STUDIES ON MORTALITY AND HISTOPATHOLOGICAL ALTERATION ON THE GILLS OF *Oreochromisniloticus* JUVENILES FOLLOWING EXPOSURE TO ETHANOLIC EXTRACT OF *Phramentheracapitata* UNDER LABORATORY CONDITIONS

Abstract
The study was conducted using two replicate (Batch A and B) for 96 hours under controlled laboratory conditions, with five different concentrations of the extract ranging from 0 to 40 mg/l. The results of the study revealed that the extract had a concentration-dependent toxic effect on the test organisms, with induced behavioural changes such as abnormal and uncoordinated swimming movement, restlessness, respiratory difficulties, and attempts at jumping out. The alterations of physico-chemical parameters of the test water were also observed, indicating the adverse effects of the extract on the aquatic environment. The 96 hours LC50 for *Oreochromisniloticus* was determine to be 22.124 mg/l representing a log-transformed concentration of 1.345 mg/l. This is the concentration where 50% of the test organisms is expected to die at the end of the 96 hours' bioassay. The histopathological examination of the gills revealed pathological changes in the test organisms at concentrations of 20, 30, and 40 mg/l whereas no histomorphological changes was observed at 0 and 10 mg/l concentrations of the extract. The result of the present findings suggests that ethanolic extract of *P. capitata* had severe impacts on the test organisms resulting in mortality and gill damage.
Keywords: *Phramentheracapitata*, Mortality, Gills, Histomorphological, Oreochromis*niloticus*,

1.0 Introduction

The study aims to assess the toxic impacts of ethanolic extract of mistletoe on the survival and histopathology of the gills of Nile tilapia (*Oreochromisniloticus*). This research is important because it helps determine the safety of using mistletoe extract as a medicinal substance and also as a plant-based additive in fish feed formulation. By evaluating the survival rate and examining the gill tissue of tilapia exposed to different concentrations of the extract, the study provides valuable information on the toxicity of mistletoe extract on aquatic species.

Mistletoe is a parasitic plant commonly used in traditional medicine for its medicinal properties. However, the safety and efficacy of using mistletoe extract as a therapeutic agent have not been thoroughly studied. In particular, its potential impact on the gill, a vital organ for respiration, is not well understood.

Phragmentheracapitata is a species of plant in the family Loranthaceae, commonly known as the African mistletoe. It is native to Africa and temperate Asia. The genus contains over 35 species. (Flanagan, 1993; Cernusak, 2004). It is a hemi-parasitic plant that grows on various host trees, mostly in tropical and subtropical regions of Africa. (Flanagan, 1993; Cernusak, 2004). *Phragmentheracapitata* has oval-shaped leaves that are about 1-3 cm long and 0.5-1.5 cm wide. Its flowers are small and inconspicuous, with greenish-yellow petals that are fused at the base. The plant produces small, spherical berries that are about 0.5 cm in diameter and contain one or two seeds (Flanagan, 1993; Cernusak, 2004). In traditional African medicine, various parts of the *Phragmentheracapitata* plant are used to treat various ailments, including diarrhea, dysentery, stomach pains, and skin diseases. The plant also has cultural significance in some African communities and is used in various rituals and ceremonies.

Nile tilapia is a widely cultivated and consumed fish species, making it a valuable model organism for studying the toxic effects of substances in aquatic environments. The study of the toxic impacts of ethanolic extract of mistletoe on the survival and histopathology of the gills of Nile tilapia (*Oreochromisniloticus*) aims to fill this gap in knowledge and contribute to the safe use of mistletoe extract as a feed additive. In addition to monitoring survival and mortality, the study also examines the gill tissue of the fish to determine the effects of the extract on the gill morphology. This includes evaluating changes in the structure and function of the gill's cells and the presence of any pathological conditions.

