

Sub-lethal toxicity of unripe akee apple (*Blighia sapida*) fruit extract on *Clarias gariepinus* fingerlings

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ABSTRACT

The experiment was carried out to know the accumulative effect of unripe ackee apple fruit on the fingerlings of *Clarias gariepinus*. Range finding test (96-hour bioassays) was conducted to determine the concentration to be used for acute and sub-lethal toxicity test of unripe ackee apple fruit on catfish, *Clarias gariepinus* fingerlings. Acute test was conducted to determine its toxicity using the 96-hour bioassays under laboratory condition and the LC₅₀ was determined graphically as 3.40ppt. Mean mortality was 0, 40, 60, 70 and 80% in the concentrations of 0, 3.33, 3.47, 3.60 and 3.73ppt. *Clarias gariepinus* showed erratic swimming pattern, loss of reflex which increased with increase in concentration. Statistical analysis of the results showed that there were significant differences ($P < 0.05$) on the effect of concentration. Mortality increased with increase in concentration of toxicant and duration of exposure. Sub-lethal test was carried out and it lasted for 14 days. The concentrations used were 0, 1.50, 2.00, 2.50 and 3.00ppt. The introduction of the toxicant did not result in high mortality but was chronic through accumulation. The highest mortality recorded was 30% and it occurred between 8th and 14th day of the experiment. Histopathological changes were revealed in the gills, skin and heart of the fish used.

Keywords: Ackee apple, *Clarias gariepinus*, toxicity, sub-lethal

INTRODUCTION

Blighia sapida of the family Sapindaceae is one of nature's gifts which have been highly utilized for various purposes by man. It is both known for its food value and its poisonous properties (Morton, 1987). For many years, it was believed that the unripe ackee fruit may be poisonous containing a natural toxin. This is being used by farmers to harvest fish obnoxiously, and when the fish is taken, could lead to vomiting, gastro intestinal contamination and seizure. There is no standard method of treating

this problem (Atolani *et al.*, 2009). As reported by Akintayo *et al.* (2002), the clinical and chemical studies carried out on ackee revealed that it contains toxic substance called hypoglycin A (HGA) and hypoglycin B (HGB). It is known that the unripe arils contain hypoglycin A (α -amino- β (2 methylene cyclopropyl) propionic acid in a concentration 100 times higher than those in the ripe ackee fruit (Golden, 2002) and it is believed that the toxic substance is dispelled by light as the jacket of the ripe fruit opens (Barennes *et al.*, 2004).

Onusiriuka and Ufodike (2000) reported the effect of sub-lethal concentration of ackee apple on the tissue chemistry of African catfish which was damaged to the liver and gills. They also reported that Hypoglycin A is a water-soluble liver toxin that induces hypoglycaemia by inhibiting glucogenesis and limiting the activity of cofactors that are required.

Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of the pollutants entering the water or the lethal effects on aquatic organisms (Fagbenro, 2002). Water extract of the floral of *Blighia sapida* have been reported to be toxic to adult *Clarias gariepinus* causing increased opercular and tail beats and mortality (Onusiriuka and Ufodike, 2000). The result of this study showed that unripe ackee apple fruit is highly poisonous and more lethal to *Clarias gariepinus* fingerlings because of the age and size.

MATERIALS AND METHOD

Clarias gariepinus fingerlings ($10\text{g}\pm 0.2$) were acclimatized in 20 litres capacity plastic tanks for two days prior to the commencement of the study. They were starved for 48 hours to empty their gastro-intestinal tract. Unripe ackee apple concentrate was prepared by dissolving one pod of fruit containing three large arilli (100 ± 0.9) in 75cl water at room temperature. The concentrate was sieved using a 0.1mm sieve. The solution was filtered to remove particles. Range finding test was carried out to determine the concentration used in the definitive test. The fish were distributed randomly in triplicate treatments into 15 plastic tanks (10 fish per tank) and the exposure treatments were 0.00, 3.33, 3.47, 3.60 and 3.73ppt. The introduction of the concentrate was followed by swirling the water with Teflon to disperse the test material. The exposure

lasted for 96 hours and the experiment was monitored at 3hours interval (Ward and Parrish, 1982). Mortality of the fish was recorded and failure to respond to external stimuli was used as criteria for death. Sub-lethal test was carried out and it lasted for 14 days. The concentrations used were 0, 1.50, 2.00, 2.50 and 3.00ppt. The temperature of the water used was measured using mercury-in-glass thermometer, pH with pH meter, dissolved oxygen with a digital dissolved oxygen meter and dissolved carbon dioxide with dissolved carbon dioxide meter. The water analysis was carried out every six hours for the 96hours of the experiment.

