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INTESTINE HISTOLOGY OF JUVENILE *CLARIAS GARIEPINUS* ACUTELY EXPOSED TO *PARKIA BIGLOBOSA* HUSK EXTRACT

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ABSTRACT

This study was carried out to determine the phytochemical contents, 96-hours LC_{50} and the histological alterations in the intestine of Clarias gariepinus juveniles exposed to acute concentrations of Parkia biglobosa husk extract. The 96h bioassay experiment was carried out using 108 mg/L, 101 mg/L, 94 mg/L, 87mg/L, 80 mg/L and 0 mg/L (control) of the P. biglobosa husk extract in triplicates. Each tank was stocked with ten (10) C. gariepinus juveniles (18.26 ± 0.04g). The findings of this study revealed that the 96-hour LC_{50} for African catfish exposed to Parkia biglobosa husk was 89.1 mg/L. Clear evidence of hepatic tissue damage was observed in fish after the exposure period. The intestines of C. garipinus juveniles exposed to different concentrations of aqueous extract of P. biglobosa was altered.The major finding from this work was that aqueous husk extract of P. biglobosa is toxic to African catfish (C. gariepinus). This research work therefore proves that the indiscriminate introduction of P. biglobosa into water bodies is toxic and would threaten the existence and wellbeing of fish and other aquatic organisms causing a disruption in the ecosystem.

Keywords: Intestinal alterations, toxicity, *Parkia biglobosa*.

INTRODUCTION

Some plants are extolled for their medicinal and antimicrobial values, some others are however noted for theirpesticidal, acaricidal, trypanocidal, pisicidal properties depending on the type and concentrations of the bio-active compounds present (Atawodi, 2005). This is because plants contain structurally diverse biological substances with varying properties (Istvan 2000, Geidam*et al*., 2007).

Clarias gariepinus belongs to the family Clariidae, is widely distributed and readily available in African waters (Abalaka *et al.*, 2013). It is widely cultured throughout Africa in both natural and artificial habitats (Nguyen and Janssen, 2002; Adeyemo, 2008).This fish is known for its hardiness and this contributes to its position as an important fish species in Nigeria. There is a general acceptance for its taste, which consequently drives up the market price resulting in higher interest among artisanal fisherfolk. Some of these fisherfolk engage in unconventional means of harvesting, this includes the use of piscicidal plants (Ogundiran *et al.*, 2009, Kori-Siakpere and Ubogu, 2008). Some plants contain bioactive chemicals which have traditionally been used to harvest fish in many parts of the world (Ayoola, 2008; Suswam, 2015; Abalaka *et al.*, 2013). Farmers in Nigeria have persistently and indiscriminately abused these natural plant piscicides by using much higher concentrations than necessary to achieve a quick kill (Abalaka *et al.*, 2013; Suswam, 2015). *Parkia biglobosa* is one of such plants, it belongs to the family Mimosaceae and is widely distributed within the savannah belts of Nigeria where it is highly sought after for its medicinal and antimicrobial properties (Ajaiyeoba, 2002; Agunu *et al.*, 2005; El-Mahmood and Ameh, 2007). Traditionally, it is also used in local fishing communities to stun fish. The toxic parts of plants known to contain piscicidal properties include the bark, pods, fresh seeds and the pulp (Abalaka *et al.*, 2013). The toxicity of plants to fish arises from their phytochemical constituents and these stunning effects usually wear off within short time (Ojogu et al., 2017; Abalaka *et al.*, 2013). However, extremely high doses have been applied and consequently extract residues have been reported in exposed fish and even unintended species. Consequently, the indiscriminate use of high piscicidal plant concentrations could lead to total fish kill in an area and bioaccumulation by the surviving fish which poses a health risk to the final consumers (Van Andel, 2000). Fish are an excellent model for determining aquatic ecosystem health (Min and Kang, 2008).The African catfish has a fast growth rate and superior tolerance to deranged water quality, it remains the choice fish for research on aquatic ecotoxicity (Mahmoud et al., 2009). On acute toxicity Suswam (2015) reported that gill tissue is most sensitive to water pollution since gills are the primary site for osmoregulation and respiration. They are highly vulnerable to lesions due to their immediate contact with aquatic pollutants as they enter the body, the toxins exert their effect on the exposed surfaces of the fish especially the gills. Suswam (2015) found out that; there were mucus accumulations on the body surface and gill filaments of dead fish exposed to Roger. Butnariu, *et al., (*2016) reported that mucus accumulation results from increase in the activity of mucus cells subsequent to pollutant exposure. The accumulation of mucus on the gill epithelium might have impaired osmoregulation and gaseous exchange leading to suffocation. The acute toxicity of a chemical determines the end point from an ecological point of view. The survival, growth, reproduction and spawning success provide a positive feedback on adoption to environmental parameters regardless of whether they are natural or man-made.