However, the safety and efficacy of mistletoe extract as a therapeutic agent have not been thoroughly studied, and more research is needed to fully understand its potential health benefits and toxic effects. Despite its widespread use in traditional medicine, the use of mistletoe extract in medical practice should be done with caution, and its safety and efficacy should be established through rigorous scientific investigation. It is important to carryout independent toxicity studies on the extract of *Phramentheracapitata*to ascertain its level of toxicity and make useful recommendations for its effective use in the treatments of ailments and as a source of additive in fish feed formulation.

2.0 Materials and Methods

2.1 Collection of Test Organism

Juveniles of *Oreochromisniloticus* were collected from AkwaIbom State University fish farm, ObioAkpaAkwaIbom State, Nigeria located within 4^0 57'52" N and $7^0$45'29'' E. The climate of the area is tropical and is characterized by distinct wet and dry seasons. The vegetation of the study area is a 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) Juveniles were collected and used for the toxicity study.

2.2 Acclimatization of Specimen's

The juveniles were acclimatized in a re-circulatory glass aquarium measuring 96 x 50 x 29 cm containing fresh water for 24hours in the fisheries and aquaculture laboratory of AkwaIbom State fish farm. This enhanced the stability of the juveniles from stress of collection and transportation (Smith 1982, Udo *et al*, 2006).

2.3 Collection of Plant Sample

Fresh leaves of mistletoe (*PhragmentheraCapitita*) growing on Avocado Leaf (*Persea Americana*) was collected for the study. The collection site of the plant was IkotUdota in AfahaEket Local Government Area, AkwaIbom 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, AkwaIbom State University, IkotAkpaden, MkpatEnin 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 ponder 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, 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). In the due date of filtration, the mixture was filtered with Muslim cloth to acquire the filtrate. The extract was stored in 250ml 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 ×10 × 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 Kaglo (2001).

2.7 Stocking of Specimen

Each of the Ten (10) plastic aquaria was filled with two liters of fresh tap water and 10 juveniles of *Oreochromisniloticus* was stocked in each aquarium. The ethanolic extract of mistletoe (*P. capitata*) with varying concentrations was added to each stocked aquarium 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.

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 mistletoe (*P. capitata*) on the juveniles of *O. niloticus* was monitored on a 24 hours' basis for 96hours as recommended by Udo *et. al.,* (2006) and Ekanem and Ekpo (2008).

2.9.1 Determination of Mortality and Survival Rates of Juveniles

The percentage mortality and survival rates of the juveniles of *O. niloticus* in the different concentrations of the ethanolic extract of *P. capitata* 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 juveniles 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 (*Phragmantheracapitata*) on the juveniles of *O. niloticus* 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 juveniles that are expected to die was determined from the graph. Similarly, the concentration that will kill 5% of the stocked juveniles 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 Collection of Sample for Histopathology Examination

The gill's tissues of *O. niloticus* juveniles 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 *Phragmantheracapitata* was presented in tables. Two-way analysis of variance (ANOVA) was used to test for significant (P=.05) difference considering the extract concentration and mortality time. Also, the LD_{50} was determined using Probit analysis. All statistics were carried out using SPSS version 20.0.

3.0 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^{o}C for Temperature and 6.77 for pH.

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
Oreochromisniloticus	**5.2**	**29.8**	**6.77**

3.2 Variation in water Quality (Physico-chemical parameters) in the test media with *Oreochromisniloticus* as test organism (Batches A and B) during the experimental period (96 Hours)

Table 2 shows the variation recorded in the different physico-chemical parameters for *O. niloticus* (Batches A and B) in the different concentration of the toxicant and time.

Dissolved oxygen concentrations ranged between 4.00 – 5.2 mg/l in the Omg/l concentration of the toxicant. The highest DO value was recorded at the 24 hours of test with the least value recorded during the 96 Hour of Test.

In the 10 mg/l concentration of toxicant, dissolved oxygen ranged between 2.8 – 4.8 mg/l. the least value was recorded during the 96 hours of test while the highest value was recorded during the 24 hours of test.

In the 20 and 30 mg/l concentration of toxicant, dissolved oxygen ranged 3.4 – 4.1 mg/l and 2.8 – 3.8 mg/l respectively. The highest and least value of DO were observe at the 24 hours of test and 96 hours of test for both concentrations during the study duration.

