the plants. This was done at the Herbarium in the Department of Botany, University of Uyo, Uyo with herbarium voucher No: (Olugbade, UUH 4418 (Uyo).

2.2 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.

2.3 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, 1000ml of 99% Concentrated Ethanol was used to Macerate 240g of the plant materials in an airtight container and kept in the laboratory under room temperature for 72 hours (3 days). The ethanolic suspension was filtered using a filter net and filter paper and the extract was evaporated in a water bath at 40° Celsius for 48 hours and stored in a beaker covered with aluminum foil for bioassay immediately after the evaporation was complete.

2.4 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 latitude 5°17′N and 7°27′N, Longitude 7°27′E and 7°58′E. The climate of the area is tropical and is characterized by distinct wet and dry seasons (George *et.al*., 2023a, b). 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.

2.5 Acclimatization of Specimen's

The fingerlings were acclimated in a re-circulatory plastic aquarium measuring 25 ×13 × 8.3 Cm^3 containing hatchery water for 24hours 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 (Udo *et al*, 2006).

2.6 Preparation of Experimental Aquaria

Ten (10) rectangular plastic aquaria measuring 25 ×13 × 8.3 Cm^3 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 (2001).

2.7 Stocking of Specimen

Prior to commencement of actual experiment, a preliminary test or range finding test with varying concentration (0, 5, 10, 15, 20 mg/l) 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. Each of the Ten (10) plastic aquaria was filled with two liters of hatchery water and 10 *Clarias gariepinus* fingerlings was stocked in each aquarium. The ethanolic extract of *Cana indica* with varying concentrations (0,21, 22, 23, 24 mg/l) was added to each stocked aquaria and allowed to stand for 96 hours for mortality examination.

2.8 Monitoring of Water Quality

Water Quality Parameters was monitored prior to commencement of the experiment and also periodically according to Standard Method (APHA,1998). Parameters that were monitored include dissolve Oxygen (DO), pH, And Temperature (^{0}C). Temperature and pH were 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 ethanolic extract of *C. indica* on the fingerlings was monitored on a 24 hours' basis for 96 hours as recommended by Udo *et. al.,* (2006) and Ekanem and Ekpo (2008).

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 *C. indica* during the period of study was determine using the formula;

% mortality =n/N × 100 (Chan, 1977).

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) (Udo *et. al*., 2006).

2.9.2 Determination of Mortality Lethal Median Concentration (96 Hours Lc_{50})

The effects of the various concentrations of the ethanolic extract of plant (*C. indica*) on the fingerlings of *C. gariepinus* was determined by graphical method (Probit Level Determination as recommended by Omoregie (2002), Omoregie and Ufodike (2000), Ekanem and Ekpo (2008) and Udo *et.al*. (2006). At Lethal Median Concentration LC_{50}, 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 50% of the stocked fingerlings at the end of the test (96 hours) was determined at the probit level (Omoregie, (2002) Omoregie and Ufodike (2000), Udo *et. al*., (2006); Ekanem and Ekpo (2008).

2.10 Behavioral Assessment of the Test Organisms in the Toxicant

Behavioral cum morphological responses of *C. gariepinus* fingerling exposed to *C. indica* leaf extract were monitored and measured daily according to OECD (2014). In addition to the acute doses, the control group without toxicant exposure were also observed as a guide for the assessment of any behavioral and morphological changes in the experimental groups. The responses monitored included irregular swimming, excessive mucus secretion, increased air gulping, vertical erection and respiratory distress Each experimental tank was observed for the concentrations and exposure durations

2.11 Collection of Samples for Histopathological Examinations

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.

2.12 Data Analysis

The results of the respective concentration effects of the ethanolic extract of *C. indica* was presented in tables. One-way analysis of variance (ANOVA) was used to test for significant difference between the varying concentrations in both batches (batch A and batch B) at the probability level of (P>0.05). Probit analysis was done using SPSS version 20.0.

3. Results

3.1 Initial Water Quality Parameters

The initial water quality parameters prior to commencement of the toxicity studies are shown in Table 1. The values of dissolved oxygen (5.8 mg/l), temperature (29.5 ^{0}C) and pH (6.40) observed were within the acceptable range for aquaculture operations.