Macroscopically, signals of toxicity are almost always preceded by change in organs, tissue, cellular and molecular levels (Lohiya *et al.*, 2002; Pathak *et al.*, 2000). Therefore, this study

focuses on the acute toxicity and phytochemical screening of *P. biglobosa* husk on *C. gariepinus* juveniles. It will provide background information related to frequent fish losses resulting from the use of toxic substances during fish harvest. Common domestic practices along riverbank in rural Nigeria include washing the pods of *P. biglobosa* off the seeds for local fermented dish (dawa-dawa) preparation. This usually leads to an unintended buildup to toxic concentrations in natural water, causing mass mortality of fish, contaminating the freshwater bodies and non-target organisms (Ayoola, 2008). C. *gariepinus* is a common fauna in the tropical freshwaters where it is widely used in aquaculture in several African countries. The use of piscicides as a tool in pond management during pond preparation to get rid of predators before fish stocking is an important tool. However, these chemicals have negative effects on the environment, farmers and health. There is paucity of information on the histopathologic and histomorphometric changes in the intestine of *C. gariepinus* fish acutely exposed to graded concentrations of *P. biglobosa* husk extract. Thus, the aim of this study is to evaluate the phytochemical contents, toxicity and histopathology of *P. biglobosa* husk extract on juvenile *Clarias gariepinus.*

MATERIALS AND METHODS

The study was carried out at the Hatchery complex, Department of Fisheries and Aquaculture Kogi State University, Anyigba, Nigeria.

Source and Preparation of *Parkia biglobosa*

Fresh samples of *P. biglobosa* seed were obtained from an open market in Dekina. They were identified and authenticated at the Department of Forestry and Wildlife Kogi State University Anyigba. The seeds were then boiled for 2 hours, after which cold water was added and pilled manually to collect the husks. The husks were collected and washed thoroughly with running distilled water. The husks were then oven-dried in the laboratory to a constant weight. The dried samples were ground into fine powder with a grinder in the laboratory, sifted with 0.25 mm sieve and then stored in an air-tight bottle.

Experimental Design

180 healthy, active juveniles of African Catfish (*Clarias gariepinus)* were collected from a reputable fish farm in Anyigba. The fishes were transported in oxygenated plastic bags to the Hatchery, Department of Fisheries and Aquaculture, Kogi State University Anyigba. The fishes were acclimatized in the Hatchery for two weeks during which they were fed commercial floating feed (Coppens) at 5% BW. Unconsumed feed and feces were removed, and the culture water replenished twice a week.

Preparation of Aqueous Extract of *Parkia biglobosa*

250 g of the resultant fine powder of *P. biglobosa husk was* weighed and dissolved in 1L of distilled water at a room temperature (25±5°C) in 1L sample bottle separately. The mixture was mixed by thorough shaking and allowed to stand for 24 h after which it was decanted and filtered using Whatman filter paper (125 mm). The filtrate was stored in an air-tight bottle and used for the bioassay tests.

Phytochemical Screening of *Parkia biglobosa*

This experiment was carried out in the Department of Biochemistry, Bayero University, Kano**.** Qualitative and quantitative phytochemical screening were carried out to determine the presence or absence of saponins, tannins, hydrocyanic glycoside, steroids, terpenoids, flavonoids, alkaloid as well as their concentrations using the standardized chemical method described by AOAC (2010).

Physicochemical analysis

This experiment was carried out in Fisheries and Aquaculture Laboratory, Kogi State University Anyigba. Temperature and Dissolved Oxygen were measured using a combined digital meter (Model JPB 607). pH was determined using pH meter (pH model 2602). Electrical Conductivity (EC) and Total Dissolved Solids (TDS) were measured using Hanna "Combo" portable metre (Hi 98129, Hanna Instruments, Inc Mauritius).