In the 40 mg/l concentration of toxicant, dissolved oxygen value ranged from 2.4 – 3.2 mg/l. The highest value was recorded during the 24 hour of test and the least value was observed during the 96 hours of test.

Temperature value were observed to range between 27.1 – 27.7 ^{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 40 mg/l concentration of the extract while the highest value of 27.7 ^{o}C was recorded during the 24 hours of test in the 0 mg/l concentration of the toxicant (control).

The value of pH was observed to range between 5.80 – 6.31 during the 96 hours' experimental bioassay. The least value of 5.80 was recorded during the 96th hours of test in the 20 mg/l concentration of the toxicant while the highest value of 6.31was recorded during the 24 hours of test in the 0 mg/l concentration of the toxicant (control).

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

Parameters	Conc.	BATCH A				BATCH B			
	(mg/l)	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Dissolved	O	**5.2**	**4.7**	**4.4**	**4.0**	**5.2**	**4.7**	**4.4**	**4.0**
Oxygen (mg/l)	20	**4.8**	**4.0**	**3.2**	**2.8**	**4.8**	**4.0**	**3.2**	**2.8**
	30	**4.1**	**3.6**	**3.6**	**3.4**	**4.1**	**3.6**	**3.6**	**3.4**
Initial: 5.2 mg/l	40	**3.8**	**3.6**	**3.4**	**2.8**	**3.8**	**3.6**	**3.4**	**2.8**
	50	**3.2**	**3..0**	**2.6**	**2.4**	**3.2**	**3..0**	**2.6**	**2.4**
Temperature	O	**27.7**	**27.6**	**27.5**	**27.6**	**27.7**	**27.6**	**27.5**	**27.6**
(^{o}C)	20	**27.5**	**27.4**	**27.3**	**27.2**	**27.5**	**27.4**	**27.3**	**27.2**
	30	**27.6**	**27.5**	**27.3**	**27.3**	**27.6**	**27.5**	**27.3**	**27.3**
	40	**27.4**	**27.4**	**27.2**	**27.3**	**27.4**	**27.4**	**27.2**	**27.3**
Initial: 29.8 ^{o}C	50	**27.4**	**27.3**	**27.1**	**27.1**	**27.4**	**27.3**	**27.1**	**27.1**
pH	O	**6.31**	**6.25**	**6.22**	**6.12**	**6.31**	**6.25**	**6.22**	**6.12**
	20	**6.26**	**6.22**	**6.12**	**6.00**	**6.26**	**6.22**	**6.12**	**6.00**
Initial: 6.77	30	**6.21**	**6.20**	**6.00**	**5.80**	**6.21**	**6.20**	**6.00**	**5.80**
	40	**6.21**	**6.22**	**6.22**	**6.12**	**6.21**	**6.22**	**6.22**	**6.12**
	50	**6.16**	**6.05**	**6.02**	**6.00**	**6.16**	**6.05**	**6.02**	**6.00**

3.3Summary of the Percentage Mortality and survivors of *O. niloticus* in the different concentrations of theethanoic extract of *P. capitata* at the end of the experiment (96 hours).

The percentage mortality and survivors of *O. niloticus* 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. Similar observations was recorded for 10 mg/l concentration of the extract in both bathes.

During the 96-hour bioassay for 20 mg/l concentration of the extract, 40 % mortality and 60 % survivors were recorded for both batch A and B.

In the 30 mg/l concentration of the extract, 80 % mortality and 20 % survivors were recorded while in the 40 mg/l concentration of the extract all the test organisms were observed dead leaving 0 % survivors in both batches (Table 3). Statistical Analysis using Anova (SPSS 20.0) showed that there was no significant difference (P = .05) in mortality between the two batches.

