**ACUTE TOXICITY OF AQUEOUS *Morinda lucida* LEAF EXTRACTS TO**

**NILE TILAPIA, *Oreochromis niloticus* (LINNAEUS 1857)**

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***ABSTRACT***

Acute toxicity tests (range finding and definitive) using aqueous extracts of *Morinda lucida* on *Oreochromis niloticus* fingerlings (mean wt., 6.2g ± 1.2g) were conducted in a static bioassay inside plastic tanks. In the range-finding test, the concentration range tested was 20, 40, 60, 80 and100g *M. lucida/*L of water while 70, 72, 74, 76 and 78 *M. lucida*/L of water was used in the definitive test. The LC50 at 24 hours was 1.869g *M. lucida* g/L of water. There were five concentrations with control and each treatment was replicated twice. For each test, 15 *O. niloticus* fingerlings were used in each plastic tank. The responses exhibited by *O. niloticus* fingerlings subjected to the toxicant include erratic swimming, loss of reflex, colour change, weakened motion and vertical swimming. These were enhanced by the increase in concentration of the toxicant and the duration of exposure. During the 24 hours of range finding test, no mortality occurred at concentrations 20, 40, 60, 80 and 100 g *M. lucida*/L. No mortality was recorded in the 96 hours at concentration 20.0g*M.lucida*/L. Histological changes occurred in the gills and liver of the fish in the definitive test as gill alterations (hydropic degeneration of the gill rays, degeneration of the gill lamellae and necrosis) which were usually related to gills function disorders. Liver shows hepato-cellular architecture, hydropic degeneration, vacuolation of the liver cells and spaces within the cell protoplasm filled with fluid. This effect intensified with increasing *M. lucida* concentration.

**INTRODUCTION**

The Nile tilapia, *Oreochromis niloticus,* is an important cultured fish species in Nigeria. The main advantage of tilapia is relatively low cost of production, mainly for fry and seed, and the quality of its flesh. The attributes that make Nile tilapia so suitable for fish farming, are its resistance against harsh conditions, ease of breeding, rapid growth rate, ability of efficiently convert organic and domestic wastes into high quality protein, and good taste (de Graaf *et al*., 1999). Other advantages are its herbivorous nature and its mouth brooding habits, tolerance of poor water quality and fast growth at warm temperature.

*Morinda lucida* is a species yielding dyes, timber, fuel and traditional medicines. The leaves are use as the remedies against different type of fever. *M. lucida* known as ‘Oruwo’ in south-western Nigeria, it is a medium-sized tree at maturity. The stem bark infusion is used as an anti-malarial and antidiabetic (Burkill, 1997), anti-malarial activity (Tona et al., 1999; Agomo et al., 1992; Asuzu and Chineme, 1990); Makinde and Obih, 1985; Koumaglo et al., 1992), anti-Salmonella typhi activity (Akinyemi et al.,2005),effect on contractivity of isolated uterine smooth muscle of pregnant and non pregnant mice(Elias et al.,2007),toxicity and mutagenic studies (Sowemimo *et al.,* 2007; Akinboro and Bakare, 2007; Koumaglo *et al.,* 1992; Raji *et al.,* 2005); and anti-diabetic property(Olajide *et al*., 1999) of *Morinda lucida* extracts have all been reported

*M. lucida* is a multipurpose species yielding dyes, timber, fuel and traditional medicines (Abbiw, 1990). The useful parts of *M. lucida* are mostly collected from wild plants. Only occasionally are plants grown in home gardens. Propagation is possible by seed and cuttings. The genus *Morinda* comprises about 80 species and occurs throughout the tropics. In Africa five species are found. The comparatively small flowering and fruiting heads on long slender peduncles are distinctive characteristics of *M*. *lucida*. The growth and development of *M. lucida* is from February to May, fruiting from April to June. *M. lucida* grows in grassland, exposed hillsides, thickest, forests, often on termite mounds, sometimes in areas which are regularly flooded, from sea-level up to 1300m altitude. In West Africa, *M. lucida* is an important plant in traditional medicine. *Morinda lucida* is an important plant used in traditional medicine. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever. *M. lucida* is one of the four most used traditional medicines against fever. *M. lucida* grows in grassland, exposed hill sides, thickest forest often on the mite mounds. The useful parts of *M. lucida* are mostly collected from wild plants only occasionally are they grown in home gardens (Adesida and Adesogan, 1972). In West Africa, the roots of *M. lucida* are sold in load shops and markets, both as dyestuff and medicine. Leaves and twigs are sold in markets as a medicinal tonic for young children in Africa.