Range Finding Test/Preliminary Test

After the acclimatization period of 14 days, series of range finding tests were carried out to determine the toxicity level or suitable concentration of the prepared *P. biglobosa* aqueous extract of the husks that were used for the acute toxicity test.

Acute Bioassay Test

Eighteen ($1m^3$) concrete tanks were used for the 96h bioassay experiment, each containing 30l of dechlorinated municipal water. The acute lethal concentrations used were based on the result obtained from range finding test.108 mg/L, 101 mg/L, 94 mg/L, 87mg/L, 80 mg/L and 0 mg/L (control) in triplicate and a micro–pipette was used to introduce the aqueous *P. Biglobosa* husk extract into the tanks. Each tank was stocked with ten (10) *C. gariepinus* juveniles (18.26±0.04g). The fishes were selected randomly and used for experiment and were starved 24h prior to the experiment and during the exposure period. The tanks were continuously aerated using battery powered aerators and were covered with plastic nets to prevent the fish from jumping out. Behavioural changes and consequently mortality were observed and recorded 6h, 12h, 24h, 48h, 72h and 96h. During the exposure period, dead fishes were immediately removed to avoid polluting the culture media and were recorded. This test was conducted in accordance with ASTM (2002) methods for acute toxicity tests.

The 96–hour LC_{50} (lethal concentration that can cause 50% mortality) was determined using a probit analysis, arithmetic method of percentage mortality data, log concentration, graph and slope function, upper and lower confidence limits of the LC_{50} for acute toxicity test. D = LC_{84} + LC_{50}/ LC_{50} + LC_{16}/2. Where LC_{50} Probit value = 5.00, LC_{84}, probit value = 5.99, LC_{16} probit value = 4.01, D = the log dose concentration values, while Log10 D = 1.21, Log10 f = Log10 D ^ (2.77)/√. Where, N = numbers of individuals tested between the range of conc. corresponding to LC_{16} to LC_{84}, Ƒ = Frequency of individuals that are in the LC_{84}& LC_{16} range. N = 30+30+30+30+30, N = 150, Upper Limit = LC50 x f, Lower Limit = LC_{50} ÷ f LC_{50} = LC_{50} (Lower limit to Upper limit; 95% Confidence Limit).

Histological Assessment of the Intestine

Histological assessment of the intestine was carried out according to the method described by AOAC, (2000) at the Department of Pathology, Aminu Kano Teaching Hospital, Kano. The plates were read under a microscope at X400 magnification to observe changes in the architecture of the intestine and then microphotographs were taken.

Data Analysis

Data collected from the various experiments were subjected to analysis of variance (ANOVA) using Microsoft excel package 2013 and the treatment means were compared using Minitab 14. Significant means were set at $p<0.05$ and separated using Duncan new multiple range test.

RESULTS

The phytochemical screening of *P. biglobosa* extract revealed the presence and quantity of certain secondary metabolites in the plant extract as shown in Table 1. Steroids, Cardiac glycoside and terpernoids were not quantitatively detected by the phytochemical screening but were detected at medium and low levels respectively by quantitative analysis.

Table 1: Phytochemical Analysis of *P. biglobosa*

Chemical constituent	**Quantitative Analysis (mg/g)**	**Qualitative Analysis**
Saponin	4.15±0.03	++
Flavonoid	6.54±0.01	+++
Cardiac Glycoside	ND	++
Tannins	2.03±0.01	+
Alkaloid	3.01±0.01	+
Phenol	2.31±0.01	++
Steriods	ND	++
Carbohydrate	1.43±0.02	+++
Terpernoids	ND	+

KEYS
ND= Not Detected
+ = Low
++ = Medium
+++ = Relative

Table 2: Physicochemical parameters of toxicant water during the acute toxicity test of aqueous extract of *P. biglobosa* to *Clarias gariepinus* juveniles.