Table 3:Summary of the Percentage Mortality and survivors of *0. niloticus* in the different concentrations of theethanolic extract of *P. capitata* 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
10	0	0	10	100	0	0	10	100
20	4	40	6	60	4	40	6	60
30	8	80	2	20	8	80	2	20
40	10	100	0	0	10	100	0	0

3.4 96 Hours LC_{50} Determination

The 96 hours LC_{50} for *O.niloticus* exposed to the different concentrations of the ethanoic extract of *P. capitata* is shown in Table 4 for both batches. The 96 hours LC_{50} is given at 22.124 mg/l representing a log transformed concentration of 1.345 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment.

Table 4: LC_{50} determinationfor *O. niloticus* at the end of the 96-hours bioassay.

Plant	Species	Probit	S. E	LC_{50} (mg/l)	Log Con. (mg/l)
Phragmentheracapitata	*Oreochromisniloticus*	P= -10.735 + 7.982X	0.111	22.124	1.345

3.5 Histopathology of the gill of *O. niloticus* Exposed to the different concentrations of the ethanolic extract of *Phragmentheracapitata.*

The gills of the control group displayed histological features within the normal range. The primary filament epithelium, secondary lamella, supporting cartilage epithelium, muscle fibers, and the capillary-rich apical part of the filament were all observed to be in a healthy state. The primary filament epithelium exhibited its characteristic structure and integrity, while the secondary lamella maintained its normal morphology. The supporting cartilage epithelium provided structural support, and the presence of well-developed muscle fibers contributed to the functionality of the gills. Additionally, the apical part of the filament exhibited a high cellularity and was abundantly supplied with capillaries, indicating an active and well-perfused region. Similar to the control group, Group 2 demonstrated normal histological features. The primary filament epithelium, secondary lamella, supporting cartilage epithelium, muscle fibers, and the capillary-rich apical part of the filament all appeared within the expected range. No significant deviations or abnormalities were observed in this group, suggesting that exposure to the ethanolic extracts of *P. capitata* did not induce histological changes in the gills. In both Group 3 and Group 4, histological analysis revealed diffuse epithelial degeneration in both the primary and secondary filaments. The degeneration appeared to affect the overall integrity and structure of the epithelial layer. Moreover, the apical part of the filament in these groups showed severe epithelial degeneration. The severe degenerative changes observed in these regions suggested a compromised condition of the gill tissue. In Group 5, complete degeneration of the secondary lamella epithelium and complete cellular loss in primary lamella was observed. The complete degeneration of the secondary lamella epithelium further emphasized the severity of the epithelial damage in the gill tissue of this group.