Histopathological examination was carried out on the gills; heart and skin of dead fish using standard histological techniques (Columbia CNMTL Histology laboratory Manual, 2011-2012) after the organs had been removed and preserved in 10% formal saline solution. The LC_{50} for acute test was determined graphically using Boyd, (2005) method.

RESULTS

A definitive test (acute test), showed that mortality increased with increase in concentration of ackee apple and time of exposure (Figure 1) and the LC_{50} for acute test was 3.40ppt (Figure 2). Further studies to determine the accumulative effect of ackee apple on *Clarias gariepinus* fingerlings involved sub-lethal test and the histopathological studies is as shown in plates 1-6. The skin, gills and heart of the fish were examined histologically and these showed pathological changes except in the control experiment. Plate 1, 3 and 5 showed the normal (control) structure of the skin, gill and heart of *Clarias gariepinus* fingerlings respectively, exposed to 0.00ppt concentration of ackee apple and showed no histological alteration. Plates 2, 4 and 6 represent the histological changes observed in the fish exposed to concentration 3.00 ppt which

was the highest concentration tested. Plate 2 showed the skin of the fish at concentration 3.00 ppt revealing several lymphoid aggregations with fibrous connective tissue at the dermal and

epidermal areas of the skin and erosion of the dermal layer. Plate 4 also showed the damage of gill filament and lamellae, the gill structure was altered and this

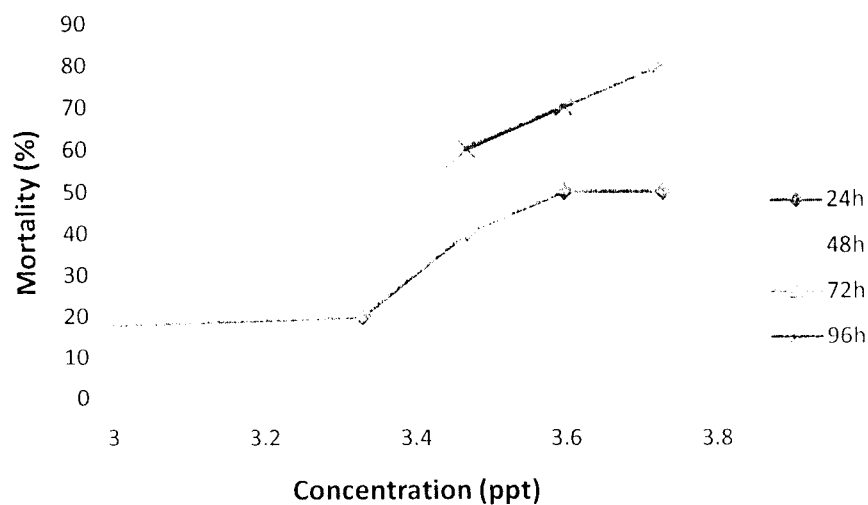


Fig. 1. Effect of time and concentration on mortality in acute test

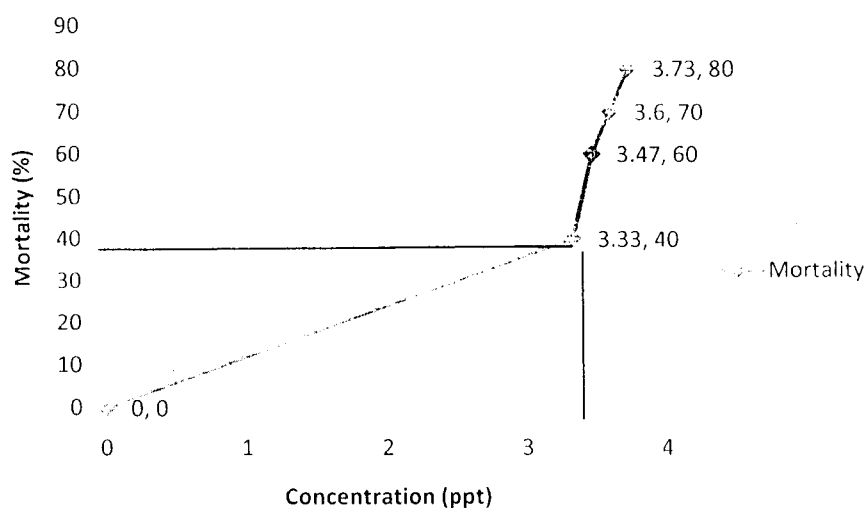


Fig. 2. LC₅₀ of acute toxicity test (3.40)

involved vacuolation, cellular infiltration, epithelial hyperplasia and mucosal erosion. Plate 6 revealed the heart of the fish showing significant level of cellular changes while there were traces of lesions on the heart tissues with degeneration in muscle bundles, focal area of necrosis, vacuolar degeneration, atrophy of muscle bundles, edema between bundles and splitting of muscles.