Treatment (mg/L)	pH	Temperature (^oC)	Electrical Conductivity (µS/cm)	Total Dissolved Solids (mg/L)	Dissolved Oxygen (mg/L)
Control	6.84± 0.00^a	27.63± 0.09^b	841.33±0.33^f	420.33± 0.33^e	4.00± 0.01^a
80	6.77± 0.01^b	27.10± 0.15^e	870± 0.88^e	434.67±0.33^d	3.64± 0.01^b
87	6.77± 0.01^b	27.77 ± 0.07^a	912.33± 0.33^d	456.00± 0.58^c	3.55± 0.01^c
94	6.68± 0.02^c	27.57± 0.12^d	929.67±0.88^c	465.33± 0.33^c	3.42± 0.01^d
101	6.41± 0.00^d	27.57± 0.12^d	953.33 ±1.66^b	47.67±1.33^b	3.26± 0.01^e
108	6.38± 0.01^e	27.60± 0.15^c	991.33± 0.67^a	496.67± 0.33^a	3.10± 0.01^f

Mean in the same column with different superscripts differ significantly (P≤0.05)

The mortality of *C. gariepinus* juveniles exposed to *P. biglobosa* for 96 hours is shown in Table 3 and Figure 2. No mortality was observed in the control bowls throughout the 96 hours exposure. The LC_{50} of the aqueous extract of *P. biglobosa* husk on *C. gariepinus* juveniles over the 96 hours exposure period was 89.8mg/l with the upper and lower confidence limit of 93.7mg/l and 86.1mg/l respectively.

Clarias gariepinus juveniles were stressed progressively with time before death. The pattern of mortality was similar to various concentrations of the botanical. The result of the mortality recorded for *C. gariepinus* juveniles exposed to *P. biglobosa* for 96 hours shows that at

concentrations of 80mg/L, 87mg/L and 94mg/L, 30, 43.3 and 56.7% mortality were recorded, respectively. At concentrations of 101mg/L and 108mg/L of 70 and 86%, respectively were recorded. There was an increase in the fish mortality with an increase in concentration of the toxicant, hence resulted in higher mortality rates.

Table 3: Mortality of *Clarias gariepinus* juveniles exposed to acute concentrations of aqueous *Parkia biglobosa* husk extract for 96 hours.

Table 3: Mortality of *C. gariepinus* juveniles exposed to *P. biglobosa* husk extract for 96 hours

Concentration	No. of fish	Total mortality	% Motality	Log Conc	Probit
0	30	0	0.0	0.00	0.00
80	30	9	30.0	1.90	4.48
87	30	13	43.3	1.94	4.82
94	30	17	56.7	1.97	5.18
101	30	21	70.0	2.00	5.52
108	30	26	86.7	2.03	6.13