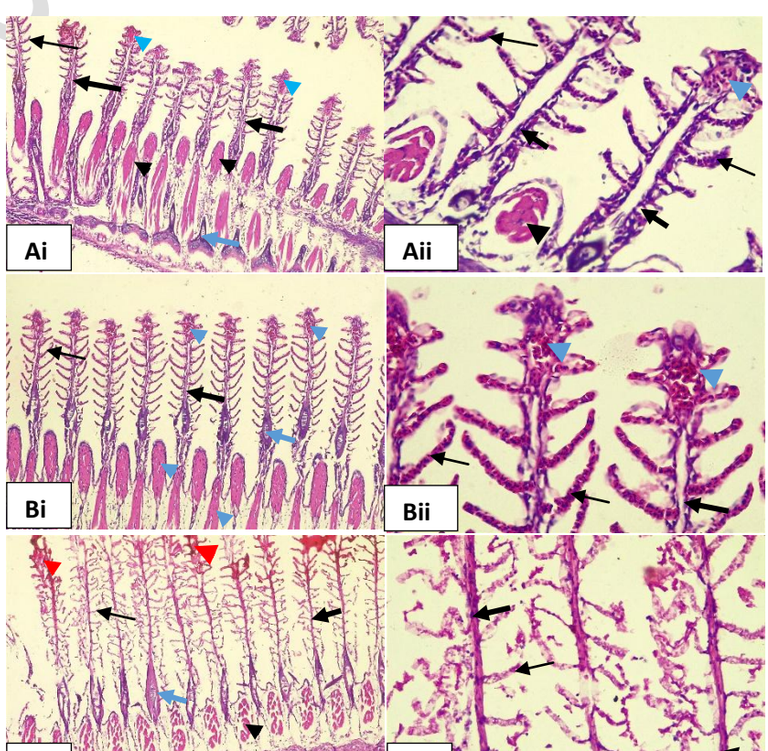

Figure 1: Photomicrograph of Gill arch tissue sections of Group 1-5. Haematoxylin and Eosin (H&E) stain. Each group was shown x100 and x400 magnification.
Control Group (Ai&Aii) showed gills with normal primary filament epithelium (thick black arrow), secondary lamella (thin arrow), the supporting cartilage epithelium (thick blue arrow), muscle fibre (black arrowhead) and the capillary-riched apical part of the filament (blue arrowhead). (Bi&Bii) showed normal histological features as the control. (Ci&Cii) and (Di&Dii) showed both primary and secondary filament but diffused epithelial degeneration, the apical part of the filament showed severe epithelial degeneration (red arrowhead). (Ei&Eii) depicted complete disruption of the secondary lamella epithelium and acellular primary lamella (thick black arrow).

4.0 Discussion

Three basic physico-chemical parameters were taken in line with standard practice in toxicological studies prior to stocking and introduction of the extract into the test media. A value of 5.2 mg/l was recorded for dissolved oxygen with a value of 29.8^{o}C recorded for temperature and a value of 6.77 was recorded for pH.

In aquaculture operations, there are standard threshold 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 ^{o}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*., 2014b; George *et.al*., 2015).

The initial values of the physico-chemical parameters of the experimental water were found to fall within the recommended threshold limits prior to the commencement of the experiment as previously reported by the authors under reference.

The unperturbed values of physico-chemical parameters observedd prior to the commencement of the experiment might be link to the absence of impurities or the toxicant and the organisms themselves (Vogels, 2000, Samabaswa and Rao, 1985; WHO, 1998). Impurities, pollutants and toxicants are known to play a role either to elevate or reduce the different physico-chemical parameters in aquatic environment (WHO, 1984; Idoho-Umeh, 2002 and Gbem*et.al*., 2001).

Variations in physico-chemical parameters were observed in the experimental aquaria in both batches during the experimental period. The values of physico-chemical parameters recorded varied depending on time and concentration of the extract. As the concentration of the toxicant increased with time, the values of the physico-chemical parameters were observed to sway when compared with the control.

This occurrence has been previously reported in the physico-chemical parameters of the test water media by Ayotunde and Ofem (2005) when reporting on the acute and chronic toxicity of *Carica papaya* seed powder to Nile tilapia (*Oreochromisniloticus*), Ayotunde*et. al.,* (2010) when investigating toxicity of *Carica papaya* seed powder to *Clariasgariepinus* fingerlings and effects on haematological parameters, Ayotunde*et. al*., (2011) when investigating the toxicity of *Carica papaya* on adult catfish (*Clariasgariepinus*), and Cagauan*et.al.*, (2004) when evaluating botanical piscides on *Oreochromisniloticus* and mosquito fish *Gambusiaaffinis.*

It is a commonly acceptable scientific finding that concentration stimulates the elevation and / or reduction in physico-chemical parameters of test water during an experiment (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, 2010).

The percentage mortality of *O. niloticus* in the ethanolic extract of *phragmentheracapitata*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 and 10 mg/l concentration of the toxicant. However, 40 % mortality was recorded in the 20 mg/l concentration in each of the batches while 80 % mortality was recorded in the 30 mg/l concentration of the toxicant. No survivor was recorded in the 40 mg /l concentration of the toxicant. The results of the present findings agrees with earlier

assertion by George *et.al*., (2013a) when reporting on the laboratory bioassay of the potential effect of rubber extract (*HeveaBrasiliensis*) on the Survival of fingerlings of *Oreochromisniloticus*; George *et.al*., (2013b) during their studies on the effect of lethal concentrations of rubber extract (*HeveaBrasiliensis*) on the survival of fingerlings of *Clariasgariepinus* under laboratory condition; George *et.al*., (2015) when working on the toxic effect of crude oil on hatchery reared *Oreochromisniloticus* fingerlings and George *et.al*., (2014) when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *Clariasgariepinus* juveniles.