Aquatic toxicity tests are used to detect and evaluate the potential toxicological effects of chemicals or toxicants which exhibit changes in the aquatic environment. Studies have revealed that organisms exposed to chemical or toxicants usually exhibit changes in opercula rate and may cause physical damages to fish particularly on the gill surfaces (Davis, 1973). Toxic substances may be introduced deliberately or accidentally into the aquatic ecosystem, impairing the quality of water and making it unsuitable for aquatic life. When the concentration of the toxic substance is higher than what the homeostasis of the fish can control, it results in death or cause damages in the fish opercula and may also cause physical damages to fish particularly on the skin, liver and gill surface.

The objectives of this study are to determine the lethal concentration (LC50) value of *O.* *niloticus* fingerlings exposed to varying concentrations of *M. lucida* leaf extract (dry) and determine the effects of acute and sub-lethal concentrations of *M. lucida* extract on histopathology of gills and liver tissues of *O. niloticus* fingerlings.

**MATERIALS AND METHODS**

*O. niloticus* fingerlings, (mean weight 6.2g ± 1.2g) were purchased and were acclimated for 48 hours prior to toxicity tests inside plastic tanks (30 L capacity). Each tank was filled with 15 litres of water obtained from the borehole. Fish were fed to satiation daily with a commercial 35% crude protein pelleted feed. Feeding was discontinued 24 hours prior to the commencement of the tests. Individual weights of the fingerlings were measured with a top-loading mettler balance and distributed randomly in duplicate treatments at 15 fingerlings/tank. Leaves of *M.* *lucida* were collected around the Federal University of Technology, Akure and sun-dried at ambient temperature, and milled into powder.

Arange finding test served as a preliminary test which was followed by definitive test. In the range finding test, five triplicate treatments using five test concentrations of 20.0, 40.0, 60.0, 80.0, 100.0g *M. lucida* /L of spring water were used. *O. niloticus* fingerlings were stocked into each tank for 24 hours prior to the introduction of *M. lucida* leaf extract (dry) to the water. The range finding test lasted for 96 hours and was checked initially at four hours intervals followed by 12 hours intervals. Mortality of the fingerlings was routinely monitored and recorded. The failure to respond to external stimuli was used as an index of death. LC50 is the concentration of *M. lucida* extract estimated to the lethal to 50 of the test organism after 96 hours of exposure using probit analysis and by graphical method. Five concentrations of *M. lucida* used in the definitive test were 70.0 72.0, 74.0, 76.0g and 78.0g *M. lucida*/L. The concentrations were prepared arithmetically and followed the results obtained from the range-finding test. The test lasted for 96 hours and *O. niloticus* fingerlings mortality was monitored at 3 hours followed by 12 hours intervals. The dissolved oxygen concentration, pH and temperature were determined every 24 hours. The behaviour and mortality of the fish were observed and recorded after 24 hours.

The 96 hour LC50 was estimated by probit analysis as described by Wardlaw (1985) and by graphical method. At the end of the definitive test, *O. niloticus* fingerlings were dissected to remove the gills and liver. The organs were fixed in 10% formalin for 72 hours, dehydrated in graded level, alcohol (50, 70, 90, 100%) after which they were cleared in 50/50 mixture of alcohol and xylene for 3 hours, then 100% xylene for three hours and impregnated in molten wax, oven-dried for 6 hours after which they were embedded in Petri dishes with wax. The specimens were mounted and sectioned 8µ thickness prior to staining in haematocylin and eosin. Photomicrographs were taken with Leitz (Ortholux) microscope fitted with camera.

**RESULTS**

During the range finding test, *O. niloticus* exhibited various reactions which included, erratic movement, vertical swimming position, colour change to dark brown, weakened swimming motions, sudden jerky swimming movement, and changes in opercula rate. All fish in the control treatment survived throughout the 96 hours duration of the experiment. Fish mortality in the varying concentrations (Table 1) increased with increasing concentration of the *M. lucida* used. In the definitive test, fish swam weakly, settling at the bottom of the tank. They showed less movement with increase in duration of the exposure, and showed increased weakness, remaining motionless most of the time. The LC50 aws determined as 1.87 mg/L. The summary of histological observations in the gills and liver of *O. niloticus* exposed to varying concentrations of *M. lucida* are presented in Tables 1 and 2, respectively.