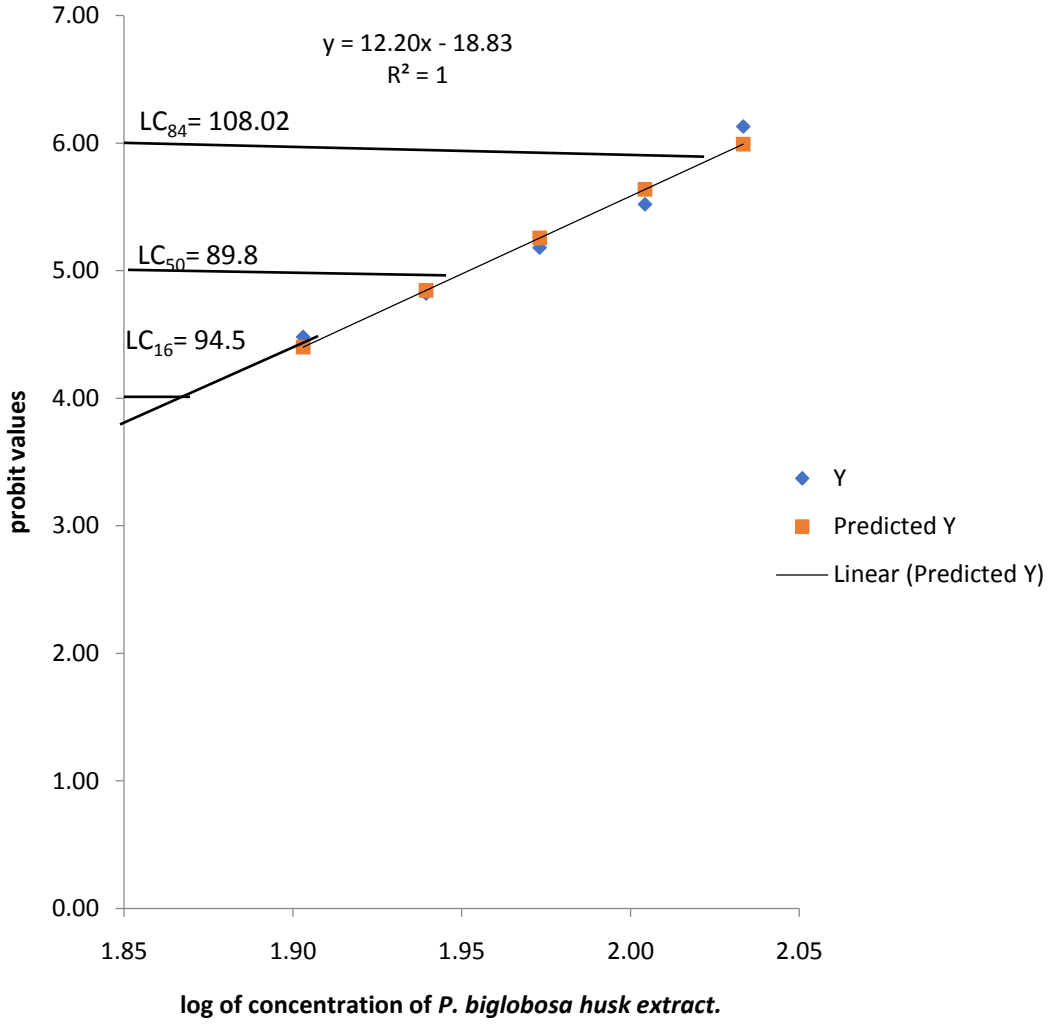

Fig. 1: Linear relationship between probit mortality and Log. Concentrations of *C. gariepinus* juveniles exposed to various concentrations of *P. biglobosa* for 96 hours.

The regression equation of the relationship estimated to be probit y = 12.208×log conc - 18.832, and on Rsquare value, R^2 =1. The expression, R^2 value indicates that, mortality rate of fish increased with increase in concentration of *P. biglobosa* pods extract. Several behavioural changes were observed ranging from excessive mucus secretion, erratic swimming, discoloration, air gulping, restlessness and settling at the bottom motionless just before death. These behavioural changes increased with increasing extract concentration. The histopathological alterations observed in the intestine of *C. gariepinus* juveniles are presented in Figs 2a-d is an indication of the toxic effect of *P. biglobosa* husk extracts.

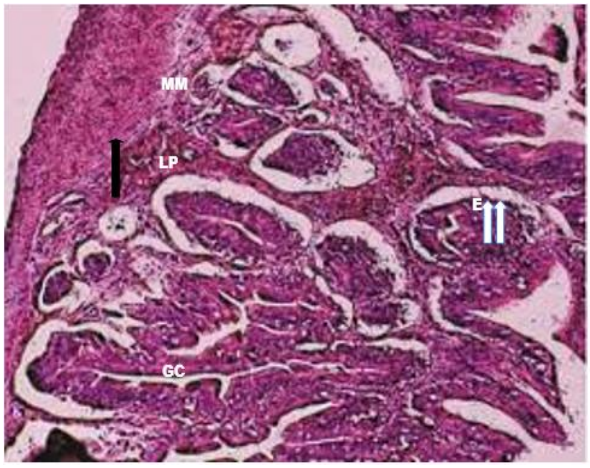

Fig 2a: Microphotograph (x400) of intestinal cells of *Clarias gariepinus* juveniles exposed to aqueous extracts of *P. biglobosa* husk at control (0.0 mg/L) showing normal architecture in control, the muscularis (black arrow), Muscularis Mucosa (MM), Epithelium (white arrow), Goblet cell (GC), Lamina Propria (LP).