Results from the present findings shows that percentage mortality was concentration dependent. The higher the concentration of the extract, the higher the percentage mortalities of the test organism. Similar results have been reported by different authors; Ogundiran*et.al.*, (2010) when investigating toxicological impacts of detergents effluents in juveniles of African catfish (*Clariasgariepinus*),Calta, *et.al.*, (2004) when studying the acute toxicity of the synthetic pyrethroid deltamethrin to young minnow carp (*cyprinuscarpio*), Ayuba*et. al*., (2002) when investigating on the acute toxicity of the root of Jimson's weed (*Daturainnoxia*) to the African catfish (*Clariasgariepinus*) fingerlings and Adedeji*et.al*., (2008) when investigating acute toxicity of diazinion to African catfish (*Clariasgariepinus*) fingerlings.

The 96 hours LC_{50} of any toxicant is the dose or concentration which kills 50 % of the stocked organisms at the end of the experimental period of 96 hours (4 days) (Samabaswa&Rao, 1985; Akpan *et. al*., 1999; Udo *et.al*., 2006; George *et.al*., 2013a; 2013b, 2014 and 2015).

The 96 hours LC_{50} is known to vary from toxicant (APHA, 1998; Samabaswa&Rao, 1985) and from concentration to concentration of the toxicant (Cagauan, *et.al*., 2004; Ayotunde*et.al*., 210).

In the present study the 96 hours LC50 was 22.124 mg/l representing a log concentration of 1.345 for both batches (A and B). The 96 hours LC_{50} of toxicants are known to vary as previously reported by the authors earlier sited above. In a related study, Ogundiran, *et. al*., (2010) reported 96 hours LC_{50} of 0.0166 mg/l and 0.0038 mg/l for batch A and B *Clariasgariepinus* 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, *et. al*., (2010) when working on the effects of soap and detergent effluents on *Clariasgariepinus* fingerlings. Again, Ayotunde, *et. al*., (2011) reported the 96 hours LC_{50} of 0,033 – 0.33 mg/l on Clariasgariepinus adults using Carica papaya extract. The varied 96 hours LC_{50} values usually obtained from different toxicants and test organisms is again reported by Ekanem*et. al*., (2011), when they reported a 96 hours LC_{50} of 5.0 ± 1.76 and 4.0 ± 1.76 mg/l for *Macrobrachiummacrobrachion* and *Macrobrachiumvollenhovenii*.

In this study the 96 hours LC_{50} of 22.124 mg/l obtained for both batch A and B may be attributed to the concentration of the toxicant finally used for the bioassay.

The effects of the ethanolic extract of *Phramentheracapitata*showed pathological effects on the gill lamellae of *Oreochromisniloticus* juveniles. However, the gill lamellae in the control (0 mg/l) and 10 mg/l concentration of the toxicant were not affected. In the 20 mg/l, 30 mg/l and 40 mg/l concentration both primary and secondary lamellae of the gills especially the apical part showed epithelial degeneration which is evident in the decreased or total loss of cellularity along the length and the apical part of the lamellae.