The water quality variables in the control and exposure concentrations did not differ. The pH was 7.2 ± 0.01 , temperature; 25.30 ± 0.13 and dissolved oxygen; 5.45 ± 0.04 . The result of this experiment revealed that fish of the same age and size react differently when exposed to different levels of ackee apple unripe fruit extract.



Plate 1. Skin of *Clarias gariepinus* exposed to 0.00ppt (Control experiment) showing no histological alteration. (Magnification= X40)



Plate 2. The skin of fish at concentration 3.00ppt showing erosion of dermal layer. (Magnification=X40)

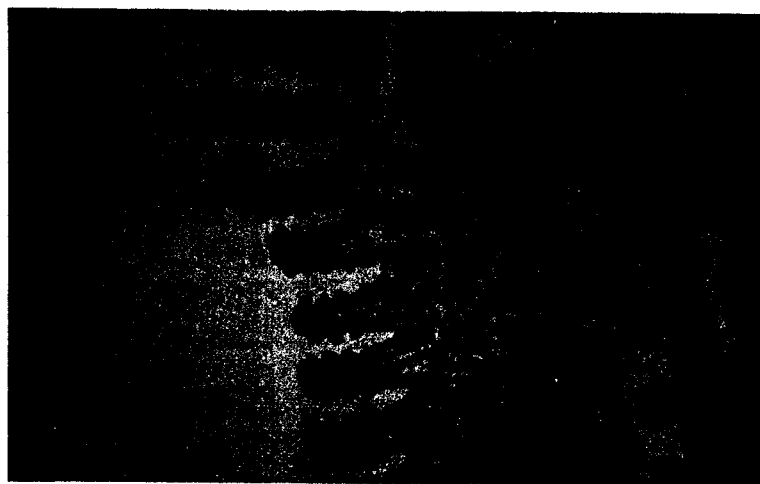


Plate 3. The gill of *Clarias gariepinus* exposed to 0.00ppt (Control experiment) showing no histological alteration (Magnification= X40)

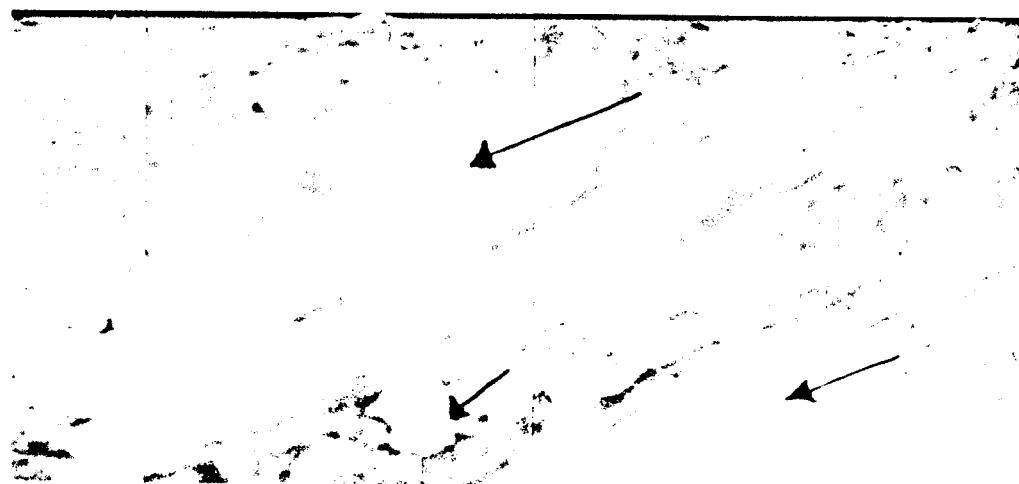


Plate 4: The gill structure showing alterations in the gill filament



Plate 5. The heart of *Clarias gariepinus* exposed to 0.00ppt (Control experiment) showing no histological alteration (Magnification= X40)



Plate 6. The heart of the fish exposed to concentration 3.00 ppt showing significant level of cellular changes with degeneration in muscle bundles. (Magnification=X40).