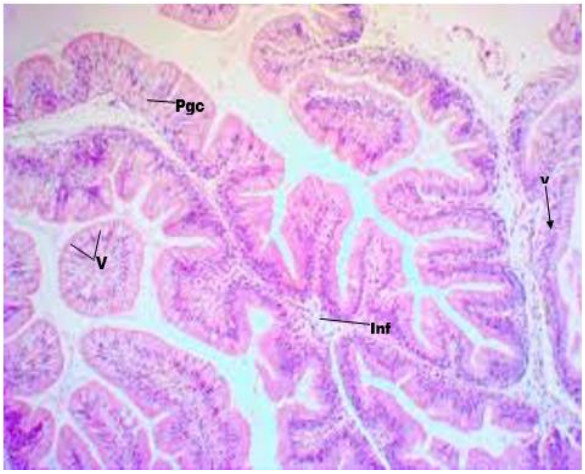

Fig 2b: Microphotograph of intestine cells of *Clarias gariepinus* juveniles exposed to aqueous extracts of *P. biglobosa* at 80mg/L showed polification of the goblet cell (pgc), vocuolation of epithelial cell (v), and inflammatory cell infiltration (inf) (x400).

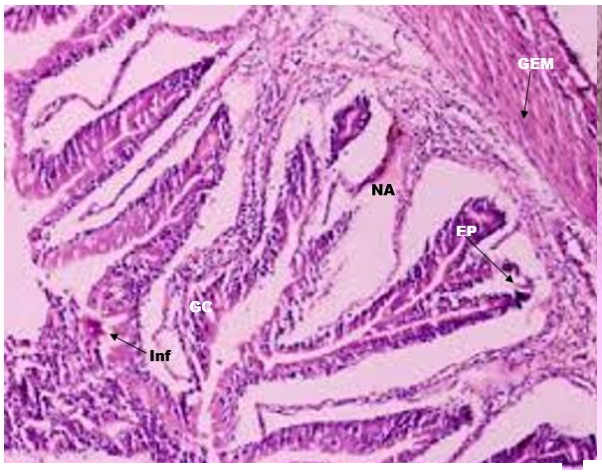

Fig 2c: Microphotograph of intestine of Clarias *gariepinus* juveniles exposed to aqueous extracts of *P. biglobosa* husk at 87 mg/L showed superficial gradual erosion of mucosa (SEM), inflammatory infiltrate (inf) epithelium linning (Ep) and necrotic area (Na).

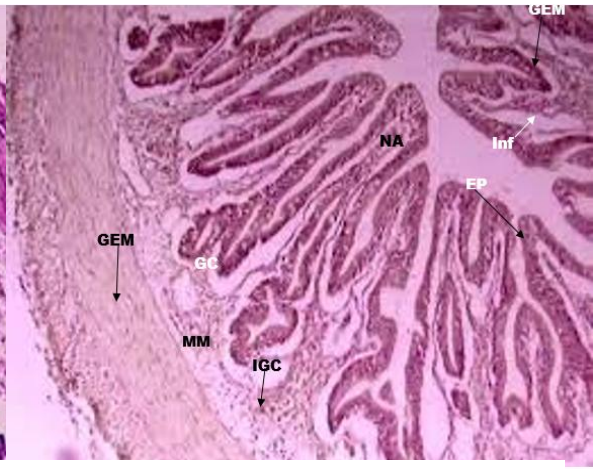

Fig 2d: Microphotograph of intestine of Clarias *gariepinus* juveniles exposed to aqueous extracts of *P. biglobosa husk* at 94 mg/L showed erosion of mucosal (GEM), irregular goblet cell (IGC), inflammatory infiltrate (inf) and muscularis mucosa (MM)(x400).

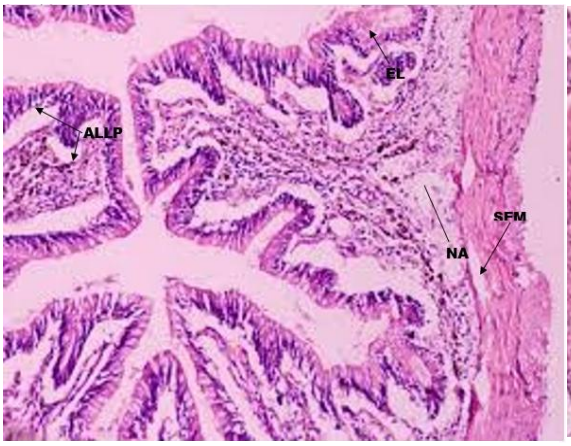

Fig 2e: Microphotograph of intestine of Clarias *gariepinus* juveniles exposed to aqueous extracts of *P. biglobosa husk* at 101 mg/L showed intestine with focal necrosis area (NA), superficial erosion mucosal (SEM), accumulation of lymphocytes in lamina propria (ALLP) and Epithelia lining (EL) (x400).