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)	Temp (^{o}C)	pH
Clarias gariepinus	5.8	29.5	6.40

3.2 Variation in water Quality parameter in the test media with *Clarias gariepinus* as test organism (Batches A and B) during the experimental period (96 Hours)

Variation in the water quality parameters of the test organisms in the different concentration and exposure time in both batches (A and B) is presented Table 2.

The lowest concentration of dissolved oxygen was observed in the 24 mg/l concentration of the extract at 96 hours of test while the highest DO level was observed in the 0 mg/l concentration during the 24 hours of test. The value of temperature was observed to range between 27.1 – 28.6 ^{o}C during the 96 hours' bioassay. The least value of 27.1 ^{o}C was recorded during the 72nd and 96th hours of test in the 24 mg/l concentration of the extract while the highest value of 28.6 ^{o}C was recorded during the 24 hours of test in the 0 mg/l concentration of extract (control).

The value of pH was observed to vary during the experimental period of 96 hours. The lowest value of pH was recorded in the 24 mg/l concentration of the extract during the 96 hours of test while the highest value was recorded in 0 mg/l concentration of the extract during the 24 hours of test.

Table 2. *Summary of the variations in the physico-chemical parameters in the test media during the experimental period.*

Parameters	Conc. (mg/l)	BATCH A				BATCH B			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Dissolved Oxygen (mg/l)	O	5.6	4.7	4.4	4.0	5.6	4.7	4.4	4.0
	21	5.2	4.2	3.4	3.2	5.2	4.2	3.4	3.2
	22	4.8	3.8	3.6	2.4	4.8	3.8	3.6	2.4
Initial: 5.8 mg/l	23	4.4	2..6	2.5	2.4	4.4	2..6	2.5	2.4
	24	4.2	2..5	2.4	2.3	4.2	2..5	2.4	2.3
Temperature (^{o}C)	O	28.6	27.5	27.5	27.5	28.6	27.5	27.5	27.5
	21	27.4	27.4	27.3	27.2	27.4	27.4	27.3	27.2
	22	27.5	27.5	27.3	27.3	27.5	27.5	27.3	27.3
	23	27.4	27.4	27.2	27.3	27.4	27.4	27.2	27.3
Initial: 29.5 ^{o}C	24	27.4	27.3	27.1	27.1	27.4	27.3	27.1	27.1
pH	O	6.31	6.25	6.22	6.06	6.31	6.25	6.22	6.06
	21	6.26	6.20	6.14	5.85	6.26	6.20	6.14	5.85
	22	6.19	6.15	6.00	5.70	6.19	6.15	6.00	5.70
Initial: 6.40	23	6.18	6.12	5.97	5.65	6.18	6.12	5.97	5.65
	24	6.14	6.08	6.02	5.45	6.14	6.08	6.02	5.45

3.3 Summary of the Percentage Mortality and survivors of *C. gariepinus* Fingerlings in the different concentrations of the ethanoic extract of *Cana indica* at the end of the experiment (96 hrs.).

The percentage mortality and survivors of *C. gariepinus* fingerlings at the end of the test period in each of the concentrations are shown in Table 3 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 21 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 22, 23 and 24 mg/l concentration of the extract leaving behind no test organisms in the test media for both batches (Table 3). Statistical Analysis using one-way Anova (SPSS 20.0) showed that there was no significant difference ($p>0.05$) in mortality between the two batches.

Table 3. *Summary of the Percentage Mortality and survivors of C. gariepinus Fingerlings in the different concentrations of the ethanoic extract of Cana indica at the end of the experiment (96 hrs.).*

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
21	7	70	3	30	7	70	3	30
22	10	100	0	0	10	100	0	0
23	10	100	0	0	10	100	0	0
24	10	100	0	0	10	100	0	0

3.4 96 Hours LC_{50} Determination

The 96 hours LC_{50} for *C. gariepinus* fingerlings exposed to the different concentrations of the ethanolic extract of *Cana indica* was determine using probit analysis. The concentrations were first transformed into log for the probit analysis (Table 4). The 96 hours LC_{50} is given at 20.5 mg/l representing a log transformed concentration of 1.31 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment (Fig. 1).