Table 1: Histological changes in gills of *O. niloticus* fingerlings.

|  |  |
| --- | --- |
| Concentration  (g/L) | Histological observation |
| 0 | No visible alteration on the gill ray |
| 70 | normal gill architecture |
| 72 | inflammation of the gill lamella |
| 74 | degeneration of the gill architecture |
| 76 | hydropic degeneration in the gill lamellae and disintegration of the gill architecture |
| 78 | erosion of the gill filaments and gill rakers |

Table 2: Histological changes in liver of *O.* *niloticus* fingerlings.

|  |  |
| --- | --- |
| Concentration  (g/L) | Histological Observation |
| 0 | Normal hepato-cellular architecture |
| 70 | normal liver architecture. |
| 72 | Normal heptocellular architecture of the liver cells. |
| 74 | Hydropic degeneration in the hepatic parenchyma of the liver cells |
| 76 | Severe alteration in the liver architecture |
| 78 | vacuolation within the cell protoplasm field with fluid |

**DISCUSSION**

Different behaviour shown demonstrated to be sensitive indicator of the physiological stress in fish subjected to sub lethal concentrations of pollutant (Davis, 1973) when the pollutant was introduced into the water containing the test fish, the fish at first displayed attempt to jump out. After being stressed, they resorted to settling at the bottom of the plastic tank and became motionless with slow opercula movement. The abnormal behaviour displayed by the fish subjected to *M.* *lucida* increased with increasing concentration of the pollutant used. The observation of fish response in this experiment agreed with Akinbulumo (2005) who reported that fish showed toxic reactions to *Derris elliptica* root powder by surfacing jaws and becoming stupefied. Also, according to Pascual *et al*., (1994) fish staying at the bottom of the plastic tank is a sign of stress or weakness.

The fish in the *M. lucida* solution showed erratic swimming, loss of reflex, vertical swimming position, colour changes (discolouration) and weakened swimming motion (Table1and 2). Fish response observed in the study agreed with similar observation made by White (1980) in Atlantic herring, *Clupea harengus*, exposed to dinoflagallate toxins. The abnormal swimming and changes in colour are indicative of stress which might lead to mortality (Chan, 1982). The test fish showed abnormal swimming and subsequently erratic movement and they finally settled to the bottom, showing signs of exhaustion, stress after which they move along the tank bottom and finally die off. This was in accordance with Liong *et al.,* 1988, moreover according to Pascual *et al.* (1994) suspected that fish staying at the bottom of the rubber tank is a sign of weakness.

*O. niloticus* fingerlings survived in low concentration of *Morinda lucida* and died at higher concentration (table 1and 2). In this study, the replicates gave different fish mortality values. This agrees with the observation of Chen and Lei (1990) who observed that juveniles of penaeus monodon showed differences in the tolerance to ammonia and nitrate solutions.

There were significant difference in the treated water in range finding test and also there were significant difference between the central medium and treated water in definitive test in terms of physico-chemical measurement (Table 4).

The results of physico-chemical parameters of the experimental water at the end of the experiment, 96 hours of introduction of toxicant are given in (table 4).Water temperature in the experimental tanks was affected by the concentration of *Morinda lucida*, also the dissolve 02 content of the samples were within the range desirable (>3ppm) for the optimum growth of *Orechromis niloticus* (Alex Bocek *et al*, 1991).

Water temperature was within the range desirable (24-280C) for the optimum growth of *O.niloticus* (Alex Bocek *et al.,* 1991). The pH of the test media obtained from the experiment indicated that the addition of *M.lucida* increased the pH.

**Histological changes**

Histological examinations of *O. niloticus* gave significant indication of toxicity of *M. lucida* (Table 5 and 6). The effects include gill alterations such as: hydropic degeneration in the gill lamellae, inflammation of the gill, and disintegration of the gill architecture and fusion which denotes gill functional disorders which may affect the fish physiology or cause death of the fish. In this study, observations showed that the damage of the liver cells increased with increasing concentration and duration of exposure to the toxicant. Liver alteration such as, normal hepato-cellular of the liver cells, hydropic degeneration in the hepatic parenchyma of the liver cells, which are usually related to liver functional disorder, which may affect the physiology and caused death and spaces within the cell protoplasm filled with fluid.

The histopathological changes detected seem to have been caused by the toxicant *M. lucida*, while the mortality recorded could be due to the malfunctioning of the gills and the disorder of the liver. The results showed that *M. lucida* is toxic to *O. niloticus* fingerlings. The results of this study showed that the survival of *O. niloticus* wasdirectly related to the concentration of *M. lucida* in solution. The water quality parameters increased concentration of *M. lucida* with time. There was a visible effect of *M. lucida* concentration on histopathological alterations/changes in the gills of the *O. niloticus* fingerlings these include; degeneration of gill architecture, disintegration of the gill and erosion of the gill filaments and gill rakes. Histopathological evidence of the gill damaged caused by *M. lucida* toxicity was evident resulting from malfunctioning of the gill.

The liver is an important centre of metabolism of various substances and supporting the stability of intra-circumstances of organism, therefore the changes that occur in the liver would interfere with the normal metabolic function of the liver cells. Mortality may be as a result of the disorder of the liver. Changes that occur in the liver include: degeneration in hepatic parenchyma, hydropic degeneration and hepato-cellular of the liver cells and necrosis. The 96 hours LC50 of *M. lucida* dry extract to *O. niloticus* was at 1.869g *M*. *lucida/*L of water at 24 hours.

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