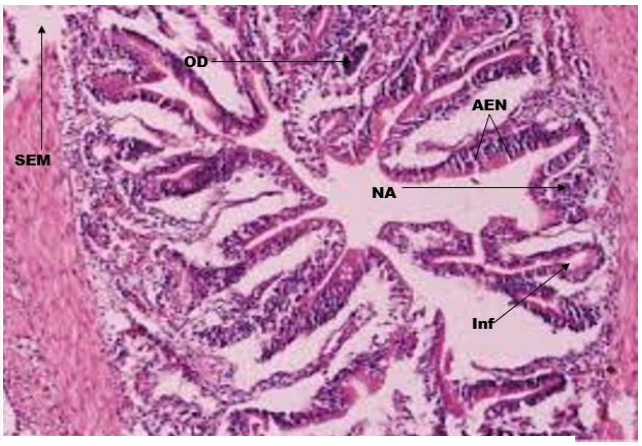

Fig 2f: Microphotograph of intestine cells of Clarias *gariepinus* juveniles exposed to aqueous extracts of *P. biglobosa* husk at 108 mg/L showed acute epithelial necrosis (AEN), Oedema degeneration (OD) and eosinophilic granules in the cytoplasm (x400).

DISCUSSION

Piscicidal plants like *Piptadeniastrium africanum, Lepidagathis alopecuroides, Balanite egyptica and Tephrosia vogelii* have been used by artisanal fishermen in obnoxious fishing practices (Akinlami *et al;* 2012; Obomanu *et al*; 2005; Absalom *et al;* 2013 and Akpa *et al.; 2010*). These researchers have identified similar bioactive compounds in several of these plants. The observed signs of toxicity including histological alterations as well as the ultimate mortality in some of the exposed fish might be due to the presence of these bioactive substances (Ologe and Sogbesan, 2007, Butnariu, *et al.*, 2015a). Tannins are reported to be cytotoxic, antineoplastic, nephrotoxic and hepatotoxic (Butnariu, *et al.*, 2016) due to their ability to precipitate exogenous and endogenous proteins. Steroids are known to inhibit oxidative phosphorylation with subsequent impairment of oxygen consumption in exposed fish (Tiwari and Singh; 2003, Butnariu and Coradini, 2012) and therefore, might have contributed to the observed signs of respiratory distress in the exposed fish. Poor water quality parameters have been reported to cause distress in *C. gariepinus* (Ayuba and Ofojekwu, 2002; Ojogu et al., 2017). The physicochemical parameters of the test water fluctuated slightly during the bioassay but were not thought to have caused fish distress nor result to mortality since they were within tolerance range (Fafioye, 2012, Butnariu, *et al.*, 2015b). The clinical signs and eventual deaths of exposed fish may be due to direct poisoning leading to pathological alterations in their tissues and organs (Abalaka and Auta; 2010). The expression of agitation by the fish correspond to both the contact and exertion phases of fish's response to toxicants exposures (Ojogu *et al.*, 2017) and it is their natural response in trying to escape from absorbing these offending extracts. The presence of excessive mucus secretion as reported by other studies (Fafioye *et al*; 2000, Abalaka and Auta, 2010, Orji *et al.*, 2014) as observed in this study was because of the increase in the activities of mucus cells subsequent to exposure to pollutants (Ojogu *et al;* 2017, Butnariu, 2012). However, such excessive mucus secretions are reported to reduce respiratory activity in fishes

(Adeogun *et al.,* 2012, Butu, *et al.,* 2014) which together with decreasing oxygen concentration constitute an established fish stressor (Ferencz, *et al.*, 2012).