Table 4. *LC50 determination for C. gariepinus Fingerlings at the end of the 96-hours bioassay.*

Concentration (mg/l)	Log Transformation	Mortality (M)	% Mortality	Survivor (S)	% Survivor
0	**0**	**0**	**0**	**0**	**100**
21	**1.32**	**10**	**100**	**3**	**30**
22	**1.34**	**10**	**100**	**0**	**0**
23	**1.36**	**10**	**100**	**0**	**0**
24	**1.38**	**10**	**100**	**0**	**0**

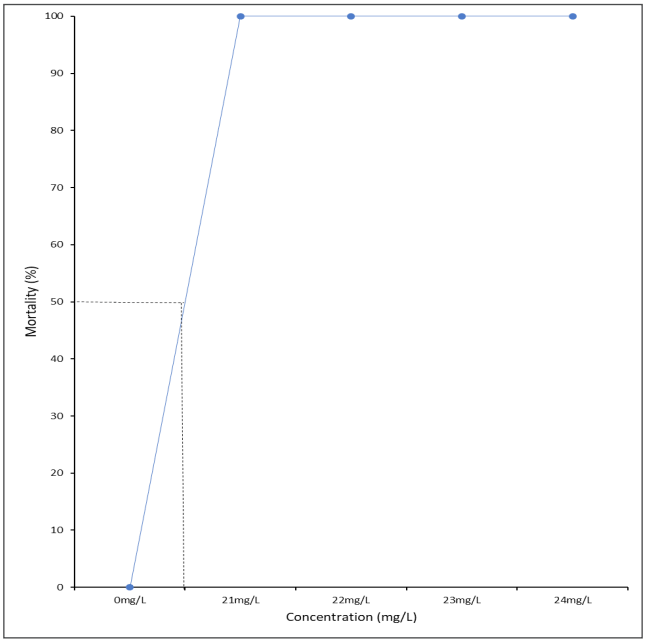

Figure 1. *Probit Graph of mortality (%) against concentration*

3.5. Behavioral Responses

Behavioral response of *C. gariepinus* fingerling exposed to concentrations of ethanolic extract of *Cana indica* leaf between 24 and 96-hours exposure durations were analyzed via a semi-quantitative measurement (Table 5). After 24 h exposure, the treated groups exposed to 21–24 mg/l concentrations of the plant extract displayed slight to moderate behavioral changes such as excessive mucus secretion, air gulping, erratic swimming, respiratory distress and vertical erection, while the control and the lowest toxicant concentration exhibited none of these behavioral changes. Further exposure (48–96 hours.) to the plant leaf extract promoted moderate to severe earlier stated behavioral abnormalities in the treated groups, in a concentration-dependent pattern, except for 21 mg/l treated group.

Table 5 *Effect of Cana indica leaf extract Acute Concentrations on Behavior of Clarias gariepinus fingerling at exposure duration intervals.*

Time in hours	Conc. (mg/l)	Gulping of air	Vertical erection	Respiratory distress	Erratic swimming	Abnormal mucus secretion
24	control	-	-	-	-	-
	21	+	-	-	-	-
	22	+	+	+	+	+
	23	++	++	++	++	+
	24	++	++	++	++	++
48	control	-	-	-	-	-
	21	+	-	-	-	-
	22	+	+	+	+	+
	23	++	++	++	++	+
	24	++	++	++	++	++
72	control	-	-	-	-	-
	21	-	-	-	-	-
	22	+	+	+	+	+
	23	++	++	++	++	+
	24	+++	+++	+++	+++	++
96	control	-	-	-	-	-
	21	-	-	-	-	-
	22	+	+	+	+	+
	23	+++	+++	+++	+++	+++
	24	+++	+++	+++	+++	+++

3.6 Histopathology of the gill of *C. gariepinus* Exposed to the different concentrations of the Ethanolic extract of *Cana indica*

The histological analysis of the gill arch in control specimens of *Clarias gariepinus* exhibited normal histological features. Abundant blood capillaries were observed with intact epithelial cells covering the lamellae. Nucleated erythrocytes were present within the blood capillaries, indicative of normal physiological function. The histological examination of tissue administered with 21mg/L of the toxicant demonstrated similar normal histological features to the control group. No significant pathological changes were observed, indicating minimal impact on gill structure and function.

In contrast, the groups exposed to higher concentrations of the toxicant (22mg/L, 23mg/L, and 24mg/L) exhibited severe alterations in gill morphology. There was a notable loss of epithelial cells covering the lamellae, leading to the exposure of underlying supporting cartilaginous tissue. This structural disruption suggests significant damage to the gill epithelium, compromising its barrier function and potentially impairing respiratory and ion regulation processes.