Pathological changes and degeneration of gill lamellae has been reported by several authors. Diana *et.al*., (2007) when investigating on the biochemical and histological effects of deltamethrim on *Carassiusauratusgibelio* with different effects such as lamellae cells hypertrophy and nuclear pycnosis in the basal cells, Gabriel *et. al*., (2007) reported histopathological changes in the gills of *Clariasgariepinus* 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*., (2015b) when reporting on the acute toxic effects of *Heveabrasiliensis* on the gills of hatchery reared *Oreochromisniloticus* fingerlings and observed histological changes in the gills of the exposed organisms which were concentration dependent, George *et.al*., (2014a) when investigating on the acute toxic effect of qua iboe light crude oil on the gills of *Clariasgariepinus* juveniles; Idowu*et. al*., (2019) when studying the effect of *Euphorbia hirta* leaf extract on histopathology of juveniles *Clariasgariepinus* and George *et.al*., (2014b) when reporting on the histopathological alterations in gills of fingerlings of *Clariasgariepinus* following sub-lethal acute exposure to *Heveabrasiliensis*,Audu*et al.* (2020) reported on the histopathological effects of unrefined water fractions of the foliage of *Balanitesaegyptiaca*on gills, kidney and liver of *Oreochromisniloticus*fingerlings. Similarly, Audu*et al.* (2017) examined histological changes in gills and liver of *C. gariepinus*intoxicated with acute concentrated grades of *Vernonia amygdalina*, Adesina, *et al.* (2013) evaluated the effect of acute toxicity of *Moringa oleifera* root extract on *O. niloticus*, Nasiruddin*et al.* (2012) investigated the histological alterations in organs of *Heteropneustesfossilis*intoxicated with extracts of three dry seed, while Oluwatoyin, (2011) studied the toxic effect of *Ipomoea aquatica* leaf extract on histopathology of *O. niloticus*. The result of the present findings on effects of toxicants on gills histomorphology agrees favourably with earlier assertion as reported by authors under reference.

4.1 Conclusion

Effects of ethanolic extract of *Phragmentheracapitata*(mistletoes) on the survival and histopathology of the gills of *Oreochromisniloticus* juveniles were investigated using static bioassay under laboratory condition. Prior to the toxicity test of the extract on the test organism physico-chemical parameters were taken before stocking of the experimental fish. Variations were observed in the physico-chemical parameters of the test media during the experimental period. The physico-chemical parameters (DO, temperature and pH) were observed to fluctuate with increased concentration of the extract with time when compared to the control. The variation observed in the test media during the 96 hours' bioassay is attributed to the introduction of the toxicant into the experimental aquaria which resulted in the mark shift or variation of these parameters as recorded within the period under study. Percentage mortality recorded in this study was observed to be concentration dependent with higher mortality recorded at higher concentrations in both batches. From the mortality's ratio the 96-hour LC_{50} was 22.124 mg/l for both batches. The results of histopathology which showed pathological changes in the gills of the test organisms in the 20, 30 and 40 mg/l concentration of the toxicant exception of 0 mg/l and 10 mg/l concentration of the toxicant which no pathological changes was observed depicts the mortality results which shows that percentage mortalities was concentration dependent with high mortality values recorded at higher concentrations. Also, histopathological results confirm the toxic effect of the extract at higher concentration which was evidence in the degeneration of the

primary and secondary gill lamellae observed during the period of the study. Based on the result of this findings, it is imperative to implement ecologically friendly methods to control invasive species within our environment. Additionally, due to the high cost of feed in aquaculture operations, further research on toxicity studies of common plants within our environment is recommended. This would help to identify which plants has the potentials to be used as a plant-based additive in the formulation of fish feed.

4.2 Recommendations

Based on the results of the findings which showed medium to high percentage mortalities when exposed to the ethanolic extract of *Phragmentheracapitata,* it is imperative that ecological friendly methods should be put in place to checkmates invasive species within our environment. However, further research is recommended on the use of *Phragmentheracapitate*in fish feed formulation at different inclusion level to confirm the results of the present findings. This recommendation stems from the fact that the toxicity level observed during the study was low except at higher concentrations. Also, based on the high cost of feed in aquaculture operations, this study recommends further research on the toxicity studies of common plant within our environment. This will help to know which of them can be used as a plant-based component in the formulation of fish feed.

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Competing Interests

Authors Declares that the article has no competing interest

Author Contribution

Author 1: 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 2: Conception, design and development of the protocol, supervision of the experiments, data analysis and reviewing the manuscript. Author 3: Student who actually played the supporting role in the experiments and reviewing the manuscript.

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