At 89.1 mg/L concentration of *P. biglobosa* husk extract more than 50% of the fish population died. The LC_{50} has been reported as the standard which culminates in the acute lethal toxicity of pollutants to fish (Absalom *et al.,* 2013). The test toxicant used in this experiment (*P. biglobosa* husk extract) resulted in restlessness, erratic movements and gasping all indicative of a hypoxic state (Usman *et al.,* 2005; Butnariu, *et al.,* 2006; Ianculov, *et al.,* 2004). These reactions which usually lead to death have been observed as the response of fish to toxicants, Oranusi and Dahunsi (2013) found the 96 hour LC_{50} for *C. gariepinus to be* 0.32 mg/L when introduced to effluents from rubber processing plants, 0.35 mg/L was reported by Orji *et al;* 2014 when exposed to *Psychotria mychrophilla leaves*, 7.35 mg/L by Abalaka *et al*.(2015) when *Adenium obesum* stem bark extract was used and 12.9 mg/L was reported by Ayotunde (2010) using *Carica papaya* seeds. On the other hand, some other toxicants have shown higher toxicity to fish than *P. biglobosa.* The 96h LC_{50} of 89.8 mg/L obtained from this study was much lower than 430 mg/L, 105.83 mg/L; and 204.17 mg/L reported by Ayotunde (2006), Abalaka and Auta (2010) and Ayuba and Ofojekwu (2002) respectively who worked with different toxins of plant origin. Expectedly, the LC_{50} varies widely across type of plants, part of the plants used, size of fish, environmental factors, water quality parameters and selective action of toxicants (Butu, *et al.,* 2014, Caunii, *et al.,* 2015). These factors come into play and influence the reaction of the fish to toxic substances encountered in the wild. The toxic effects of these piscicidal substances are far reaching, and their action on the intestine was observed in this experiment. The two major entry sites of toxicants to the bloodstream and organs of a fish are the gills and intestine (Mohamed, 2008). The intestine in its role as the site where nutrients are absorbed was partially eroded by the toxicant (Fig 2c-d). Histopathological changes occurred in a concentration-dependent manner. Clear evidence of hepatic tissue damage was observed in the test subject (*C. gariepinus*).The feed and water a fish consume are the media through which pollutants enter its digestive tract, causing structural and functional deterioration of organs like the intestine (Younis *et al.,* 2015). This damage was observed by comparing the intestine of the control (fish to which the toxicant was not applied) and the test fish. The intestine from the control experiment had a normal architectural makeup of intestinal morphology, the Muscularis, Muscularis Mucosa, Epithelium, Goblet cell and Lamina propria (Fig 2a) were intact. The intestine of the treated fish showed histo–pathological changes (Figs 2b-f). These alterations can cause several physiological stresses, leading to eventual suffocation and death of the fish. The intestines showed specific responses to the presence of the toxins which included inflammatory cell infiltration, vacuolation of epithelial cell, proliferation of goblet cells superficial erosion of the mucosa, acute epithelia necrosis, oedema degeneration and after 96 hours of exposure, necrotic areas were evident (Figs 2b-f). Braunbeck and Appelbaum (1999) also found that in the intestine, exposure to endosulfan is associated with changes in the epithelial lining, which indicates disturbance of the intestinal absorption capacity. Cengiz *et al.* (2001) reported oedema, degeneration, accumulation of lymphocytes in the lamina propria, pycnotic state of nuclei, and necrosis in the intestine of *Gambusia affinis* exposed to endosulfan. The intestine of *Cyprinus carpio* exposed to chlorinated pesticides aldrin, dieldrin, BHC and DDT showed fusion of intestinal folds and acute epithelial necrosis (Satyanarayan *et al.,* 2012). Das and Gupta (2013a) found superficial erosion of mucosa, dense lamina propria, chronic inflammatory cell infiltration, vacuolation and haemorrhage of mucosa and submucosa in *Esomus danricus* exposed to sublethal concentrations of

endosulfan. This goes to show that the more toxic the toxicant is, the more changes occur to the intestinal tract.

In conclusion this study has shown that, the aqueous husk extract of *P. biglobosa* was acutely toxic to African catfish (*C. gariepinus)* when exposed to 89.8 mg/L or higher concentrations. The toxic effects were reflected in the behavioural changes, intestinal pathological alterations and mortality of the fish.

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