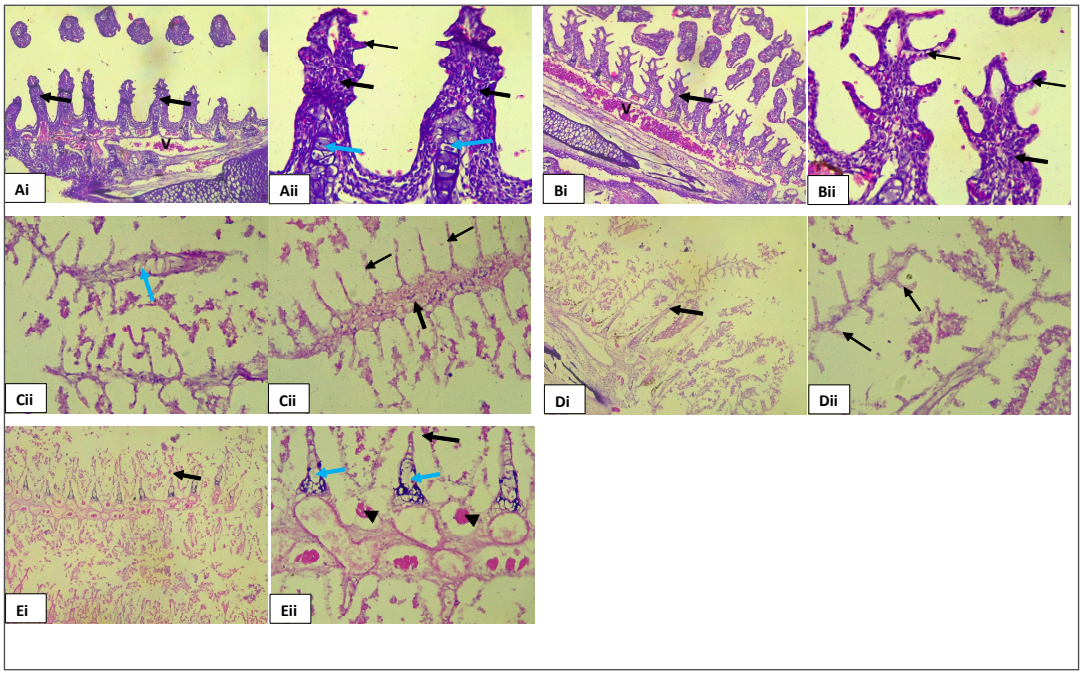

Figure 1. *A (i&ii) Showing Normal gill lamellae (primary filament (**thick arrow**) and the secondary (**thin arrow**) with no pathological changes at 0 mg/l of the extract. B(i&ii) showed normal gill arch, no pathologic changes seen. C(i&ii) and D(i&ii) showed increased loss of epithelial cells of the primary and secondary lamella across the gill arch at 22 mg/l and 23 mg/l concentration of the extract respectively. E (i&ii) Gill section showing diffused epithelial degeneration of the primary and secondary lamella at 24 mg/l concentration of the extract at X 100 and 400 magnifications.*

4. Discussion and Conclusion

4.1 Discussion

4.1.1 Initial Values of the Phsico-chemical parameters of the Experimental water

Prior to commencement of the experiment, the physico-chemical parameters measured (dissolved Oxygen = 5.8 mg/l, temperature = 29.5°C and pH = 6.97) were within the acceptable range for aquaculture operations. This could be attributed to the absence of impurities or contaminant in the test water (Vogels, 2000). Impurities, pollutants and contaminants are known to play a role either to elevate or reduce the different physico-chemical parameters in aquatic environment (Gbem *et.al*., 2001 and Idoho-Umeh, 2002).

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 (Udo, 2007, Ajah 2007, George *et.al*., 2013a; George *et.al*., 2013b; George *et.al*., 2014a; George *et.al*., 2015a, George, *et. al*., 2015 c).

4.1.2 Variations in water quality in the test media during the experimental period

The introduction of *Cana indica* into the test media led to fluctuations in water quality parameters over the course of the 96 -h bioassay period. Theses variations could have influenced the overall toxicity observed in the experimental groups. Water quality is critical to the growth and physiological processes of aquatic organisms (George, *et. al*., 2015a). Variations in the physico-chemical properties of water can alter physiological and metabolic processes of aquatic animal species, therefore it can be said that the activities of aquatic animals reflect the water quality conditions of the aquatic environment. Variations in dissolved oxygen, temperature and pH of the test media could be attributed to the pollution potential of *C. indica* leaf extract. Presence of contaminants / toxicants in different forms can affect the living conditions and physico-chemical properties of an aquatic ecosystem (George, *et. al*., 2015 a). Oribhabor and Akanse (2020) reported considerable changes in water quality such as temperature, ammonia, conductivity levels, following the application of *L. cuanescans* leaf extract in an experimental test media.