The behavioural changes and mortality in *Clarias gariepinus* fingerlings when exposed to different sub-lethal concentrations of unripe *Blighia sapida* unripe fruit extract are presented in Table 1. Loss of reflex was present in concentrations 2.00 ppt and 2.5 ppt but highly present in concentration 3.00 ppt. Erratic swimming pattern and peeling of skin was present in concentration 2.50 ppt but

very high in concentration 3.00 ppt. Discolouration and hyper-ventilation was noticed in concentration 1.50 and 2.00 ppt but very high in 2.50 and 3.00 ppt. Mortality was recorded in concentrations 2.50 and 3.00 ppt which was 20% and 30% respectively. The varied morphological changes recorded were more evident at higher concentrations and increased with time of exposure.

Table 1: Behavioural changes and mortality of *C. gariepinus* during exposure to ackee apple unripe fruit extract.

Behaviour	0.00	1.50	2.00	2.50	3.00
Loss of reflex	-	-	+	+	++
Erratic Swimming	-	-	-	+	++
Peeling of skin	-	-	-	+	++
Discolouration	-	+	+	++	++
Hyper-ventilation	-	+	+	++	++
Mortality(%)	0	0	0	20	30

Key: Not present, + Present, ++ Highly present

DISCUSSION

The study clearly revealed that *Blighia sapida* unripe fruit is poisonous to *Clarias gariepinus* fingerlings as shown in the histopathological changes of the fish organs such as the Liver which is the main organ for detoxification that suffered serious morphological alteration at higher concentrations. The same effect was reported by Fafioye *et al.*, (2004) who studied the acute toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles.

The fruit aril of ackee apple is edible when fully ripped but when unripe; it is believed to be poisonous, containing a natural toxin (Atolani *et al.*, 2009). The unripe fruit of ackee is considered to be responsible for the vomiting sickness, an acute condition, frequently observed in Jamaica, which is characterized by v, omiting and a coma (Meda, 1992), these could be the reason for a change in behaviour of *Clarias gariepinus* immediately the toxicant was introduced into the water used. Larson *et al.*, (1994) reported additional symptoms associated with chronic ackee apple fruit ingestion including cholestatic jaundice, abdominal pain and elevated liver function values.

Clarias gariepinus fingerlings were stressed progressively with time before death and at higher concentrations, rate of mortality increased, this was also reported by Fafioye, (2012) who carried out an acute and sub-lethal test of five plants (Almond- *Terminalia catappa*, Pawpaw- *Carica papaya*, Neem- *Azadirachta indica*, Tobacco- *Nicotiana tobacum* and Cassava- *Manihot esculenta*, on white tilapia, *Oreochromis niloticus* (Trewavas). Fish of the same age and size reacts differently to the toxicant and mortality occurred at different periods, this was similar to the findings of James and Cheryl, (1993) who showed that hybrid of striped bass of the same age

exhibited differential tolerance to lethal ammonia concentration.

Dissolved oxygen level reduced greatly within the 96 hour bioassay and the great alteration in gill structure may reveal loss of oxygen-carbon dioxide exchange, these may act as criterion for mortality. These histopathological changes in gills are similar to epithelial damages caused by cadmium (Oronsaye, 1997). Highest percentage of mortality was recorded within the first 24 hours of acute test; this was similarly reported by Eileen (1991) who recorded total mortality within 24 hours when copper concentrations of 2.61 and 5.60 mg/l were used on juvenile red drum, *Sciaenops ocellatus* and 97% mortality within 24 hours when 1.36 mg/l of copper was used. A very significant observation was the change in colour of the catfish to light yellow which may be taken after the colour of toxicant used. A thin layer of the toxicant was observed covering the whole surface of the water used for the experiment, this retarded the rate of dissolved oxygen uptake by water and so caused a lower dissolved oxygen concentration and mortality of the fish, this was also reported by Oronsaye (1997). Water quality variables were within acceptable ranges for toxicity test (APIA, 1985), they may not have acted synergistically with the toxicant to affect the behaviour as well as mortality recorded in fish. The same observation was made by Keremah *et al.*, (2010) in relative toxicity of aqueous leaf extracts of *Lepadogathis alopecuroides* (vahl) to the clariids, *Clarias gariepinus* and *Heterobranchus bidosalis* fingerlings.

In conclusion, the use of plant poison to catch fish is still used in many places in the world today. Most fish poisons, also called ichthyotoxics or piscicides, occur in several plant species and a variety of chemicals found in these plants will stun fish when it passes through the gills or in some cases ingested. The fish then floats to the

surface for easy catch. The results has shown that unripe ackee apple extract is toxic and kills *Clarias gariepinus* fingerlings (the recruits) faster than the adult fish at all concentration therefore, avoidance of its use in harvesting fish in the wild is advocated.

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