During the experimental period variations in the physico-chemical parameters were generally observed in the experimental aquaria in both batches. The values of physico-chemical parameters varied depending on time and concentration. As the concentration of the toxicant increased with time, the values of the physico-chemical parameters were observed to fluctuate when compared with the control. Similar observations have been reported in related studies by (George *et.al*.,

2013a; George *et.al*., 2013b; George *et.al*., 2014a; George *et.al*., 2015a, George *et. al*., 2023a, b).

It is a generally acceptable scientific finding that concentration influences the elevation and / or reduction in water quality parameters of test water during toxicity test (Heijerick *et.al*., 2003; Ayotunde *et.al*., 2011) couple with the fact that the organisms will also spend their absorbing oxygen in particular for survival (Ogundiran *et.al*., 2010; Adewoye, 2010a).

4.1.3 Preliminary Test

The primary objective of range finding test is to established a range of concentrations of *Cana indica* extract to be tested on *C. gariepinus*. This help in determining the concentration range where observable toxic effects occur. Though toxicity range values are usually found to be different for each toxicant and organisms (Bossayt and Jansen, 2005; George, *et. al*., 2023 c, d).

No mortality was recorded in the 0 mg/l (control) concentration of the toxicant used for the bioassay. Similarly, 100 % survivors were recorded in the 5, 10, 15 and 20 mg/l concentration of the extract with no mortality recorded. This provided crucial information on the actual range of doses to be used for the final toxicity test

4.1.4 Percentage Mortality and Survivors

The experimental groups which were exposed to the different concentrations of *C. indica*, exhibited mortality rates, with the exemption of the control group. This indicates that *cana indica* had a toxic effect on the fingerlings of *C. gariepinus*. Percentage mortality of *C. gariepinus* in the ethanolic extract of *C. indica* 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 21 mg/l concentration in each of the batches while 100 % mortality was recorded in each of the 22, 23 and 24 mg/l concentration of the extract. The results of the present findings are in consonance with the earlier reports by (George *et.al*., 2013a; George *et.al*., 2013b; George *et.al*., 2015b; George *et.al*., 2014a).

In the present study, the severity of the toxic effects observed in the fish is directly related to the concentration of the *C. indica* extract to which they were exposed. As the concentration of the extract increases, the adverse effects on the fish become more pronounced resulting in mortality as observed. The results of the present findings agree favourably with reports by different authors; (Ayuba *et. al*., 2002; Calta, *et.al.*, 2004; Adedeji *et.al*., 2008; Ogundiran *et.al.,* 2010, Essien-Ibok, 2020).

Oh *et.al*., (1991) gave three factors for the selective toxicity of toxicants for various fish species as: different inhibition of acetylcholinesterase, different detoxification and absorption. The above factors might have probably been responsible for the different toxic reaction showed by the fish in the different concentration and time during the period of experiment. The reactions are usually more pronounced at higher concentrations due to increased inhibition of acetylcholinesterase (Oh *et.al*., 1991) which eventually results in the death of the test organisms (Adedeji *et.al*., 2008; Ayotunde *et. al*., 2011; George *et al*., 2014a).

4.1.5 96 Hours LC_{50}

The 96 hours period is a standardized duration used in many toxicity tests, allowing for consistent comparisons between different substances and organisms. It represents a short-term exposure period, which can be particularly relevant for aquatic organisms that may experience acute toxicity effects over a relatively brief time frame. LC_{50} values helps in assessing the potential risks posed by substances to aquatic ecosystems and human health, allowing informed decision-making regarding their use and management (Udo *et.al*., 2006; George *et.al*., 2013a; 2013b, 2014 a and 2015a). The 96 hours LC_{50} is known to vary from toxicant (APHA, 1998) and from concentration to concentration of the toxicant (Laguan *et.al*., 2004; Ayotunde *et.al*., 210).

In the present study the 96 hours LC_{50} was 20.5 mg/l representing a log transformed concentration of 1.31 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment for both batches (A and B). The 96 hours LC_{50} of toxicants are known to vary for different toxicant. For instance, Ogundiran *et. al*., (2010) reported 96 hours LC_{50} of 0.0166 mg/l and 0.0038 mg/l for batch A and B *Clarias gariepinus* fingerlings under the toxicity effects of detergent effluents, 96 hours LC_{50} of 0.1 mg/l and 0.03 mg/l was reported by Adewoye, (2010b) when working on the effects of soap and detergent effluents on *Clarias gariepinus* fingerlings. Again, Ayotunde *et. al*., (2011) reported the 96 hours LC_{50} of 0,033 – 0.33 mg/l on *Clarias gariepinus* adults using *Carica papaya* extract.

In this study the 96 hours LC_{50} of 20.5 (1.31) 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 range finding test.

4.1.6 Behavioural Response to Toxic Effects of the Extract

The harmful effects of several range of toxicants can be measured via the behavioral expressions of the test organisms. In ecotoxicological studies, animal behavioral measurements are effective to assess environmental cum toxicant-induced stress. The present study showed that the test animal was highly sensitive to the toxic effect of *C. indica* leaf extract, following the behavioral aberrations exhibited, particularly in a concentration and duration-dependent pattern. Toxicant-induced stress such as excessive mucus secretion, air gulping, erratic swimming, respiratory distress and vertical erection, were evident in the treated groups and could further impair physiological processes and subsequent mortality (George, *et. al*., 2023 a, b, c, d). Plant piscicides have been reported to induce behavioral abnormalities in aquatic species such as hyperactivity, reduced swimming, loss of equilibrium, air gulping, incessant jumping in L. Camara exposed *C. gariepinus* fingerlings (George, *et.al*., 2023 a, d) and *P. capitata* exposed C. gariepinus fingerling (George, *et.al*., 2023 b, c), excessive mucus accumulation and jerky movement in both Crude oil (George, *et. al*., 2014 b) and *H. brasiliensis* (George, *et. al*., 2013 a) exposed *C. gariepinus* juveniles and fingerlings respectively.

4.1.7 Effects of the Extract on the gills of the test Organisms

Histopathology is an essential marker that reflects pathological changes in the tissues of the test's organisms due to exposure to contaminant or toxicant. In the present study, gill tissues of *C. gariepinus* exhibited prominent concentration-dependent histopathological changes after 96 hours exposure to ethanolic extract of *Cana indica*. The gills in fish undertake important physiological activities such as osmoregulation, ion regulation, gas exchange and excretion of nitrogenous waste (Arman, 2021). Gills are considered important indicators for measuring aquatic-based exposure to environmental pollutants (Arman, 2021), due to its direct and primary contact with pollutants. In the gills of the exposed groups, the plant piscicide induced lesions like cellular degeneration, hyperplasia, erosion of primary and secondary lamellar and epithelial degeneration. The evident histologic distortions in the gill could imply obvious disruption of respiration activities with primary impact on gaseous exchange across the lamellar epithelium of the gill (George, *et. al*., 2023 a, b c). Severe histopathological lesions such as degenerated epithelial cells and eroded lamellar were reported in the gill of *C. gariepinus* exposed to *Euphorbia hirta* leaf extract (Idowu, *et.al*., 2019); Audu *et al*. (2017) reported dose-dependent distortions in the gill of *C. gariepinus* exposed to acute concentrations of *Vernonia amygdalina*, George *et. al*., (2023 a) reported a dose-dependent distortions in the gills of *C. gariepinus* exposed to ethanolic extract of *Latana camara* and George *et. al*., (2023 b) reported severe pathological changes in gills of *C. gariepinus* exposed to ethanolic extract of *Phragmenthera capitata*.

4.2 Summary and Conclusion

Based on the findings of this study, we infer that *C. indica* leaf extract adversely affected the behavior of *C. gariepinus*. In addition, the plant extract induced variations in water quality parameters, prominent histopathological lesions in the gill tissues of the exposed bio model and subsequently mortality. Therefore, fisher folks are warned to apply caution and avoid indiscriminate application of the plant material during fishing as both targeted and non-targeted aquatic species may be harmed when in contact with the plant substance.

4.3 Recommendations

Further studies on the aspects of neurotoxic, molecular and genotoxic tendencies of *C. indica* to aquatic species are encouraged.

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